SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

Proceedings of a meeting held at

Nairn, Inverness, Scotland

on the 30th, 31st March and 1st April 2005

Edited by D.J. Mellor, A.M. Russell and J.L.N. Wood

ISBN 0 948073 69 1

ACKNOWLEDGEMENTS

The following bodies provided financial support for the conference and the publication of these proceedings:

Inverness and Nairn Enterprise

Merial Animal Health

Pfizer Animal Health

Scottish Agricultural College

CONTENTS

***

PLENARY PAPER

TOWARDS ERADICATION OF BOVINE TUBERCULOSIS IN IRELAND:

A CRITICAL REVIEW OF PROGRESS

S.J. MORE[1]

SUMMARY

There has been a national bovine tuberculosis eradication programme in Ireland since 1954. Initial progress was rapid, but has subsequently stalled despite the implementation of each of the accepted elements of disease control. Based on results from the East Offaly and four area projects, there is now conclusive evidence that wildlife (specifically transmission of infection from badgers to cattle) are a key constraint to disease eradication in Ireland, with cattle-to-cattle transmission of relatively lesser importance. Ireland is currently implementing a comprehensive strategy to address this constraint, whilst maintaining existing measures to control cattle-to-cattle transmission. In the short-term, a national programme of wildlife control has been implemented in areas of high disease prevalence. In the longer term, Ireland is committed to the development of an effective badger vaccine and the implementation of a strategic programme of badger vaccination.

AN OVERVIEW OF THE ERADICATION PROGRAMME

Initial progress

There has been a national bovine tuberculosis eradication programme in Ireland since 1954. During the initial stages of this programme, progress was rapid leading to a considerable reduction in the prevalence of the disease by the mid 1960s. At this point, however, progress stalled (Sheehy & Christiansen, 1991), although disease prevalence has subsequently remained low (Fig. 1). From the mid 1960s to the late 1980s, national attention focused on issues relating to quality control and biosecurity, and testing standards were subject to intense scrutiny. During this period, pre-movement testing was made mandatory, animal identification and the ability to trace animals was improved, strategic disease control measures were introduced in areas of high prevalence, and data analysis was enhanced following the advent of computerisation.

[1] Simon More, Centre for Veterinary Epidemiology and Risk Analysis, Faculty of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland. Email: simon.more@ucd.ie

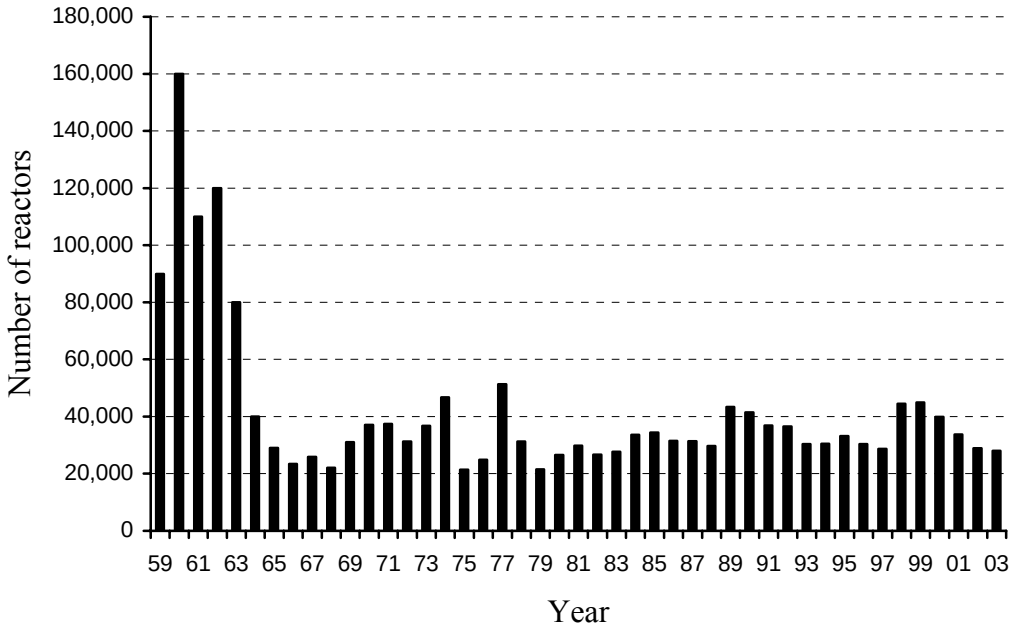


Fig. 1 The number of bovine tuberculin reactors detected in Ireland each year, between 1959 and 2003

The establishment of ERAD

In 1988, a major initiative was undertaken with the launch of the Eradication of Animal Diseases Board (ERAD), a new executive agency to oversee the management of the eradication programme. Over the following four years, an exhaustive programme of tuberculin testing (44 million tests on the 7 million cattle in the national herd over 4 years) was implemented, together with an extensive range of new and improved support measures. These measures included the refinement of a programme management system, a reactor collection service and improved compensation/hardship grants; random sample testing of herds by government veterinarians; the establishment of a specialised epidemiological research and tuberculosis investigation unit together with supporting laboratory services; continuation of a pre-movement test; improved control of dealers; depopulation of problem herds; improved cattle tags and checking of cattle at factories and marts; extended restriction and de-restrictions; the establishment of local ERAD committees and a TB farm advisory service; the development and implementation of a farmer awareness campaign, covering a range of issues including disease-proof fencing and cleansing and disinfection; improved *post-mortem* procedures during factory surveillance; establishment of badger research and control services; improved control of slurry/factory waste; control of calf movements; attention to the cleaning of trucks of the reactor collection service; and improved District Veterinary Office (DVO) procedures (Downey, 1990, 1992). However, despite these intensive measures, and a substantial investment of financial and human resources, no substantive progress was observed.

To the present day

Following this period and to the present day, Ireland continues to implement a comprehensive disease control programme. As part of the ERAD legacy, a detailed research programme was initiated to identify and address constraints to disease eradication in Ireland. There has also been significant epidemiological input into policy formulation.

CATTLE-TO-CATTLE TRANSMISSION

The importance of cattle-to-cattle transmission has been reviewed by Griffin & Dolan (1995), based on information from experimental and observational studies. There is evidence of bacterial excretion from some infected animals (Pollock & Neill, 2002), and transmission of infection has been demonstrated under experimental conditions (Griffin & Dolan, 1995). However, based on evidence from the field, cattle-to-cattle transmission (measured indirectly using incidence of disease – but not necessarily infection – among in-contact animals) may be relatively uncommon under Irish conditions. Although brought-in animals have been identified as an important cause of herd breakdowns, there is generally little evidence of transmission from each primary case (Flanagan et al., 1998; Griffin, 1991). Similarly, substantial breakdowns are not common, despite very close contact during winter housing. Nonetheless, it is frequently difficult to determine the specific cause of herd breakdowns, and therefore the relative importance of cattle-to-cattle transmission and other sources of infection. This is particularly problematic in situations where higher rates of within-herd prevalence are found, and it is often difficult to distinguish between lateral spread and a common source, such as wildlife (Griffin, 1992). For this reason, a weighting system is now used to enable field staff to rate the relative importance of a series of infection sources (O'Keeffe & Higgins, 2003). Given this background, and in order to quantify the relative importance of cattle-to-cattle transmission under Irish field conditions, it is essential first to understand the importance of transmission of infection from wildlife.

THE IMPORTANCE OF WILDLIFE

Building evidence

Ongoing disease problems, despite comprehensive eradication programmes to prevent cattle-to-cattle transmission, have been experiences in a number of countries, notably New Zealand, the United Kingdom and, more recently, the state of Michigan in the USA. In each of these countries, the existence of a wildlife reservoir of *Mycobacterium bovis*, the causal agent of tuberculosis in cattle, has impeded, or has been perceived to impede, eradication of this zoonotic disease (Krebs et al., 1997; Morris & Pfeiffer, 1995; O'Brien et al., 2002).

In Ireland, evidence has been building of the potential role of badgers *(Meles meles)* in bovine tuberculosis, including recognition that badgers are highly susceptible to *M. bovis* infection (Gormley & Costello, 2003), with tuberculosis being endemic within the badger population in Ireland (O'Boyle et al., 2003). Indeed, a prevalence approaching 50% was identified in a recent survey (L. Corner, unpublished). *Mycobacterium bovis* infection in Irish badgers was first reported in 1975 (Noonan et al., 1975). Further, there has been a range of observational epidemiological evidence linking badgers and tuberculosis in cattle, including: (1) an association between the risk of herd breakdown and distance to badger setts in co. Offaly, with risk increasing in association with the number of infected badgers that were captured in the

nearest infected sett (Martin et al., 1997). However, results from later work, using data from co. Kilkenny, were less certain, using detailed methods to quantify badger exposure, including the period of time that cows had access to housing and to specific farm fragments (during grazing) (Olea-Popelka et al., unpublished); (2) the identification of identical strains of *M. bovis* in local cattle and badger populations (Costello et al., 1999); and (3) ongoing disease problems, despite intensive disease control efforts in Ireland aimed at early detection and prevention of cattle-to-cattle transmission.

Definitive evidence

The above-mentioned information, on its own, is not sufficient to prove disease causation. In particular, it provides little direct evidence in support of a temporal relationship (providing evidence for transmission of *M. bovis* from badgers to cattle). To illustrate, it is possible to have coincident disease (with identical strains) in local badgers and cattle but without badgers being the source of infection. This could occur, for example, if cattle were to infect badgers (and not *vice-versa*). Given this context, a field trial offers the best opportunity to assess critically the impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Therefore, the East Offaly and four area projects have been studies of major national importance. Each has sought to provide conclusive evidence of the contribution, or otherwise, of badgers to bovine tuberculosis.

The East Offaly project was conducted during 1989 to 1995, with badgers being proactively removed under licence from a central inner Project Area (528 km^2) and outer Buffer Zone (210 km^2), but not from the surrounding Control Area (1456 km^2). These areas, which were centred in county Offaly, were similar in terms of cattle husbandry, land-type and land-use, badger densities at project start, and tuberculin testing regimens (both in terms of frequency and interpretation). A total of 1,264 badgers (an average of 0.34 badgers/km^2/year; with 12% disease prevalence based on lesion detection at *post-mortem*) were removed from the Project area during the 7-year study period, with a removal intensity during the first 2 years of the study (when 71.0% of badgers were captured (Dolan et al., 1994)) of 0.85 badgers/km^2/year. Based on multivariable analyses, there was a significantly lower proportion of new confirmed tuberculous herd restrictions among cattle in the Project Area as compared the Control Area (Ó'Máirtín et al., 1998a; Ó'Máirtín et al., 1998b). According to Eves (1999), the most striking change was the absence of large outbreaks of disease in cattle in the Project Area in later years of the project. This effect has continued to the present day, with the rate of herd restrictions within the Project Area generally remaining at approximately one-third of the national average (Bob Hammond, personal communication). Although concern has been raised about the use of 'doughnut-type' design and the potential for continuing migration of badgers from the Control to the Project Area (Phillips et al., 2003), this will have had the effect of making it harder to detect a treatment effect, if one was present.

The four area project sought to build on the East Offaly project, and to determine the effect of badger removal at a number of sites representing a wider range of farming environments. The study was conducted from September 1997 to August 2002 in matched removal and reference areas (average area of 245.1 km^2) in counties Cork, Donegal, Kilkenny and Monaghan. Badger removal was intensive and proactive throughout the study period in the removal areas (removal intensity of 0.57 badgers/km^2/year during the first 2 years of the study), but reactive (in response to major tuberculosis outbreaks in cattle) in the reference areas (removal intensity during equivalent period of 0.07 badgers/km^2/year). During the study period, and after accounting for

all key confounders, there was a significant difference between the removal and reference areas in all four counties in both the probability of, and the time to, a confirmed herd restriction due to tuberculosis. To illustrate, in the final year of the study the odds of a confirmed herd restriction in the removal (as compared to the reference areas) were 0.25 in Cork, 0.04 in Donegal, 0.26 in Kilkenny and 0.43 in Monaghan, and the hazard ratios (removal over reference) ranged from 0.4 to 0.04 (a 60–96% decrease in the rate at which herds were becoming the subject of a confirmed restriction) (Griffin et al., in press). Some concerns have been raised regarding the validity of this study (Donnelly et al., 2003); however, these have proved unfounded following critical evaluation (Griffin et al., In press).

CURRENT UNDERSTANDING OF DISEASE EPIDEMIOLOGY IN IRELAND

The key role of badgers, within a context

The East Offaly and four area projects have provided compelling evidence of the key role of badgers as an infection source for cattle herds. However, it is critical that these studies are considered within a clearly defined context. The Irish bovine tuberculosis eradication programme is comprehensive, incorporating each of the accepted elements of disease control, including mandatory annual tuberculin testing of all animals in the national herd and early, ongoing removal of infected animals. It has long been suspected that another source of the bovine tubercle bacillus is involved, given the lack of national progress towards eradication despite these efforts. These suspicions have now been confirmed, with the results from these two studies clearly highlighting wildlife (and specifically transmission of infection from badgers to cattle) as a key constraint to disease eradication in Ireland. These findings are of national importance and provide compelling evidence of the linkage between proactive badger removal and tuberculosis in Irish cattle. Note that this linkage (and the consequent impact of proactive badger removal) would not have been evident if the national control programme were less effective. To illustrate, if cattle-to-cattle transmission were still common, differences in disease incidence between the removal and reference areas would not have been as marked.

The relative importance of cattle-to-cattle and badger-to-cattle transmission

On the basis of these studies, cattle-to-cattle transmission is believed to be of relatively lesser importance than badger-to-cattle transmission in Ireland. Although it is not yet possible to quantify the relative importance of these routes of transmission (this is currently under investigation; Paul White, personal communications), there is a range of evidence to support this view. First, there has been a lack of progress since the 1960s, and particularly during the ERAD era, despite the implementation of control measures that are known to eliminate cattle-to-cattle transmission. In the absence of wildlife reservoirs, these measures proved effective in the eradication of disease from Australia (Neumann, 1999). Secondly, the results from the east Offaly and four area projects clearly demonstrate a substantial – and significant – reduction in tuberculosis in cattle herds following proactive badger removal (Griffin et al., in press; Ó'Máirtín et al., 1998a; Ó'Máirtín et al., 1998b). Further work is currently underway to determine the source of infection in the removal area breakdowns. Based on preliminary information, at least some of the breakdowns in the removal area can be attributed to effects outside the removal area, including the purchase of infected cattle from outside the removal areas and ongoing badger activity at the periphery of these areas (Barrett & More, unpublished). In Ireland, there is also a substantial disparity between disease prevalence in badgers and cattle.

In a recent survey of captured badgers, using improved post-mortem technique and laboratory support, the prevalence of infection approached 50% (L. Corner, personal communications). In contrast, the apparent incidence of infection in cattle during 2002 was 0.4% (29,162 bovine reactors, from a population of approximately 7 million cattle)(Anon., 2003). Finally, as a result of mandatory annual testing and early and ongoing removal of infected animals (Good et al., 2003), there is limited opportunity for Irish cattle to become infectious (that is, capable of transmitting infection) prior to detection. This view is supported by field evidence, where singleton reactor breakdowns (that is, breakdowns involving only a single reactor) accounted for between 38.3 and 44.4% of all breakdowns each year during 1987-1997 in Ireland (O'Keeffe & Crowley, 1995), despite very close contact between animals throughout winter housing. It is important to emphasise that disease transmission is affected by the number of contacts per unit time, the transmission potential per contact and the duration of infectiousness (Halloran, 1998). Therefore, there are a range of factors that will influence the relative importance of cattle-to-cattle and badger-to-cattle transmission, including the property of the particular infectious agent, and significant host and environmental factors (including efficiency and frequency of testing, and methods of management, including stocking density) (Baldock, 1997). Therefore, although these conclusions are relevant to the Irish situation, they should be extrapolated to other regions with care.

The mechanism of badger-to-cattle transmission

Although the importance of badgers in the epidemiology of bovine tuberculosis in Ireland is now clear, the mechanism of badger-to-cattle transmission remains uncertain. Based on the pathological evidence (Costello et al., 1998; O'Boyle, 1999, 2000, 2002; O'Boyle et al., 2003; O'Keeffe et al., 1996), lesions in infected Irish badgers are most-common in the thoracic cavity (bronchial and mediastinal lymph nodes and lung tissue) and head region (pharyngeal, parotid and submandibular lymph nodes), confirming the respiratory route as an important route of exit (O'Boyle, 2002). In cattle, the aerogenous route, rather than ingestion, is believed to be the main route of entry (Phillips et al., 2003), noting that infection with *M. bovis* can be established in cattle following the inhalation of one or a small number of tubercle bacilli in an aerosol droplet (Pollock and Neill, 2002). Consequently, transmission of infection may be most efficient when cattle and infected badgers are sharing the same airspace. There is evidence of badgers frequenting housing, with farmers being unaware of their presence (Cheeseman & Mallinson, 1981). Although attention to this point has particularly focused on the terminally-ill badger (Phillips et al., 2003), infected, but apparently healthy badgers, may also be infectious and a risk to cattle, albeit at a lower level (L. Corner, unpublished). Our understanding of the interface between cattle and badgers is complicated by results of several recent Irish studies. Although cattle and badgers tended to have similar *M. bovis* strains within broad geographical areas, badger strains were not strongly clustered within an area, leading the authors to speculate about the dynamic nature of badger movements (Olea-Popelka et al., in press; Olea-Popelka et al., submitted).

MOVING FORWARD

In order to eradicate tuberculosis from the Irish cattle population, Ireland will need to control tuberculosis in badgers, with which cattle may come in contact, in a sustainable fashion (Gormley & Collins, 2000). However, this presents significant challenges for scientists and policy-makers (Gormley & Costello, 2003), including the international legal protection, and

national status, afforded to badgers; the potential for increases in badger numbers, as a consequence of agricultural intensification and increase in productive pastures; the close physical proximity of badgers and cattle, given the preference for Irish badgers to locate setts in hedgerows (Hammond et al., 2001); and the high prevalence of infection among Irish badgers (L. Corner, unpublished). Ireland is currently implementing a comprehensive strategy to address these challenges, whilst maintaining existing measures to control cattle-to-cattle transmission. In the short-term, the Department of Agriculture and Food is implementing a national programme of wildlife control when and where wildlife are implicated in on-farm breakdowns of bovine tuberculosis (O'Keeffe et al., 2002). These activities are focused in areas of high disease prevalence. In these areas, badger removal will form the basis of temporary disease control (by minimising contact between cattle and infected badgers), and will also provide potential locations for vaccination trials and (later) usage (O'Keeffe et al., 2002). In the longer-term, Ireland is committed to the development of an effective badger vaccine and the implementation of a strategic programme of badger vaccination, with the aim to reduce *M. bovis* transmission between infected badgers and susceptible cattle (Gormley & Costello, 2003). The feasibility of such an approach was first considered in 1994, with input from scientists from Ireland and Northern Ireland (Ellis et al., 1994). Current work is focusing on the use of a live vaccine based on *M. bovis* BCG (L. Corner, unpublished), which might persist in the host and continuously prime the protective cellular immune response (Gormley & Costello, 2003). Results from early experimental studies have been promising (L. Corner, unpublished).

In association with these efforts, epidemiological research is being conducted by, or in partnership with, the Centre for Veterinary Epidemiology and Risk Analysis in a number of complementary areas. With respect to the national control programme, there is ongoing database development, as well as investigations concerning the benefit-cost of a targeted pre-movement test and the efficiency of factory surveillance. More generally, work has commenced on breakdowns instigated by a factory lesion detection, on aspects of badger ecology in Ireland (including diet, reproduction and overall population size), and on work to support vaccine development (including bait-uptake and disease transmission studies).

ACKNOWLEDGEMENTS

I gratefully acknowledge the many collaborators who have contributed, through epidemiological research, to progress towards the eradication of bovine tuberculosis in Ireland, including the staff of the Centre for Veterinary Epidemiology and Risk Analysis (Damien Barrett, Tracy Clegg, Isabella Higgins, Guy McGrath, James O'Keeffe, Paul White); the staff of the Department of Agriculture and Food, particularly Michael Sheridan, Martin Blake, Margaret Good, John Griffin, Ian O'Boyle, Eamonn Costello, Leonard Dolan, John Eves, and the staff of the District Veterinary Offices of Cork, Donegal, Kilkenny, Monaghan and Offaly; Leigh Corner, Eamonn Gormley and Sandrine Lesellier from the Faculty of Veterinary Medicine, University College Dublin; Wayne Martin and Francisco Olea-Popelka from the University of Guelph, Canada; David Williams, Gabrielle Kelly and Joe Condon from the Department of Statistics and Actuarial Science, University College Dublin; Klaas Frankena, Ilse van Grevenhof and Mart de Jong from Wageningen University, The Netherlands; Paddy Sleeman from University College Cork; John Pollock, Robin Skuce, Owen Denny, Paddy McGuckian & others from DARDNI, Northern Ireland; Glyn Hewinson and others from VLA in the UK; Nicola Marples and students from Zoology, Trinity College Dublin; Dirk Pfeiffer from the Royal Veterinary College, UK; and Mark Stevenson from Massey University, New Zealand.

REFERENCES

Anon. (2003). Bovine tuberculosis statistics: annual summary, 2002. *In* 'Selected Papers 2002-2003', pp. 72-87. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin

Baldock, C. (1997). The concept of herd immunity in vaccination programs. *In* 'Proceedings of Epidemiology Program, 10th FAVA Congress, 24th-28th August 1997, Cairns, Australia' (S. J. More, ed.), pp. 39-45

Cheeseman, C. L., and Mallinson, P. J. (1981). Behaviour of badgers (*Meles meles*) infected with bovine tuberculosis. J. Zool. 194, 284-289

Costello, E., Quigley, F., O'Grady, D., Flynn, O., Gogarty, A., McGuirk, J., O'Rourke, J., and Griffin, J. (1998). Laboratory examination of tissues from badgers and cattle from the four-area study for evidence of tuberculosis. *In* 'Selected Papers 1997', pp. 32-36. Tuberculosis Investigation Unit, University College Dublin, Dublin

Costello, E., O'Grady, D., Flynn, O., O'Brien, R., Rogers, M., Quigley, F., Egan, J., and Griffin, J. (1999). Study of restriction fragment length polymorphism analysis and spoligotyping for epidemiological investigation of *Mycobacterium bovis* infection. J. Clin. Microbiol. 37, 3217-3222

Dolan, L. A., Eves, J. A., and Bray, D. (1994). East Offaly Badger Research Project (EOP): Interim report for the period, January 1989 to December 1994. *In* 'Selected Papers 1994', pp. 1-3. Tuberculosis Investigation Unit, Dublin

Donnelly, C. A., Woodroffe, R., Cox, D. R., Bourne, J., Gettinby, G., Le Fevre, A. M., McInerney, J. P., and Morrison, W. I. (2003). Impact of localized badger culling on tuberculosis incidence in British cattle. Nature 426, 834-837

Downey, L. (1990). 'Ireland's TB problem - What can and must be achieved.,' ICOS with the support of the Agricultural Trust

Downey, L. (1992). Bovine TB Programme. Review of ERAD Measures - Technical Audit. Eradication of Animal Disease Board, Dublin

Ellis, W., Collins, J. D., Feore, M., Neill, S., Sheridan, M., Sleeman, D., Hughes, S., and Rogers, M. (1994). 'The feasibility of developing a vaccine against tuberculosis for use in the badger *(Meles meles)*.' Department of Agriculture, Food and Forestry (Republic of Ireland) and Department of Agriculture for Northern Ireland

Eves, J. A. (1999). Impact of badger removal on bovine tuberculosis in east County Offaly. Irish Vet. J. 52, 199-203

Flanagan, P., Finn, M., Flynn, O., Costello, E., O'Grady, D., Quigley, F., and Griffin, J. M. (1998). A study of within herd transmission of *Mycobacterium bovis* from newly purchased infected cattle, using DNA fingerprinting techniques. *In* 'Selected Papers 1998' (J. D. Collins and R. F. Hammond, eds.), pp. 51-57. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin

Good, M., O'Boyle, I., O'Keeffe, J., and Maher, P. (2003). Handbook for the veterinary nanagement of herds under restriction due to tuberculosis. pp. 88, Department of Agriculture and Food, Dublin

Gormley, E., and Collins, J. D. (2000). The development of wildlife control strategies for eradication of tuberculosis in cattle in Ireland. Tubercle Lung Dis. 80, 229-236

Gormley, E., and Costello, E. (2003). Tuberculosis and badgers: new approaches to diagnosis and control. J. Appl. Microbiol. 94, 80S-86S

Griffin, J. M. (1991). The role of bought-in cattle in herd breakdowns due to tuberculosis in part of County Cavan during 1989. *In* 'Selected papers 1990-1991', pp. 12-15. Tuberculosis Investigation Unit, University College Dublin, Dublin

Griffin, J. M. (1992). Analysis of epidemiology reports on outbreaks of bovine tuberculosis involving 504 herd in 22 counties. *In* 'Selected papers 1992', pp. 28-32. Tuberculosis Investigation Unit, University College Dublin, Dublin

Griffin, J. M., and Dolan, L. A. (1995). The role of cattle-to-cattle transmission of *Mycobacterium bovis* in the epidemiology of tuberculosis in cattle in the Republic of Ireland: a review. Irish Vet. J. 48, 228-234

Griffin, J. M., Williams, D. H., Kelly, G. E., Clegg, T. A., O'Boyle, I., Collins, J. D., and More, S. J. (in press). The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Prev. Vet. Med

Halloran, M. E. (1998). Concepts of infectious disease epidemiology. *In* 'Modern Epidemiology, Second Edition' (K. J. Rothman and S. Greenland, eds.), pp. 529-554. Lippincott-Raven, Philadephia

Hammond, R. F., McGrath, G., and Martin, S. W. (2001). Irish soil and land-use classifications as predictors of number of badgers and badger setts. Prev. Vet. Med. 51, 137-148

Krebs, J., Anderson, R. M., Clutton-Brock, T., Donnelly, C. A., Morrison, W. I., and Young, D. (1997). 'Bovine Tuberculosis in Badgers and Cattle.,' Ministry of Agriculture, Fisheries and Food, London

Martin, S. W., Eves, J. A., Dolan, L. A., Hammond, R. F., Griffin, J. M., Collins, J. D., and Shoukri, M. M. (1997). The association between the bovine tuberculosis status of herds in the East Offaly Project Area, and the distance to badger setts, 1998-1993. Prev. Vet. Med. 31, 113-125

Morris, R. S., and Pfeiffer, D. U. (1995). Directions and issues in bovine tuberculosis epidemiology and control in New Zealand. New Zeal. Vet. J. 43, 256-265

Neumann, G. B. (1999). Bovine tuberculosis - an increasingly rare event. Aust. Vet. J. 77, 445-446

Noonan, N. L., Sheane, W. D., Harper, W. R., and Ryan, P. J. (1975). Wildlife as a possible reservoir of bovine tuberculosis. Irish Vet. J. 29, 1

O'Boyle, I. (1999). Review of Badger (*Meles meles)* research licenses in 1998. *In* 'Selected Papers 1998', pp. 10-14. Tuberculois Investigation Unit, University College Dublin, Dublin

O'Boyle, I. (2000). Review of Badger (*Meles meles)* research licenses in 1999. *In* 'Selected Papers 1999', pp. 15-21. Tuberculosis Investigation Unit, University College Dublin, Dublin

O'Boyle, I. (2002). Review of Badger (*Meles meles)* research licenses in 2000 and 2001. *In* 'Selected Papers 2000-2001', pp. 19-25. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin

O'Boyle, I., Costello, E., Power, E. P., Kelleher, P. F., Bradley, J., Redahan, E., Quigley, F., Fogarty, U., and Higgins, I. (2003). Review of Badger (*Meles meles)* research licenses in 2002. *In* 'Selected Papers 2002-2003', pp. 13-18. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin

O'Brien, D. J., Schmitt, S. M., Fierke, J. S., Hogle, S. A., Winterstein, S. R., Cooley, T. M., Moritz, W. E., Diegeld, K. L., Fitzgerald, S. E., Berry, D. E., and Kaneene, J. B. (2002). Epidemiology of *Mycobacterium bovis* in free-ranging white-tailed deer, Michigan, USA, 1995–2000. Prev. Vet. Med. 54, 47-63

O'Keeffe, J. J., and Crowley, M. J. (1995). 'Episode classification in the eradication of bovine tuberculosis: a different perspective,' Tuberculosis Investigation Unit, Dublin

O'Keeffe, J. J., O'Boyle, I., and Fogarty, U. (1996). Observations on the pathology of tuberculosis in badgers. *In* 'Selected Papers', pp. 23-26. Tuberculosis Investigation Unit, University College Dublin, Dublin

O'Keeffe, J. J., Hammond, R. F., and McGrath, G. (2002). Density maps highlight areas with chronic bovine tuberculosis and enable targeting of resources to eradicate disease. *In* 'Selected Papers 2000-2001', pp. 41-44. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin

O'Keeffe, J. J., and Higgins, I. (2003). Analysis of epidemiology reports on selected herd breakdowns of tuberculosis, 1994-1998. *In* 'Selected Papers 2002-2003' (J. D. Collins and R. F. Hammond, eds.), pp. 46-52. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin

Olea-Popelka, F. J., Costello, E., McGrath, G., Collins, J. D., O'Keeffe, J., Kelton, D. F., Berke, O., and Martin, S. W. (in press). Spatial relationship between *M. bovis* strains in cattle and badgers in four areas in Ireland. Prev. Vet. Med

Olea-Popelka, F. J., Phelan, J. A., Eves, White, P. W., McGrath, G., Collins, J. D., O'Keefe, J., Duggan, M., Collins, D. J., Kelton, D. F., Berke, O., and Martin, S. W. (submitted). Quantifying badger exposure and the risk of bovine tuberculosis for cattle herds in county Kilkenny, Ireland. A case study

Ó'Máirtín, D., Williams, D. H., Dolan, L., Eves, J. A., and Collins, J. D. (1998a). The influence of selected herd factors and a badger-intervention tuberculosis-control programme on the risk of a herd-level trade restriction to a bovine population in Ireland. Prev. Vet. Med. 35, 79-90

Ó'Máirtín, D., Williams, D. H., Griffin, J. M., L., D., and Eves, J. A. (1998b). The effect of a badger removal programme on the incidence of tuberculosis in an Irish cattle population. Prev. Vet. Med. 34, 47-56

Phillips, C. J. C., Foster, C. R. W., Morris, P. A., and Teverson, R. (2003). The transmission of *Mycobacterium bovis* infection to cattle. Res. Vet. Sci. 74, 1-15

Pollock, J. M., and Neill, S. D. (2002). *Mycobacterium bovis* infection and tuberculosis in cattle. Vet. J. 163, 115-127

Sheehy, S. J., and Christiansen, J. H. (1991). 'Cost/benefit analysis of Irish bovine tuberculosis eradication schemes', University College Dublin, Dublin

TUBERCULOSIS

RISK OF BOVINE TUBERCULOSIS BREAKDOWNS IN POST-FOOT-AND-MOUTH DISEASE RESTOCKED CATTLE HERDS IN GREAT BRITAIN

J.J. CARRIQUE-MAS[1], G.F. MEDLEY AND L.E. GREEN

SUMMARY

The 2001 foot-and-mouth disease (FMD) epidemic in Great Britain (GB) led to the depopulation of hundreds of cattle herds. Many farms were later restocked and tested for bovine tuberculosis (bTB). Movement and bTB testing data for these farms were analysed to determine the effect of cattle movements on the time to bTB breakdown. There was a marked increase in hazard of breakdown associated with late testing of these farms, and therefore Weibull survival was used. Variables independently associated with increased hazard of breakdown were: 1) Purchasing over 32 animals from a high bTB incidence county; 2) Purchasing a large proportion of a source herd; and 3) History of bTB in the restocked herd itself. Purchasing from many sources was protective. Results highlight the risk from the source herds' surrounding geographical area, and emphasise the importance of timely testing to control bTB. They suggest that measures limiting or controlling cattle movements (e.g. pre-purchase testing) may have some impact by reducing the introduction of infection.

INTRODUCTION

Mycobacterium bovis is the major cause of bTB, a zoonotic infection affecting cattle and other species worldwide (Pollock & Neill, 2002). In most industrialised countries, the incidence of bTB in cattle has declined over the last few decades, as a result of national elimination programmes. In GB, there was a marked reduction in bTB incidence from the 1930s, and by 1960 most farms were attested free of bTB. (Evans, 1981). However, since the late 1980s there has been a steady increase in the number of breakdowns (Bourne et al. 2000; Pollock & Neill, 2002).

Control of bTB in cattle is based on regular testing of herds. Herds disclosing reactors are placed under movement restrictions, which are only lifted after all animals in the affected herd have passed one or two (depending on whether the incident is confirmed or not) consecutive herd tests 60 days apart. In GB, during the 1960s and 1970s the herd testing interval was increased from annual to biennial and subsequently to triennial in areas of low incidence (Evans 1981). The current testing frequency of herds in GB is annual, biennial or quadrennial, depending on the herd's perceived risk of infection.

Some case-control studies have reported a significant association between cattle purchasing and bTB. For example, an Irish study found an association between the importation of bulls and

[1] Juan J. Carrique-Mas, Ecology and Epidemiology Group, Department of Biological Sciences, University of Warwick, CV4 7AL Coventry, UK, Email: J.J.Carrique-Mas@warwick.ac.uk

chronic bTB (Odds ratio (OR) = 3.9) (Griffin et al., 1993). An Italian case-control study reported a strong risk associated with the introduction of cattle since the last bTB test carried out in the herd (OR = 5.8) (Marangon et al., 1998). In contrast, another study in the Republic of Ireland failed to detect an association between the purchase of animals and the occurrence of bTB breakdowns at the subsequent test (Griffin et al., 1996), perhaps due to the fact that pre-purchase bTB testing was compulsory in that country. Further evidence of the link between cattle purchases and bTB comes from a study in Canada involving 995 cattle and deer herds investigated following nine breakdowns. The study reported the highest reactivity in trace-out tests, that is in herds that had purchased animals from the index herds (Munroe, et al., 1999).

An analysis of reports between 1972 and 1988 concluded that between 8-10% of confirmed breakdowns in the south west of England, and 50-64% in the rest of the country could be attributed to purchase of infected cattle (Goodchild & Clifton-Hadley, 2001). Moreover, there is evidence of intra-herd transmission following the introduction of new animals (Griffin, 1993).

The 2001 foot-and-mouth-disease (FMD) epidemic in the UK had a considerable impact on the British cattle livestock industry, with more than 6,000 cattle herds depopulated between 20th February and 30th September 2001 (Davies, 2002). After the epidemic, most affected farms were re-populated, purchasing stock from a variety of sources.

The objective of this study was to determine the effect of cattle purchasing patterns on the risk of herd breakdown with bTB. Information derived from this study may potentially be useful to inform policy as to whether any movement control measures may help to control the spread of bTB. It will also give an insight into the interaction between testing, movement and infection in determining the risk of herd breakdown.

MATERIALS AND METHODS

Data sources

A total of 894 depopulated farms were available for study. Cattle purchases following depopulation were identified from the Cattle Tracing System (CTS) database, which registers all cattle movements in GB from birth to death. Full movement data are available for animals born after September 1998. For older animals, the database contains all movements of cattle since January 2001. Data on bTB testing history both for restocked herds and for herds from which purchased animals were sourced (source herds) came from VetNet.

Study population and study period

The 894 farms were located in 26 counties, and were classified by their latitude into: north of 54º 30’ 42’’ (479 herds in 10 counties), and south of 54º 30’ 42’’ (415 herds in 17 counties). Two counties, Cumbria and Devon, had over 50% of the farms, with 300 and 182 farms respectively in each county. The farms were selected for further analyses based on the fulfilment of two conditions: 1) Having been tested first with either a check test (VE-CT), a routine herd test (VE-RHT) or a whole herd test (VE-WHT) between 30th September 2001 (end of the FMD epidemic) and 23rd June 2003; and 2) Having logical movement records indicating the purchase of animals following FMD.

Thirty-eight (4.2%) of the 894 farms had no movement data, and 141 (15.8%) either had not been tested during the study period or had been tested using a different type of test. Finally, eight herds were excluded because they had incongruous movement data. The remaining 730 farms are referred to from here on as restocked herds. Of these, 425 (58.2%) were located north of 54º 30' 42'' (91% of which in Cumbria and Scotland) and 305 (41.8%) south of that latitude.

Statistical analyses

Proportional hazards Cox (Cox, 1972) and parametric Weibull parametric (Collett, 1994) survival modelling were used. The first outcome of interest was time to test, since the understanding of the factors affecting early testing of herds was essential for the interpretation of results of the test itself. Further models dealt with the outcome of bTB detection, with different variations: a) with reactor/s detected at first test; b) with reactor/s detected at first test or with inconclusive reactor becoming a reactor at retest (referred to as 'breakdown'); or c) as a result of visible lesions or positive *M. bovis* culture from one of the reactors detected during the course of the breakdown ('confirmed breakdown'). The time variable was the period between the first movement into the restocking herd and the herd test. Kaplan-Meier survival curves were plotted to investigate survival from restocking to breakdown; these were used to estimate the change of hazard rate with time. A plot of log (cumulative hazard) versus log (survival time) was used to assess visually the change in the hazard of breakdown function. Here follows a list of the variables considered for the analyses; their values are shown in Table 1: 1) No. animals purchased present on the test date (No. animals purchased-tested); 2) No. animals purchased but sold prior to herd test (No. animals purchased-left); 3), 4) Mean age in months and standard deviation (SD) of purchased animals (at time of purchase); 5) Percentage of animals purchased aged more than 3 years (% animals >3 yrs); 6) Percentage of animals purchased aged less than 1.5 years (% animals < 1.5 yrs); 7) Percentage of animals purchased of dairy breed (% dairy animals); 8) No. animals purchased from counties with incidence ≥4.0 reactors/1000 animals tested during 2002 (No. animals from high incidence county); 9) No. animals purchased from source herds with bTB history; 10) No. animals purchased from source herds bTB tested when the animals were present (No. animals tested in source herd); 11) No. animals purchased from source herds bTB tested less than a year before the purchase (No. animals from source herd tested <1 yr); 12) No. of source herds; 13) Average size of source herds; 14) Average percentage of source herd purchased; 15) Maximum percentage of a source herd purchased; 16) Pre-FMD bTB history in restocked herd (Restocked herd bTB history); 17) Over 50% of animals purchased during December 2001 to February 2002 (Over 50% purchased winter 2001/02); 18) Restocked herd location: South of 54º 30' 42''. The values of the dichotomous variables used were: 425 herds (South of 54º 30' 42''); 100 herds (Restocked herd bTB history); 305 herds (Over 50% cattle purchased winter 2001/02).

All continuous variables except Mean and SD of age of purchased animals, Average and Maximum % of source herd purchased were log10 transformed and centred (by subtraction of their mean value). Candidate variables for multivariable modelling were those associated with a significance value <0.2 in the univariable analysis. Multivariable survival regression models were fitted in a stepwise forward fashion. The significance of new variables was assessed by the likelihood test. Variables were left in the multivariable model if either: a) they remained significant; b) their removal affected the estimates of other variables in the model; or c) their inclusion in the model was judged necessary due to their biological relevance. After fitting the final model, variables dropped during the model building process were again included one at a time to investigate residual confounding, and retained in the model if they became significant.

The assumption of linearity for continuous variables was assessed visually by replacing them in the final model by categorical variables defined by quintiles of the variables as cut-off points. Assessment of the overall fit of the model was achieved by plotting deviance residuals versus the rank order of survival times. Overly influential observations on model variables were detected by computing the delta-betas for each relevant variable versus the rank of survival time. For each variable in the multivariable model, a test of the proportional hazards assumption of the Weibull models was carried out as described by Collett (1994). All statistical analyses were carried out using S-Plus 6.1 (Insightful Corp, Seattle).

Table 1. Description of the (untransformed) continuous variables used for the study (730 herds)

Variable type	Variable name	Median	1st Quartile	3rd Quartile
Herd composition	No. animals purchased-tested	112	65	182
	No. animals purchased-left	2	0	11
	Mean age of purchased animals (months)	21	11	38
	SD age of purchased animals (months)	20.2	9.5	27.7
	% animals >3 yrs	24.6	2.8	27.6
	% animals <1.5 yrs	35.0	15.3	38.3
	% dairy animals	19.9	0	84.6
bTB and testing in source herds	No. animals from high incidence county	17	0	97
	No. animals from herds with bTB history	1	0	29
	No. animals tested in source herd	9	0	32
	No. animals tested in source herd <1 yr	0	0	8
	No. animals from source herd tested <1 yr	10	0	42
Purchasing behaviour	No. source herds	6	3	11
	Average size source herd	178	127	242
	Average % of source herd purchased	12.9	7.9	20.2
	Maximum % of source herd purchased	38.6	20	63.2

RESULTS

Restocking and testing

Most farms began to restock in November 2001. December 2001 was the month when the largest number of animals was purchased.

Animals: A total of 120,471 animals were purchased, but 13.6% of these animals had left (by sale or death) the restocked herds before the test date. The source herd could be determined for 92.7% of them. Of those, 38.8% were purchased from a source herd located in the same county as the restocked herd and 74% animals came from herds in the same geographical region (defined by latitude 54º 30' 42'') as the restocked herd. This latter proportion was greater for herds located south of 54º 30' 42'' (95.3%) than for those north of 54º 30' (61.2%), reflecting a net transfer of animals from the south to the north. At the time of testing, restocked herds had carried out a median of seven purchasing events (determined by the number of dates of purchasing) (interquartile range = 4-12). The median age of purchased animals was 21 months, the majority of which (69.7%) had been born in the source herds.

Source herds: A total of 6,506 source herds supplied animals to restocked herds. Of these, 82.6% supplied animals to only one restocked herd. The median number of animals supplied by each source herd was 6 (interquartile range = 2-19). There was a record of previous bTB testing for 96.0% of source herds. Only 26.2% of the source herds had been tested one year or less before they started selling animals to restocked herds, and these herd supplied 21.5% of all animals purchased. The number of animals that had potentially been tested in source herds (i.e. they were present in the herd when a herd test was conducted) was 17,560 (15.7%). For 7,525 (6.7%) animals, this test had occurred less than one year before the purchase.

Testing: Testing of restocked herds started in December 2001, and peaked in March-April 2002. Herds north of 54º 30' 42'' were tested considerably earlier (median time to test = 140 days, interquartile range = 108-214) than those located south of this latitude (median = 252 days, interquartile range = 162-399) ($P < 0.001$). A Kaplan-Meier plot showing time to test for herds in these two geographical areas is presented (Fig. 1).

A Cox survival model was fitted stepwise with all study variables to investigate whether they were associated with early testing. Three variables were independently associated with longer time to test: No. animals purchased (log) (HR = 0.80, P = 0.001), No. animals tested in source herd (HR = 0.88; P = 0.05) and South of 54º 30' 42'' (HR = 0.39, $P < 0.001$).

Breakdowns

Reactors were disclosed in 57/730 (7.8%) tested restocked herds. A further 95 herds had one or several inconclusive reactors (IRs), but no reactors. At the ensuing VE-IR tests, reactors were disclosed in 20 of these herds. Therefore, a total of 77 breakdowns were available for the study, 56 south of 54º 30' 42'' and 21 north of this latitude (Table 2). Of all breakdowns, 37.7% (29/77) were confirmed. This percentage was greater in herds south of 54º 30' 42'' (23/56, 41.1%) than in those north of 54º 30' 42'' (6/21, 28.6%), although the difference was not significant (P = 0.2). In herds north of 54º 30' 42'', all breakdowns except one were disclosed between January and September 2002, a date by which only 10 (2.3%) herds remained untested. In contrast, 94 (31%) herds south of 54º 30' 42'' had begun restocking and remained untested by the end of September 2002. Testing of these herds resulted in 27 breakdowns (Fig. 2).

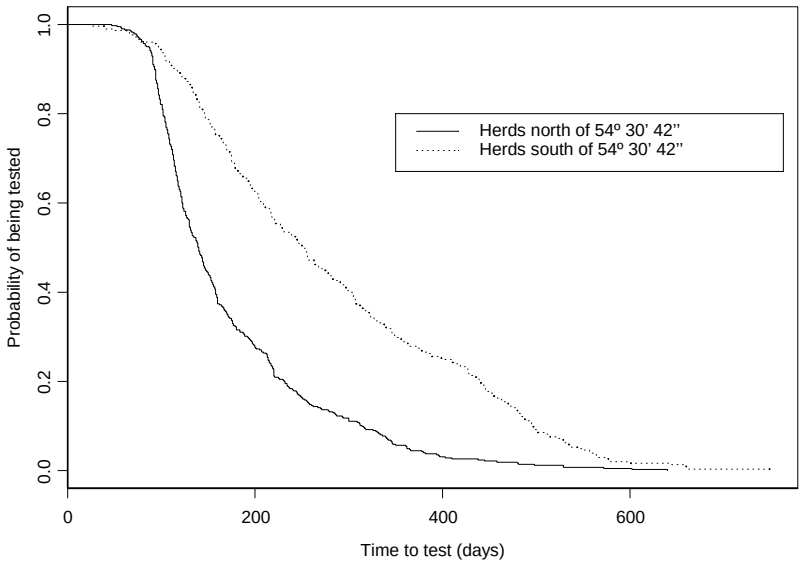


Fig. 1 Kaplan-Meier plot of time to test for restocked herds north and south of 54º 30' 42'' (425 and 305, respectively)

Table 2. Breakdowns in restocked herds (30th September 2001-23rd June 2003) (730 herds)

	All herds (%)	North of 54º 30' 42''	South of of 54º 30'42''
No. herds (%)	730 (100%)	425 (100%)	305 (100%)
No. herds with reactors at first test (%)	57 (7.8%)	13 (3.1%)	44 (14.4%)
No. herds with only IRs at first test (%)	95 (13.0%)	51 (12%)	44 (14.4%)
which become reactors at the VE-IR test/s	20 (2.7%)	8 (1.9%)	12 (3.9%)
No. herds with breakdown (%)	77 (10.5%)	21 (4.9%)	56 (18.4%)
No herds with confirmed breakdowns	40 (5.5%)	6 (1.4%)	23 (7.5%)

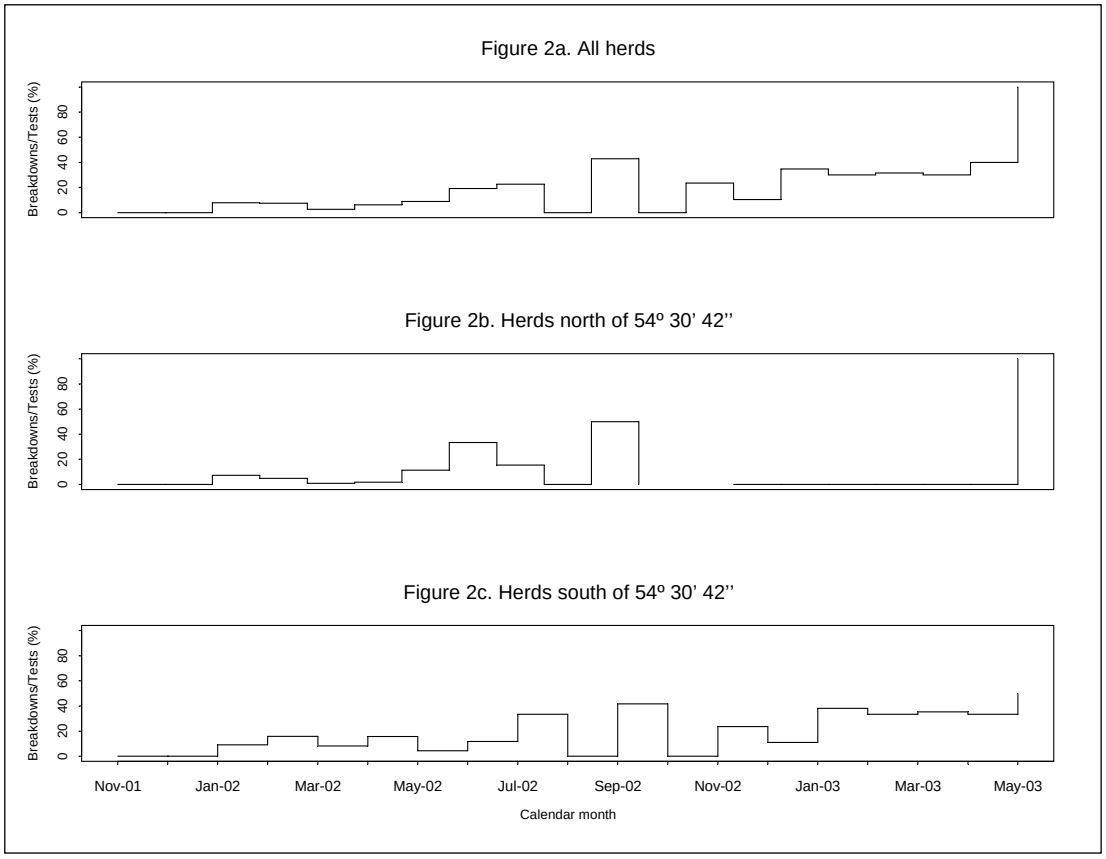


Fig. 2 Percentage of tests leading to breakdown (N = 77) by calendar month in all herds (N=730) (2a), in herds in the north of latitude 54º 30' 42'' (N = 425) (2b), and in the south of latitude 54º 30' 42'' (N = 305) (2c).

Hazard rates and cumulative hazard

Crude hazard rates calculated at failure times for the outcome 'breakdown' (77 herds) are shown in Fig. 3a. The plots of hazards at different failure times for the outcomes reactor/s at first test and confirmed breakdown were very similar (data not shown). A plot of log(cumulative hazard) versus log(time) resulted in an approximately straight line with a slope of approximately 2.5, suggesting that the hazard rate was increasing with time at an increasing rate (Fig. 3b). Therefore, a Weibull model with shape parameter of 2.5 was considered appropriate to model the survival experience. In subsequent modelling, the shape parameter was determined by maximum likelihood. The hazards were initially assumed to be proportional.

Survival models: Breakdown and reactor outcomes

Univariable hazard ratios (HRs) from the Weibull models are shown in Table 3. Candidate variables for multivariable modelling for the outcome breakdown were: 1) No. animals purchased-left; 2), 3) Mean and SD of age of purchased animals; 4) % animals >3yrs; 5) No. animals from high incidence county; 6) No. animals from herds with bTB history; 7) No. animals tested in source herd <1 yr, 8) No. sources; 9) Average % source herd purchased; 10) Maximum % of source herd purchased; 11) History of bTB in restocked herd; and 12) Over 50% cattle purchased in the winter 2001/02. The coefficients of the variables and their statistical

significance for the outcome reactor/s at the disclosing test were reasonably similar. For this reason, the main model was built in a forward stepwise fashion using breakdown as the outcome.

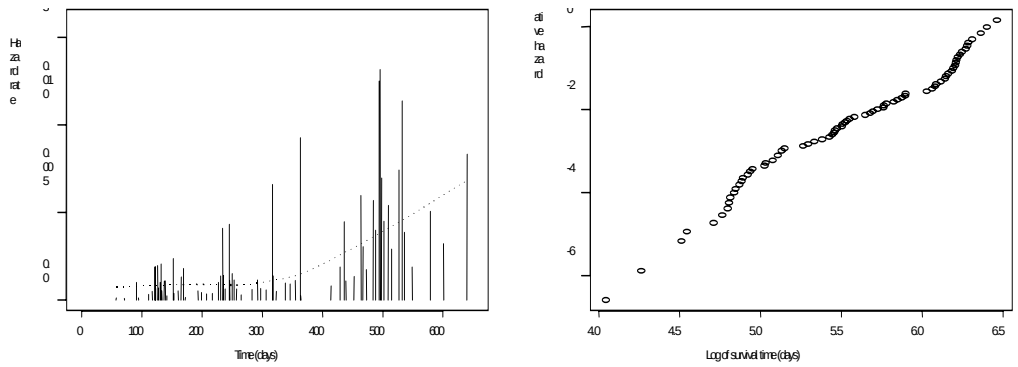


Fig. 3 Left (a): Plot of crude hazard rates of breakdown (730 herds, 77 breakdowns). A smoothed line has been added. Right (b): Plot of log-cumulative hazard (log (-log(survival probability) versus log (time).

After examining the relationship between the outcome and quintiles of the continuous variables, the variables No. sources and No. animals from high incidence county were respectively transformed into the dichotomous variables: Over 8 sources and Purchased over 32 animals from high incidence county. Variables independently associated with increased HR in the multivariable model with the outcome breakdown (Model 1) were: 1) Purchased over 32 animals from high incidence county; 2) Maximum % of source herds purchased, and 3) History of bTB in restocked herd (borderline significant). The variable Over 8 sources was protective. The variable No. animals purchased-tested, although non significant, was forced into the model (Table 4).

Multivariable models with the outcomes reactor/s detected at first test (Model 2) and confirmed breakdown (Model 3) were built using the explanatory variables of Model 1 (Table 4). Mean age of purchased animals and % animals >3 yr were not significant and therefore excluded from the multivariable models, since they were largely confounded by No. sources: herds restocking from fewer sources also bought older stock overall (data not shown). The variable South of 54º 30' 42'' was not significant when forced into the model. Hazard ratio estimates for Models 2 and 3 were reasonably similar to those of Model 1, except for a higher HR for Purchased over 32 animals from high incidence county (which increased from 2.2 in Model 1 to 3.72 and 4.05 in Models 2 and 3, respectively).

Model fit and adequacy: The plotting of deviance residuals indicated a better fit of the model for herds south of 54º 30' 42'' (data not shown). Consequently, Model 1 was re-run with data from the 305 herds south of 54º 30' 42'' only. Hazard ratios were similar to those obtained for the equivalent model using data from all herds. There was no indication that the shape parameters changed across subsets of the data, therefore the proportional hazards assumption was considered to be correct.

Model-derived hazard estimates: The scale (λ) and shape (γ) parameters from Model 1 were λ = 1.178*E-7 and γ = 2.38, respectively. The baseline hazard of breakdown (×1000) (per day) computed at times 100, 200, 300, 400, 500 and 600 days (from equation $h_o(t) = \lambda\gamma t^{\gamma-1}$) was 0.1613, 0.419, 0.735, 1.093, 1.487, and 1.912, respectively. These hazards correspond

theoretically to a herd with no bTB history, with 112 purchased animals present in the farm on the test date, which made up a maximum of 44% of a source herd, from over 8 sources, none of which located in a high incidence county.

Table 3. Univariable hazard ratio estimates from Weibull survival modelling (730 herds). 1) Outcome: Reactor/s detected at first test; 2) Outcome: Reactor/s detected at first test or inconclusive reactor becoming a reactor at retest

Outcome	*Breakdown*			*Reactor/s detected at first test*		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
No. animals purchased	1.34	0.81-2.20	0.256	1.58	0.87-2.88	0.137
No. animals purchased-left	0.77	0.57-1.05	0.095	0.81	0.57-1.15	0.232
Mean age of purchased animals	1.28	1.07-1.53	0.016	1.24	1.01-1.52	0.040
SD age purchased animals	1.45	1.13-1.85	0.003	1.30	0.98-1.71	0.07
% animals >3 yrs	1.01	1.01-1.02	0.019	1.01	1.0-1.02	0.050
% animals <1.5 yrs	0.99	0.98-1.0	0.335	1.0	0.99-1.01	0.332
% dairy animals	1.00	1.00-1.01	0.392	1.00	0.99-1.01	0.504
No. animals from high inc. county	1.35	1.02-1.80	0.038	1.67	1.14-2.43	0.008
No. animals from herds with bTB history	1.22	0.91-1.62	0.182	1.30	0.92-1.83	0.131
No. animals tested in source herd	0.90	0.66-1.23	0.501	0.89	0.61-1.29	0.525
No. animals tested in source herd <1 yr	0.74	0.52-1.04	0.087	0.72	0.48-1.08	0.112
No. animals from source herd tested <1 yr	0.95	0.71-1.27	0.738	1.00	0.71-1.40	0.98
No. sources	0.51	0.31-0.83	0.007	0.51	0.29-0.90	0.019
Average size source herd	0.84	0.35-2.03	0.696	0.61	0.23-1.61	0.317
Average % of source herd purchased	1.03	1.02-1.04	<0.001	1.03	1.02-1.05	<0.001
Maximum % of source herd purchased	1.011	1.003-1.019	0.006	1.02	1.01-1.03	0.001
History of bTB in restocked herd	2.10	1.27-3.45	0.003	2.31	1.29-4.11	0.005
Over 50% cattle purch. in the winter 2001/02	1.38	0.86-2.22	0.180	1.27	0.73-2.23	0.390
South of 54º 30’ 42’’	1.19	0.70-2.02	0.522	1.47	0.76-2.87	0.252

Table 4. Multivariable (proportional hazards) Weibull hazard ratio model estimates (730 herds)

Model		1			2			3	
Outcome		*Breakdown*			*Reactor/s detected at first test*			*Confirmed breakdown*	
Shape parameter		2.38			2.43			2.79	
Intercept (SE)		6.717 (0.149)			7.005 (0.210)			7.142 (0.287)	
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Maximum % of source herd purchased	1.013	1.004-1.023	0.008	1.020	1.008-1.032	0.0010	1.015	0.998-1.032	0.078
No. animals purchased-tested	1.05	0.55-2.01	0.875	1.05	0.49-2.23	0.9090	1.12	0.39-3.23	0.835
Purch. over 32 animals from high incidence county	2.20	1.18-4.10	0.013	3.72	1.54-8.95	0.0034	4.05	1.08-15.18	0.038
History of bTB in restocked herd	1.58	0.96-2.62	0.073	1.55	0.88-2.74	0.1310	1.60	0.72-3.58	0.250
Over 8 sources	0.41	0.24-0.70	0.001	0.33	0.18-0.62	0.0005	0.36	0.15-0.86	0.022

Reactor data: source herds and county incidence

The number of sources located in high and low incidence areas were 2,891 and 3,615 respectively, supplying 47,621 and 64,112 animals to restocked herds, respectively. A total of 180 reactors disclosed in the first test or at VE-IR retest were reported in all 77 breakdowns. The source herd could be determined for 157 (87%) reactors, which were supplied by 96 herds. None of the source herds supplied reactors to more than one restocked herd. Eighty-three (86.5%) of these source herds were located in high incidence counties. Only one source from a low incidence county supplied more than one reactor (Table 5).

Table 5. Source herds supplying reactors to restocked herds (96 source herds)

No. source herds	No. reactors supplied per herd	No. source herds in high incidence county	No. source herds in low incidence county	Total No. reactors supplied
64	1	52	12	64
15	2	14	1	30
10	3	10		30
4	4	4		16
1	5	1		5
2	6	2		12
96		83	13	157

DISCUSSION

The 2001 FMD epidemic in GB provided an ideal natural experiment to study the effect of purchasing on the occurrence of bTB breakdowns in restocking herds. This allowed comparison of the effects of purchasing animals from different regions, bTB status, adjusting for other factors related to the restocked herd itself (i.e. historical bTB presence, region, size). Infection transmitted from infectious animals on the farm that escaped detection at previous bTB tests could be ruled out, and it was safe to assume that all infection present was either due to one or several infected purchased animals, to the acquisition from the environment or from a contiguous herd. Further, because much of the routine testing was halted during 2001, a greater proportion of purchased animals were untested, compared with normal circumstances. This allowed examination of the consequences of delayed testing on the occurrence of bTB.

Time to test

To understand the reasons for early testing following restocking, a survival model was fitted to the data (730 herds), for which the outcome was time to test. One key assumption in survival modelling is that censored herds (with negative bTB test result) must have the same probability of failure as the remaining (untested) herds. It was suspected that early bTB testing of restocked herds may have been motivated by a perceived higher risk in those herds (i.e. due to their pre-FMD bTB history, or the origin of the purchased animals). This would violate this assumption. However, it was found that the main important predictor for earlier testing was geography. Most of these differences could be explained by regional testing behaviour as determined by the

herd's Animal Health Division Office (AHDO). The addition of this variable made all other variables non-significant (data not shown). Presumably, availability of human resources may well explain these differences. The other two significant variables negatively associated with early testing were No. animals purchased and No. animals tested in source herd. This is likely to be a reflection of the dynamics of the herds during the time of restocking: herds tested later were generally larger in size (since they had more time to restock and may have allowed for more births) and had a larger proportion of the stock born in the herd. Also, since bTB testing resumed after the FMD impasse in the rest of the country, the probability of purchasing cattle tested in the source herds increased as time went on.

Increase in hazard rates

There was an increase in the hazard rate (probability of breakdown per unit time) associated with late testing. This is likely to have happened for two reasons: 1) Intra-herd transmission in herds that purchased originally infected animals; and 2) increasing likelihood of infection from the environment and/or from an infected contiguous herd. Note that these two reasons are not strictly independent in that infection can theoretically be passed from one animal to another via the environment. In herds purchasing only one infected animal, intra-herd transmission would have resulted in the presence of two or more infected animals. As a consequence of this, herd test sensitivity is increased. The individual animal test sensitivity has been estimated to range between 0.68-0.9 (Costello et al., 1997; Monaghan et al., 1994). For example, assuming a sensitivity of 0.8 for one infected animal, the presence of two truly infected animals would increase the herd sensitivity to 1-(0.2^2) = 0.96. Moreover, and given that bTB infections are characterized by an un-responsive period of varying length depending on the stage of infection (Quirin et al., 2001), a delay in testing also means that herds with already infected animals were more likely to show a positive skin test reaction. Given the above factors, it is not surprising that the hazard rates increased with time in our study. This is consistent with the observed bTB testing data for GB, which showed a clear increase in the proportion of tested herds with bTB during 2002 compared to 2000, both north and south of 54º 30' 42'', although this increase was greater for restocked herds (data not shown). This may partly be as a result of the decreased testing during 2001, which implied the failure to remove potentially infected animals for one year. The additional risk observed among restocked herds (compared to non-restocked) is likely to be a consequence of the importation of infection through movement of animals. These results emphasise the importance of timely testing of bTB, especially in high-risk areas or in herds that purchased animals from them. It is not known to what extent a build-up of infection in the environment following restocking may have contributed to the observed increase in the hazard rate.

Purchasing of animals from high incidence counties

Testing data of the GB counties from 2002 was used to define 'high incidence', setting 4 reactors per 1,000 animals as a threshold. Data from 2001 were greatly reduced and targeted only at high risk herds. After accounting for the increasing baseline hazard and other covariates, it was found that purchasing more than 32 animals from high incidence counties led to a greater than two-fold increase in risk, although the precise magnitude is not likely to be informative. The fact that this coefficient for this predictor becomes even greater in the model with confirmed breakdowns (from 2.20 to 4.05) reinforces the importance of the geographical location of the herd source on the risk of importing bTB. Further evidence of this is the fact that the majority of

source herds supplying reactors were located in high incidence counties. A history of bTB in the source herd was not a predictor of bTB in restocked herds. This suggests that the testing regime adequately removed past bTB infection in the herds, but new infection was later acquired from the surrounding area (i.e. at county level), as has been suggested by Green and Cornell (2005). This may have been exacerbated by the lack of testing during 2001.

Testing in source herds

An unexpected finding was the lack of effect of any variables related to testing in the source herd. This may partly be explained by the low proportion of animals purchased that had potentially been tested in source herds (up to 15.7%). This means that the vast majority of animals were purchased without having ever been tested, and would consequently be expected to have a small impact on the outcome.

History of bTB in restocked herds

A history of bTB prior to FMD in restocked herds was an independent risk factor for bTB. The increase in risk associated with a bTB history may reflect a number of factors, which include an environmental reservoir, herd management factors and a contiguous herds effect. Certain types of herd management have been associated with an increased risk of bTB (Griffin et al., 1993). However, an effect associated with the history of bTB in the source herd was not found in this study. This is probably a reflection of the few animals purchased from herds with history of bTB (median=1), a fact which would make any potential effect difficult to detect.

Number of sources and maximum percentage of source herd purchased

Both the number of sources and the maximum percentage of source herd purchased were factors significantly associated with bTB breakdown, the former a protective effect and the latter a risk factor. Further work is currently being done to interpret these results, but a plausible explanation is that it is a reflection of test performance. Since the test has a less than perfect sensitivity, and assuming that most herds are uninfected but that most infected herds have more than one infected animal, it is then likely that for an equal number of purchased animals, farms restocking from few sources have a greater probability of purchasing more than one infected animal compared with farms restocking from many sources. This would consequently translate into an increased probability of breakdown detection. However, it also may partly be explained if purchasing from several sources reflected a more careful selection of the stock (i.e. animals more fit or resistant to infection), whereas farmers purchasing from few sources may have potentially included sub-standard animals as part of the wholesale deal.

The risk associated with the maximum percentage of source herd purchased may be explained by the same reason. This variable was highly correlated with the size of the source herd, since purchases from small source herds generally involve a larger percentage of them being bought. Another plausible explanation would be if the ‘per animal’ risk is greater in smaller herds. However, existing published data suggest the opposite; an increase in risk with herd size at both animal and herd levels (Goodchild & Clifton-Hadley, 2001).

The data pertaining to 6,200 restocked herds are currently being analysed, which are considered to be the majority of cattle herds affected by the 2001 FMD epidemic. Results

presented here suggest that the performance of the test (sensitivity and specificity) is a critical component in the interpretation of breakdown data. Additionally, the numbers of reactors disclosed at the breakdowns may be informative in this regard.

CONCLUSIONS

In summary, strong evidence was found of an association between bTB breakdowns in restocked herds and the level of bTB in the region where the source herds are located, suggesting a failure of the testing regime in preventing the acquisition of new infection in those herds. Consequently, measures limiting or controlling the movement of animals from high to low incidence regions (e.g. by pre-purchase testing) may have an impact by limiting the introduction of bTB. In addition, there was also an association between breakdowns and a history of bTB in the restocked herd itself, which is consistent with an environmental reservoir of infection. Further, restocking from many source herds decreased the risk of bTB breakdown. Given the imperfect test performance, herds purchasing from many sources (or regularly from the same source) may be at an increased risk of importing infection without detection.

ACKNOWLEDGEMENTS

The authors wish to thank Alan Aldridge of CTS and Andy Mitchell of VLA for respectively supplying cattle movement and bTB testing data for restocked herds. We are also indebted to Prof. David Cox (University of Oxford) for helpful criticism and advice. The current project has been funded by DEFRA (SE3026).

REFERENCES

Bourne, J., Donnelly, C.A., Cox, D.R., Gettinby, G., McInerney, J.P., Morrison, I. and Woodroffe, R. (2000). Bovine tuberculosis: towards a future control strategy, Vet. Rec. 146, 207-210

Collett, D. (1994). Modelling survival data in medical research, Chapman & Hall/CRC, Boca Raton, USA. 347p

Costello, E., Egan, J.W., Quigley, F.C., and O'Reilly, P.F. (1997). Performance of the single intradermal comparative tuberculin test in identifying cattle with tuberculous lesions in Irish herds. Vet. Rec. 141, 222-224

Cox, D. R. (1972). Regression models and life tables. Journal of the Royal Statistical Society B-74, 187-220

Davies, G. (2002). The foot and mouth disease (FMD) epidemic in the United Kingdom 2001. Comp. Immunol. Microbiol. Infect. Dis. 25, 331-43

Evans, H. and Thompson, H. (1981). Bovine tuberculosis in cattle in Great Britain. I: Erradication of the disease from cattle and the role of the badger (*Meles meles*) as a source of *Mycobacterium bovis* for cattle. Animal Regulation Studies 3, 191-216

Goodchild, A.V. and Clifton-Hadley, R.S. (2001). Cattle-to-cattle transmission of *Mycobacterium bovis*. Tuberculosis 81, 23-41

Green, L.E and Cornell M.A. (2005). Investigations of cattle herd breakdowns with bovine tuberculosis in four counties in England and Wales using VetNet data. (Submitted)

Griffin, J., Hahesy, T., Lynch, K., Salman, M., McCarthy, J. and Hurley, T. (1993a). The association of cattle husbandry practices, environmental factors and farmer characteristics with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. Prev. Vet. Med. 17,145-160

Griffin, J. (1993b). The role of bought-in cattle in herd breakdowns due to tuberculosis in part of County Cavan during 1989. Irish Veterinary Journal 46, 143-148

Griffin, J., Martin, S., Thorburn, M., Eves, J. and Hammond, R. (1996). A case-control study on the association of selected risk factors with the occurrence of bovine tuberculosis in the Republic of Ireland. Prev. Vet. Med. 27, 217-229

Marangon, S.M., Martini, M., Dalla Pozza, M. and Neto, F. (1998). A case-control study on bovine tuberculosis in the Veneto Region (Italy). Prev. Vet. Med. 34, 87-95

Monaghan, M.L., Doherty, M.L., Collins, J.D., Kazda, J.F., and Quinn, P.J. (1994). The tuberculin test. Vet. Microbiol. 40, 111-124

Munroe, F.A., Dohoo, I.R. McNab, W.B. and Spangler, L. (1999). Risk factors for the between-herd spread of *Mycobacterium bovis* in Canadian cattle and cervids between 1985 and 1994. Prev. Vet. Med. 41, 119-33

Pollock, J.M. and Neill S.D. (2002). *Mycobacterium bovis* infection and tuberculosis in cattle. Vet. J. 163, 115-27

Quirin, R., Rasolofo, V. Andriambololona, R., Ramboasolo, A., Rasolonavalona, T., Raharisolo, C., Rakotoaritahina, H., Chanteau, S. and Boisier, P. (2001). Validity of intradermal tuberculin testing for the screening of bovine tuberculosis in Madagascar. Onderstepoort J. Vet. Res. 68, 231-238

Wilesmith, J. W. (1983). Epidemiological features of bovine tuberculosis in cattle herds in Great Britain. J. Hyg. (Lond) 90, 159-176

THE IMPACT OF BADGER REMOVAL ON THE CONTROL OF TUBERCULOSIS IN CATTLE HERDS IN IRELAND

J.M. GRIFFIN[1], T.A. CLEGG, G.E. KELLY, D.H. WILLIAMS, I. O'BOYLE, J.D. COLLINS AND S.J. MORE

SUMMARY

Tuberculosis in cattle continues to be prevalent in Ireland. In Ireland and Britain, there has been evidence of a potential link between tuberculous badgers and bovine tuberculosis. This study's objectives were to assess the impact of badger removal on the control of tuberculosis in cattle herds. The study was conducted between 1997 and 2002 in matched removal and reference areas in 4 counties: Cork, Donegal, Kilkenny and Monaghan. Badger removal was proactive in the removal areas, but reactive (in response to severe tuberculosis outbreaks in cattle) in the reference areas. A significant difference was found between the removal and reference areas in all 4 counties in the time to a confirmed herd restriction due to tuberculosis. The hazard ratios (removal over reference) ranged from 0.4 to 0.04, that is a 60 to 96 percent decrease in the rate at which herds were becoming the subject of a confirmed restriction.

INTRODUCTION

Two factors have highlighted the potential role of wildlife as a constraint to eradication of bovine tuberculosis in Ireland. First, there was no observable progress towards disease eradication despite the implementation of intensive measures that have effectively eliminated cattle-to-cattle transmission in other countries (Collins, 2001). Secondly, there was increasing evidence in support of the involvement of wildlife as a reservoir of *Mycobacterium bovis*. A number of other countries, notably New Zealand, Great Britain, Northern Ireland and, more recently, the state of Michigan in the USA, have faced similar concerns regarding the progress of their eradication programmes. In each case, the persistence of an *M. bovis* infected wildlife reservoir has been considered a constraint to the control of tuberculosis in cattle. More specifically, there has been growing evidence of the role of infected badgers (*Meles meles*, a species with legal protection in Ireland since 1976) as a reservoir of bovine tuberculosis in Ireland and Britain. There was recognition that badgers were highly susceptible to *M. bovis* infection and that tuberculosis was endemic within the badger population in Ireland. However, this information on its own was not sufficient to prove that *M. bovis* is being transmitted from badgers to cattle, since it is possible to have coincident disease (with identical strains) in local badgers and cattle, but without badgers being the source of infection.

Given this background, a field trial offers the best opportunity to determine critically whether badgers are a source of infection for Irish cattle. The East Offaly project in Ireland was

[1] John Griffin, Centre for veterinary Epidemiology and Risk Analysis, Faculty of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland. Email: john.griffin@ucd.ie

the first study of this type, comprising a 738 km^2 'project' area (where badgers were removed under licence) in county Offaly and a surrounding 1,455 km^2 'control' area (Eves, 1999; Ó'Máirtín et al., 1998a; Ó'Máirtín et al., 1998b). Results from the East Offaly project provided robust evidence of the link between tuberculous badgers and tuberculosis in cattle, with badger removal resulting in a statistically significant decrease in the risk of disclosure of a tuberculin reactor in a herd (Ó'Máirtín et al., 1998b). The current study seeks to build on the East Offaly project, and to determine the effect of badger removal at a number of sites representing a wider range of farming environments in Ireland. Detailed analysis of this study has been presented elsewhere (Griffin et al., In press), the results of which are summarised in this paper along with additional descriptive findings.

MATERIALS AND METHODS

Study Areas

The study was conducted between 1st September 1997 and 31st August 2002 in matched removal and reference 'study areas' in four different geographical regions of Ireland, namely counties Cork, Donegal, Kilkenny and Monaghan. During the selection of the removal areas, a range of criteria was used, including apparent disease prevalence, the presence of natural geographical boundaries and areas considered representative of the diverse Irish landscape. Where natural boundary barriers were absent, 'buffer areas' were created, up to 6 km in width, at the periphery of each selected removal area. Likewise, the matching criteria for the reference areas used in the study included county, livestock density, herd size, farm-enterprise type, disease prevalence and selected geographical features. In addition, but only when natural barriers were absent, each reference area was separated from both the removal and adjoining buffer areas by a distance of at least 3 km.

The badger population

Badger population and habitat: On all participating farms, a comprehensive survey of badger habitat and activity was conducted on all land parcels within each removal, buffer and reference area.

Badger removal policy: In the removal and buffer areas, a proactive programme of badger removal was carried out under licence on 2 to 3 occasions each year. In each of the reference areas, badger removal was entirely reactive and was only conducted following severe outbreaks of tuberculosis (four or more standard reactors) in cattle herds.

A number of badger removal operations were carried out prior to the study. Data relating to these operations have been recorded since 1988.

Tuberculosis status: A gross *post-mortem* examination was conducted on all captured badgers following euthanasia. If no evidence of tuberculosis was found, a pool of designated tissues was sent for histopathological examination and culture. A badger was regarded as positive for tuberculosis if it was positive on histopathological examination and/or on culture.

The cattle population

Tuberculosis status: The national tuberculin testing programme was conducted, without modification, in the study areas throughout the study period. Therefore, all herds underwent at least annual testing, with animals being tested using the single intradermal comparative tuberculin test (SICTT). An animal was deemed a 'standard reactor' if the increase in skin thickness at the site of injection of the bovine tuberculin was >4 mm in excess of the increase at the site of the injection of avian tuberculin. Official Veterinary Inspectors examine all slaughtered animals during abattoir surveillance, either routinely or following a positive herd test. The herd was subject to animal movement restrictions if an animal was deemed a reactor, or tuberculosis was diagnosed in a non-reactor animal at routine *post-mortem* examination. The schedule for the tuberculin testing programme along with the test interpretation used were identical in both the removal and reference areas and corresponded to those concurrently used in the national programme.

For the purposes of this study, a confirmed tuberculosis herd restriction was defined as a herd restriction at which a tuberculous lesion was detected in one or more animals at *post-mortem* examination at the time of or during the course of the restriction.

Descriptive statistics

Confirmed restriction risk: The 'confirmed restriction risk' (CRR) is defined as the number of newly confirmed restrictions per 100 herds *per annum.* The denominator of this measure includes all herds that were officially tuberculosis-free (that is, unrestricted) prior to their first test within that year. The numerator includes all new confirmed herd restrictions within the year (that is, herds that were unrestricted prior to their first tuberculin test in that year). If a herd had a confirmed restriction more than once in a year, it was only counted once in the numerator. A year was defined as commencing on 1 September and ending on 31 August in the following year.

An average CRR was calculated for three time periods: the 'pre-study' period (from 1st September 1992 to 31st August 1997, inclusive) and two periods in the 'study' period (from 1st September 1997 to 31st August 2000 and from 1st September 2000 to 31st August 2002 inclusive). This measure was calculated by summing the number of confirmed restrictions per year and dividing by the sum of the number of herds per year, within each time period. It is therefore a weighted average.

Modelling

Details of a logistic regression analysis and survival analysis have been presented by Griffin et. al. (In press). A summary of the survival analysis carried out by Griffin et al. (In press) are presented. The outcome measure of the survival analysis was the time to a confirmed herd restriction.

A survival time for each herd was calculated as the time from September 1, 1992 to a confirmed restriction, or August 31, 2002, whichever came first. In the latter case, the survival time was censored. In cases where no herd test was carried out in the 2 years prior to the end of the study or where a between-test interval was more than 2 years, the survival time was censored at the time of the last herd test. A range of explanatory variables were included in the models

tested including: TR, a factor with 2 levels denoting the removal and reference areas; herd size (time dependent); CO a factor with 4 levels denoting the counties, and a time dependent factor PH with 2 levels denoting whether the herd had previously had a confirmed restriction. YEAR was used to denote a factor with 10 levels representing the years 92/93, 93/94, … , 01/02. A time-dependent factor PERIOD with 2 levels corresponding to the pre-study and study (i.e. from 1 September, 1997) periods was used to summarise results.

Survival analysis: The Kaplan-Meier estimate of the survival function (Collett, 1994) was computed separately for the removal and reference areas within each county, and these were compared using the nonparametric Wilcoxon test.

A Cox regression model was constructed as described by Collett (1994). Herd size and YEAR were entered into the model as time-dependent variables, changing with chronological time. To account for herds with multiple restrictions, the time-dependent factor PH was included. The counting process form of a Cox model was used with the Anderson-Gill method for treating multiple events (Therneau & Grambsch, 2000). The model included the terms TR, CO and PH and all 2 and 3 way interactions including those with YEAR. The model was checked by examining the martingale, influence and Schoenfeld residuals. A similar Cox model was also developed to model survival in the 5-year period prior to the study and the 5-year period during the study. Model terms were included and tested as in the 10-year model, with the factor PERIOD replacing YEAR. This model was used to estimate the overall hazard ratio between removal and reference areas and between periods.

RESULTS

Study areas

The total size of the removal (excluding the buffer areas) and reference areas was 1,961 km^2 (Table 1), which is approximately 3.9% of the agricultural land area of the Republic of Ireland.

Table 1. The area (km^2) of the removal, buffer and reference areas in counties Cork, Donegal, Kilkenny and Monaghan.

County	Removal	Buffer	Reference
Cork	188	119	199
Donegal	215	11	275
Kilkenny	252	61	253
Monaghan	305	63	274

Badger population

Sett survey: A total of 3,077 setts and 2,448 setts were located in the removal and reference areas respectively. Of these, 549 and 464 setts were active at the time of the initial survey in the removal and reference areas respectively.

Badger removal: Prior to the study, there was no organised badger removal prior to 1988 in Ireland. There had been limited badger removal in counties Cork and Donegal during the pre-

study period (1992 to 1997). In contrast, badger removal had occurred during the pre-study period in counties Kilkenny and Monaghan.

There was a high level of support from farmers. Only one farmer withheld permission to survey, and permission to undertake a programme of badger removal within the removal areas was only refused at 13 surveyed setts (0.42% of those setts surveyed).

There were 2,360 badgers removed in the removal and buffer areas during the study period. In the reference areas, a total of 258 badgers were removed. Figure 1 illustrates the annual removal intensity in the removal and buffer areas, and reflects the relative badger density throughout the study period, assuming uniform removal efficacy.

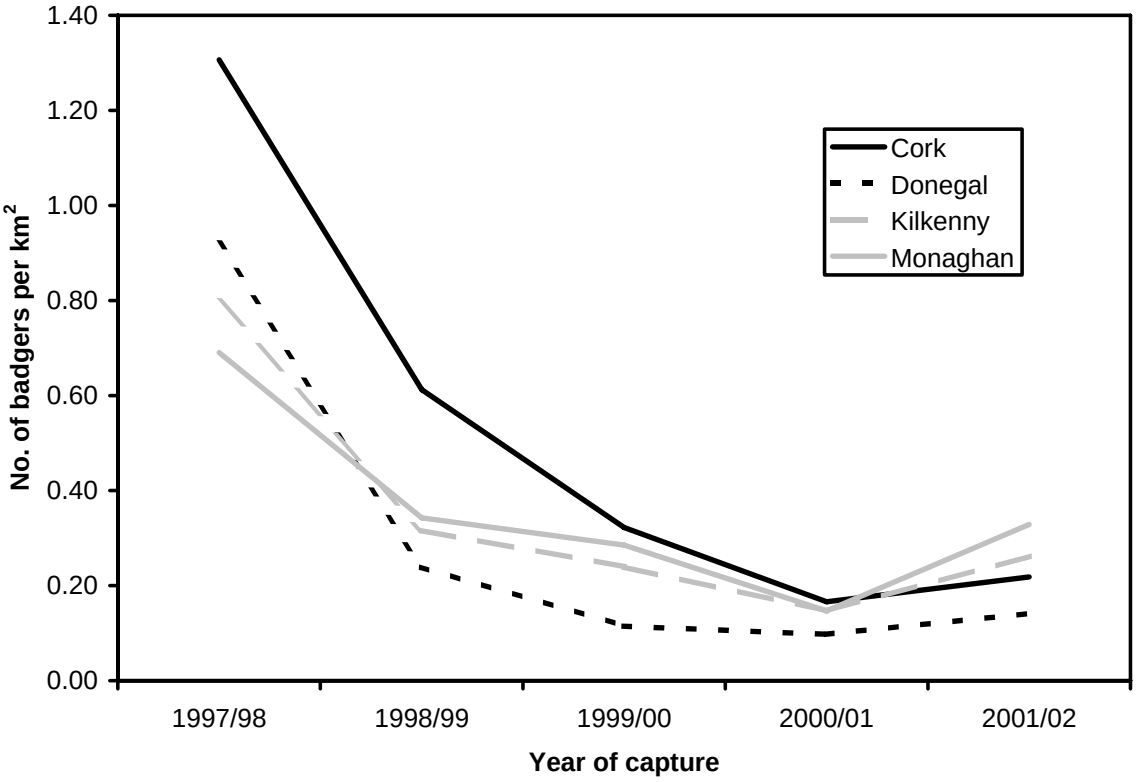


Fig. 1 Density of badger capture per km^2 in the removal and buffer areas by county and year of capture

Tuberculosis status: Of the 2,360 badgers captured in the removal and buffer areas during the study period, 2,310 (97.9%) were examined *post-mortem* with samples being forwarded for culture and/or histopathology. Of the initial 2,310 badgers, 450 (19.5%) were considered positive for tuberculosis. The prevalence of tuberculosis dropped during the study period, ranging from 9% in Monaghan to 14% in Cork in the last year of the study (Fig. 2). In Kilkenny, the prevalence was relatively stable during the course of the study, finishing at 15%. There was a significant difference between the four counties in the proportion deemed positive in the removal and buffer areas ($\chi^2 = 41.79$, $P < 0.001$). Cork had the highest proportion of tuberculous badgers (26%), whereas Donegal and Kilkenny each had the lowest infected badger population (14%).

Of the 258 badgers captured in the reference areas during the study period, 218 (84.5%) were examined at *post-mortem* with samples being forwarded for culture and/or histopathology.

Some 57 (26.1%) were positive for tuberculosis. The prevalence of tuberculosis among captured badgers in the removal and buffer areas compared to the reference areas was significantly different ($\chi^2 = 5.52$, P =0.02).

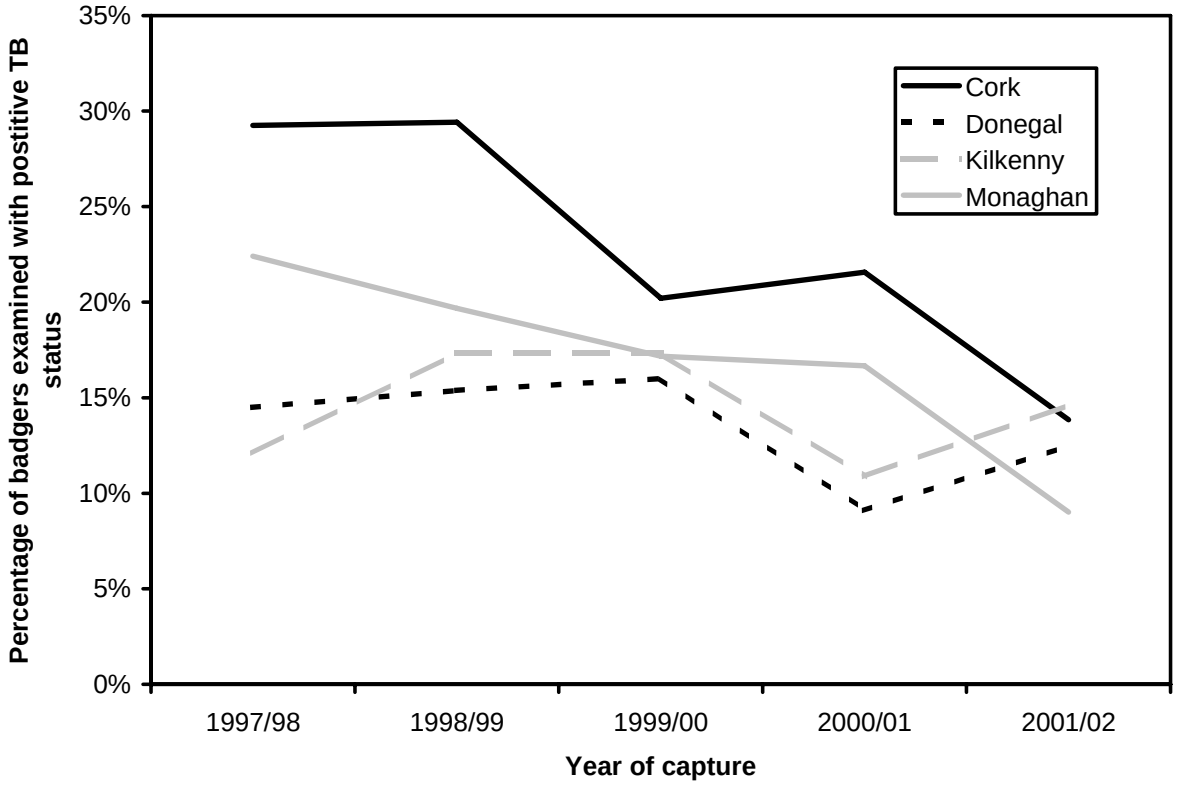


Fig. 2 Proportion of examined badgers in the removal and buffer areas deemed positive for tuberculosis, by county and year of capture

Cattle population

Descriptive statistics: *Prior to the study (1st September 1992 to 31st August 1997):* In the removal and reference areas in all counties, except Donegal, the average annual CRR was substantially higher than the national average (Fig. 3). The average CRR was higher in the removal area compared to the reference area in Cork (11.1 vs. 8.2) and Donegal (3.8 vs. 1.7), whilst the CRR was higher in the reference area in Kilkenny (7.5 vs. 7.8) and Monaghan (7.2 vs. 8.9) (Fig. 3).

During the study period (1st September 1997 to 31st August 2002): In the removal areas, a total of 193 (11.7% of all) herds were the subject of a confirmed restriction on at least one occasion. This represented a substantial decrease in the CRR. The largest change in the removal areas was observed in Cork where the average CRR fell from 11.3 in the pre-study period to 1.0 in the last two years of the study period. The smallest change was observed in Donegal where the CRR fell from 3.8 in the pre-study period to 0.3 in the last two years of the study period. In all counties, the average CRR in the removal areas during the study period was below the national average (Fig. 3). In the reference areas in the study period a total of 393 (26.7% of all) herds were subjected to a confirmed restriction on at least one occasion. In all counties, except Donegal, and in comparison to the pre-study period, the average CRR in the reference areas fell

in the last two years of the study period. The average CRR was lower in the removal area compared to the reference area in all counties during the study period (Fig. 3). The average CRR in the removal areas in the last two years of the study for all counties was almost half that in the reference area (e.g. Cork 4.7 vs. 1.0, Donegal 3.6 vs. 0.3, Kilkenny 7.0 vs. 2.5, Monaghan 6.3 vs. 3.2).

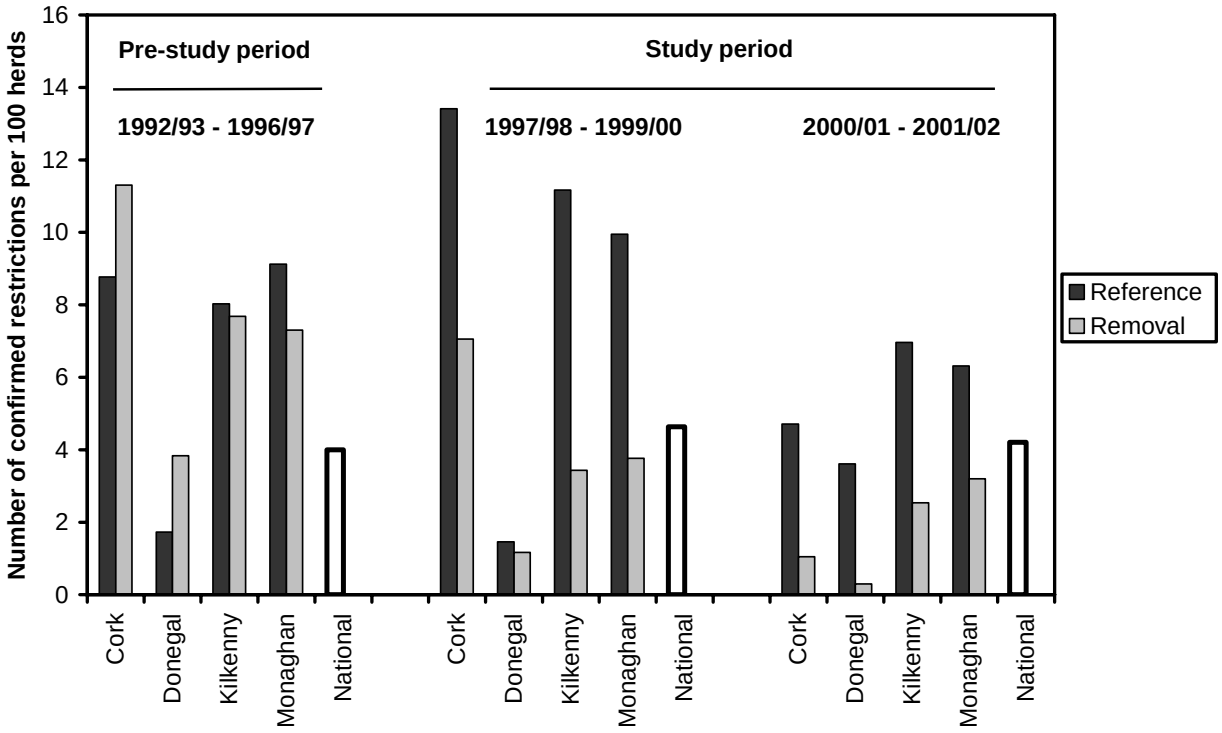


Fig. 3 Weighted average of the annual confirmed restriction risk (CRR) in the removal and reference areas and nationally in the pre-study (1st September 1992- 31st August 1997) and study (1st September 1997 – 31st August 2002) period

Survival Analysis:

In every county, there was a significant shortening of survival time ($P < 0.001$ using the Wilcoxon test) for the reference areas. Table 2 gives the estimates of the 5-year survival probability. In each removal area, the probability of surviving for 5 years without a confirmed restriction was higher than the corresponding reference area ($P < 0.001$ in all counties, Wald's test).

The final Cox model contained the terms PH, log(H), TR, CO and the 2- and 3-way interactions between TR, CO and YEAR. The TR × CO × YEAR interaction was significant ($P < 0.001$), therefore, the effect of treatment varied over county and over year. Table 3 compares the removal and reference areas for each county and each year. Cork, Kilkenny and Monaghan all show significant effects of treatment during the study period, with hazard ratios <1. Donegal shows a significant effect of treatment only in the final year of the study, although the hazard ratio is decreasing in the last 3 years. There were significant differences between removal and reference areas prior to the study period (with removal having a higher hazard) for 1 year in Cork and Kilkenny and 2 years in Donegal. In 1 year in Kilkenny, the removal area had a significantly lower hazard (Table 3).

Table 2. Kaplan-Meier probability of surviving for 5 years without a confirmed restriction, by county and treatment, in the study period

County	Reference area			Removal area		
	Survival probability	S.E.	No. in the risk set	Survival probability	S.E.	No. in the risk set
Cork	0.599	0.027	273	0.781	0.024	270
Donegal	0.897	0.016	331	0.963	0.010	370
Kilkenny	0.615	0.030	207	0.857	0.023	224
Monaghan	0.651	0.019	550	0.851	0.013	663
4 Areas	0.690	0.012	1360	0.865	0.008	1531

To summarise results, a Cox model was fitted with PERIOD replacing YEAR. This reduced model contained the terms PH, log(H), TR, CO and 2-way and 3-way interactions between TR, CO and PERIOD. Thus, the effect of treatment varied over counties and over period. The hazard ratios, removal over reference, in the study period were significantly less than 1 in every county (Table 4). In the pre-study period, the hazard ratios, removal over reference, were significantly greater than 1 in Cork and Donegal, whilst not significant in Kilkenny and Monaghan.

Table 5 examines the difference between the pre-study and study period. In the reference areas, there was no significant change between the 2 periods for any county. In contrast, in the removal areas, the hazard ratio in the study period compared to prior to the study was significantly less than 1 in every county, indicating that survival times for the removal area in every county were longer in the study period than before. There was also a significant county effect ($P < 0.001$) in the reference areas, in the pre-study period and again in the study period, and in the removal areas in the pre-study period and again in the study period.

Table 3. Hazard ratios (HR), from the Cox model, of a confirmed herd restriction in the removal area compared to the reference area in counties Cork, Donegal, Kilkenny and Monaghan

Year	Cork		Donegal		Kilkenny		Monaghan	
	P-value	HR	P-value	HR	P-value	HR	P-value	HR
92/93	0.002	3.48	0.768	1.14	0.338	0.65	0.809	0.95
93/94	0.448	1.26	0.035	3.23	0.043	0.43	0.487	0.87
94/95	0.852	1.05	0.001	5.56	0.409	1.36	0.393	0.84
95/96	0.286	1.34	0.550	0.79	0.020	2.12	0.985	1.00
96/97	0.119	1.41	0.828	0.74	0.517	1.23	0.669	1.11
97/98	0.906	1.03	0.438	0.55	0.414	0.75	<0.001	0.30
98/99	0.005	0.47	0.820	0.87	<0.001	0.15	<0.001	0.44
99/00	0.003	0.35	0.247	0.43	0.002	0.24	0.006	0.49
00/01	0.016	0.16	0.124	0.18	0.247	0.56	0.051	0.59
01/02	0.029	0.25	0.002	0.04	0.017	0.26	0.007	0.40
Log(H)[a]	<0.001	1.68						
PH[b]	<0.001	1.29						

Significant estimates (P <0.05) have been shaded.
a. Constant effect of log herd size.
b. Constant effect of previous history (PH)

Table 4. Hazard ratios (HR), from the reduced Cox model, of a confirmed herd restriction in the removal area compared to the reference area in counties Cork, Donegal, Kilkenny and Monaghan

Period	Cork		Donegal		Kilkenny		Monaghan	
	P-value	HR	P-value	HR	P-value	HR	P-value	HR
1992-1997	0.004	1.43	0.005	1.83	0.523	1.11	0.480	0.93
1997-2002	<0.001	0.52	<0.001	0.28	<0.001	0.35	<0.001	0.43
Log(H)[a]	<0.001	1.67						
PH[b]	<0.001	1.29						

Significant estimates (P <0.05) have been shaded.
a. Constant effect of log herd size.
b. Constant effect of previous history (PH)

Table 5. Hazard ratios (HR), from the reduced Cox model, of a confirmed herd restriction in the study (1st September 1997 to 31st August 2002) compared to the pre-study (1st September 1992 to 31st August 1997) period in counties Cork, Donegal, Kilkenny and Monaghan

Study area	Cork		Donegal		Kilkenny		Monaghan	
	P-value	HR	P-value	HR	P-value	HR	P-value	HR
Reference	0.363	1.16	0.653	1.13	0.565	1.12	0.363	0.86
Removal	<0.001	0.36	<0.001	0.17	<0.001	0.36	<0.001	0.40

DISCUSSION

Study justification

The four area project represents a logical progression from the East Offaly project. This earlier work was the first large-scale field trial to investigate the effect of badger removal on the control of bovine tuberculosis, and provided evidence of a statistically significant effect. Some concerns have been raised regarding the design of that project (Eves, 1999; Ó'Máirtín et al., 1998b), including the opportunity (given the doughnut design) for continuing migration of badgers into the control area, and the need for greater geographical diversity; each was addressed in the design of the current work. However, in terms of the former concerns, it is important to note that inward migration (with badgers moving from the control to the project area) will have had the effect of making it more difficult to detect a treatment effect, if one was present. The findings of the current study are consistent with the earlier results from the East Offaly project.

Study findings

Badger population: The results from this study indicate that the overall size of the badger population in Ireland may be less than the estimate of 200,000 adult badgers provided in a national survey, carried out between 1989 and 1993 by Smal (1995). This population estimate was based on the assumption that a badger social group uses only one main sett, and that the average social group size was 5.9 adults. In the removal and buffer areas we would expect 549 distinct social groups (the number of active main setts) and an estimated badger population of 3,239 adults. However, only 2,360 badgers were actually captured in these areas during the study. Since it is reasonable to assume (certainly in counties Cork, Kilkenny and Monaghan where the removal area boundaries were not entirely secure) that many of the later captures were immigrant badgers, the number captured during the first two years of the study (1,580) may be a more accurate estimate of the size of the native population at the start of the study. Smal's estimate of social group size was similar to reports from the UK (Cresswell et al., 1990; Wilson et al., 1997) and from East Offaly (O'Corry-Crowe et al., 1993), but at odds with studies from Northern Ireland (Feore & Montgomery, 1999), where average group sizes as low as 3.0 were reported. In order to reconcile these differences, further studies are currently underway to investigate the size of the badger population in Ireland.

Distribution of tuberculosis amongst the badger population: In this study, the prevalence of tuberculosis in badgers (19.5% in the removal areas and 26.1% in the reference areas) was

considerably higher than estimates from a range of previous Irish studies, including the East Offaly study (12% in the project area (Ó'Máirtín et al., 1998b)), routine examination of animals removed under licence (annual prevalence ranging from 12.2 to 13.3%; (O'Boyle, 1999, 2000, 2002; O'Boyle et al., 2003)) and a study of road casualty accidents (11.6%; (O'Boyle et al., 2003)). These differences are more likely to be a reflection of improved diagnostic methods rather than any change in actual disease prevalence (Costello et al., 1998). Several trends in disease prevalence are of particular interest. Tuberculosis prevalence was higher in the reference areas, which may reflect the selective way in which badgers were removed in these areas. Equally, the reducing rate in the removal areas may reflect changes in disease transmission with a declining population. There were also significant county differences, with levels being highest in the Cork removal area (29%) and lowest in Kilkenny (12%). Again, disease prevalence may have been influenced by population density, noting that there had been some badger removal in the two years prior to the study in Kilkenny and Monaghan. However, there is currently little understanding of the spatial distribution of tuberculosis in badgers in Ireland.

Cattle population: During the study period, there was a significant difference between the removal and reference areas in both the probability of and the time to a confirmed herd restriction due to tuberculosis (Griffin et al., in press). The estimated probability of surviving 5 years without a confirmed restriction was between 7% (Donegal) and 24% (Kilkenny) higher in the removal over the reference area (Table 2). In the final year of the study, the hazard ratios (removal over reference) ranged from 0.40 to 0.04 or less (Table 3), that is, a 60 to 96 percent decrease in the rate at which herds were becoming the subject of a confirmed restriction. In addition, the CRR in the removal area of every county was almost half that in the reference area in the study period. These effects are consistent across all 4 counties, which is remarkable given known differences between each of these 4 areas. These differences include the varying levels of badger disturbance prior to study start (minimal in counties Cork and Donegal but recent in counties Kilkenny and Monaghan), varying levels of herd restriction in the pre-study period and the diverse farming environments and likely badger densities.

Study validity

The study sites were not chosen randomly; rather, a number of criteria were used during the selection of the study areas. Consequently, the results from this study must be generalised with care. It would seem reasonable, given the absolute size of the study areas (covering 1,961 km^2 and representing 3.9% of the agricultural land area in the *Republic* of Ireland), the diversity of farming environments represented in this study and the general consistency of the key study results in each of the 4 areas, that these results can be generalised to other 'problem' areas of Ireland.

CONCLUSIONS

This study needs to be considered within a clearly defined context. The Irish control programme is comprehensive, incorporating each of the accepted elements of disease control, including mandatory annual tuberculin testing of all animals in the national herd and early, ongoing removal of infected animals. As a direct consequence of these efforts, cattle-to-cattle transmission has become relatively less important in the epidemiology of bovine tuberculosis in Ireland. The results from the four area (and earlier East Offaly) study clearly highlight that wildlife (and specifically transmission of infection from badgers to cattle) is a key constraint to

disease eradication in Ireland. The relative importance of cattle-to-cattle and wildlife-to-cattle transmission will depend on a range of circumstances, including the standard of animal husbandry, aspects of national disease control and badger ecology. Therefore, the results should be extrapolated to other regions with care.

In order to eradicate tuberculosis from the Irish cattle population, in the short-term, Ireland is implementing a national programme for wildlife control when and where wildlife are implicated in on-farm breakdowns of bovine tuberculosis. In the longer-term, Ireland is committed to the development of an effective badger vaccine and the implementation of a strategic programme of badger vaccination.

ACKNOWLEDGEMENTS

The study was designed and undertaken by the Centre for Veterinary Epidemiology and Risk Analysis (CVERA), University College Dublin. The fieldwork was undertaken by the Department of Agriculture and Food and personnel from the Farm Relief Service under the supervision of the local District Veterinary Office. The data were collated and analysed by CVERA and the Department of Statistics and Actuarial Science, University College Dublin. Expertise on ecological issues was provided by the Department of Zoology, University College Cork and Dr Chris Smal. *Post-mortem* examinations on badgers were carried out by the Central Veterinary Research Laboratory, Regional Laboratories of the Veterinary Service and the Irish Equine Centre. The study was funded by the Department of Agriculture and Food.

REFERENCES

Collett, D. (1994). Modelling Survival Data in Medical Research. Chapman and Hall, London, 347 p

Collins, J.D. (2001). Tuberculosis in cattle: new perspectives. Tuberculosis (Edinb) 81, 17-21

Costello, E., Quigley, F., O'Grady, D., Flynn, O., Gogarty, A., McGuirk, J., O'Rourke, J. and Griffin, J. (1998). Laboratory examination of tissues from badgers and cattle from the four-area study for evidence of tuberculosis, In: Selected Papers 1997. Tuberculosis Investigation Unit, University College Dublin, Dublin, 32-36

Cresswell, P., Harris, S. and Jefferies, D.J. (1990). The history, distribution, status and habitat requirements of the badger in Britain. Nature Conservancy Council, Peterborough

Eves, J.A. (1999). Impact of badger removal on bovine tuberculosis in east County Offaly. Irish Vet. J. 52, 199-203

Feore, S. and Montgomery, W.I. (1999). Habitat effects on the spatial ecology of the European badger (Meles meles). Journal of Zoology 247, 537-549

Griffin, J.M., Williams, D.H., Kelly, G.E., Clegg, T.A., O'Boyle, I., Collins, J.D. and More, S.J. (In press), The impact of badger removal on the control of tuberculosis in cattle herds in Ireland. Prev. Vet. Med. In press

Hammond, R.F. (1997). Variables used to characterise the four areas badger survey removal/reference areas. Tuberculosis Investigation Unit, Selected Papers 1996, University College Dublin, 12-18

O'Boyle, I. (1999). Review of Badger (*Meles meles)* research licenses in 1998, In: Selected Papers 1998. Tuberculosis Investigation Unit, University College Dublin, Dublin, 10-14

O'Boyle, I. (2000). Review of Badger (*Meles meles)* research licenses in 1999, In: Selected Papers 1999. Tuberculosis Investigation Unit, University College Dublin, Dublin, 15-21

O'Boyle, I. (2002). Review of Badger (*Meles meles)* research licenses in 2000 and 2001, In: Selected Papers 2000-2001. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin, 19-25

O'Boyle, I., Costello, E., Power, E.P., Kelleher, P.F., Bradley, J., Redahan, E., Quigley, F., Fogarty, U. and Higgins, I. (2003). Review of Badger (*Meles meles)* research licenses in 2002, In: Selected Papers 2002-2003. Veterinary Epidemiology and Tuberculosis Investigation Unit, University College Dublin, Dublin, 13-18

O'Corry-Crowe, G., Eves, J.A. and Hayden, T.J. (1993). Sett distribution, territory size and population density of badgers (*Meles meles* L.) in east Offaly., In: Hayden, T.J. (Ed.) The badger. Royal Irish Academy, Dublin, 35-56

Ó'Máirtín, D., Williams, D.H., Dolan, L., Eves, J.A. and Collins, J.D. (1998a). The influence of selected herd factors and a badger-intervention tuberculosis-control programme on the risk of a herd-level trade restriction to a bovine population in Ireland. Prev. Vet. Med. 35, 79-90

Ó'Máirtín, D., Williams, D.H., Griffin, J.M., L., D. and Eves, J.A. (1998b). The effect of a badger removal programme on the incidence of tuberculosis in an Irish cattle population. Prev. Vet. Med. 34, 47-56

Smal, C. (1995). The Badger and Habitat Survey of Ireland. Stationary Office, Dublin

Therneau, T. and Grambsch, P. (2000). Modelling Survival Data. Springer, New York, 350 p

Wilson, G.S., Harris, S. and McLaren, G.. (1997). Changes in the British badger population 1988 to 1997. Peoples Trust for Endangered Species, London

MODELLING

EXPLAINING CLASSICAL SWINE FEVER PERSISTENCE BY COMBINING EPIDEMIOLOGICAL AND ECOLOGICAL MODELLING

S. KRAMER-SCHADT[1], N. FERNANDEZ AND H.-H. THULKE

SUMMARY

How does Classical Swine Fever (CSF) persist in wild boar populations? An individual-based, spatially-explicit simulation model was developed and several hypotheses explaining CSF persistence were tested. The influences of different simulated factors on persistence were compared using generalised linear models to determine the causes that favour persistence of CSF. In contradiction to what would be expected, piglets partially protected by maternal antibodies were not found to be a significant contributor to disease persistence ($p < 0.001$). Effective infection probability was found to have a strong influence, with high values favouring persistence. Thus, it is very likely that most susceptibles are reached by the virus in times when there are only few susceptibles remaining. However, the strongest effect was a variable disease outcome related to the virulence of the virus. This was expressed as a parameter combination influencing individual mortality and the period of virus shedding, with a moderately virulent virus resulting in a higher number of infected animals that survive longer (i.e. chronic infections). These findings place strong emphasis on chronically infected animals as perpetuators of the disease, because the virus can survive during times when there is no reproduction in wild boar and hence no new susceptibles.

INTRODUCTION

Classical Swine Fever is a viral disease, which has recently caused very serious economic losses in the European Union (EU). It is rated as one of the most important diseases of domestic animals in Europe (Artois et al., 2002). Classical Swine Fever virus infection occurs under natural conditions in domestic pigs and wild boar, *Sus scrofa*. Although several countries, such as Australia, Canada, New Zealand and USA, succeeded in eradicating CSF, the disease is currently present in parts of the wild boar population in many geographical regions of the EU Member States, i.e. France, Germany and Italy (Moennig et al., 1999).

Classical Swine Fever infection in wild boar is thought to be the main risk factor for CSF outbreaks in domestic pigs, due to direct contact in some areas of extensive pig keeping (Laddomada et al., 1994; Zanardi et al., 2003) or to indirect contact through feeding of contaminated wild boar meat to domestic pigs (Laddomada, 2000; Teuffert et al., 1998; Wachendörfer et al., 1978). Combating CSF in wild boar populations is therefore a special challenge to protect the pig farming industry.

[1] Stephanie Kramer-Schadt, Department of Ecological Modelling, UFZ Centre for Environmental Research, Permoser Str. 15, D-04318 Leipzig, Germany. Email: Stephanie.Kramer@ufz.de

The CSF virus may be circulating in and perpetuated for years among some European wild boar populations, but it is yet unclear how the disease can remain in some foci (Fritzemeier et al., 1998 and 2000; Kern et al., 1999; Laddomada et al., 1994). The current hypotheses explaining observed persistence relate to (i) prenatal infection in piglets, (ii) piglets that are partially protected by maternal antibodies and (iii) the occurrence of moderately virulent virus strains resulting in more chronic infections in affected populations.

How can a better understanding of the disease course of CSF and the factors leading to disease cycling be achieved? Reliable field trials require long-term designs, which are costly and sometimes impossible to conduct. An alternative is model-based investigations, because models provide the opportunity to explore the influence of many epidemiological and demographic parameters (Peck, 2004). Individual-based, spatially explicit models are most adequate to investigate disease transmission, the key process in host-pathogen interactions (McCallum et al., 2001). In the case of the wild boar, its social and territorial organisation may result in different rates of contact between individuals of different social groups, and reduced rates of contact between territories separated by distance or landscape features. Therefore, a spatially-explicit, individual-based approach provides the appropriate modelling technique to tackle the two different scales of CSF in wild boars: the within-herd and the between herd aspects.

An individual-based, spatially-explicit simulation model is presented that reveals under which hypothetical scenario the disease will persist. Results are discussed in respect to wild boar being a possible reservoir for CSF and the implications this can have for the management of this species.

MATERIALS AND METHODS

Modelling methods

The model is based on female boar groups that follow individual life histories within their home ranges of 4 km², reflected by a grid cell within the landscape. The model starts with a landscape of 50 by 50 cells, which reflects an area of 10,000 km². There are initially 2500 groups with 15 individuals each, and a period of five years is allowed to elapse in order to develop a stabilised population structure before one infected animal is released into the wild boar population. The respective ages of the pigs released initially are drawn from a distribution obtained from the model after having it run for 100 years. Male groups are not modelled explicitly, because they are not the limiting factor for population survival. In addition, the effect of long distance dispersal on disease transmission is not considered. The model uses time steps of one week, as this is approximately the incubation period of the disease (Artois et al., 2002). The demographic variables for each surviving individual (i.e., age) are updated at each step.

The demographic parameters of the wild boar model mainly stem from published data from populations in France, Italy, Poland and Germany. The data are based on long-term field studies of up to 10 years, mostly of hunted populations (Table 1). For the analysis of disease persistence, parameters for the boar population dynamics were fixed according to these references.

Table 1. Wild boar population demography parameters as used in the model

	Symbol	Published values		Model parameter (range)
Carrying capacity of boars per home range	CC	normally 5-10; up to 40	[a]	5 breeding females
Maximum age [years]	Y_{max}	9 (females), 6 (males)	[b]	11
		11	[c]	
Number of piglets per female	N_{piglet}	3.2 ± 1.68 SD	[a]	3.2, sd = 1.68, range (0 – 10)
		1.5-4.5	[c]	
		4.95 ± 0.42 SE	[d]	
		6 ± 2 SD	[e]	
		6.7 ± 2.1 SD	[f]	
		1.6 – 5.5	[g]	
Survival rates of piglets	SR_{piglet}	0.48 ± 0.37 SD	[a]	0.48, sd = 0.37, range (0.1 – 1)
		0.6 – 0.65	[h]	
Survival rate of yearlings	$SR_{yearling}$	< 0.5	[b]	0.6
		0.26-0.47, 0.65	[h]	
Survival rates of adults	SR_{adult}	0.64 ± 0.24 SD	[a]	0.64, sd = 0.24, range (0.28 – 1)
		0.38 (males), 0.2 (females)	[h]	
Natal dispersal distances of subadult females	d_{group}	up to 20 km, mean 4.5 km	[i]	Up to 9 km

[a]Focardi et al., 1996, [b]Stubbe et al., 1989, [c]Jezierski, 1977, [d]Boitani et al., 1995, [e]Ahmad et al., 1995, [f]Nahlik & Sandor, 2003, [g]Andrzejewski & Jezierski, 1978, [h]Gaillard et al., 1987, [i]Truvé & Lemel, 2003

There are insufficient data available to identify the distribution function for all disease parameters. Therefore, a uniform distribution was assumed for each variable with upper and lower limits derived from literature or estimation (Table 2). Seven thousand six hundred and eighty (7680) parameter sets were generated, and for each parameter set the model was run for 40 replicates. The sensitivity of the model to the input parameters was investigated by analysing the extinction risk of the disease within 10 years. Relationships between input parameters and model output were analysed using generalised linear models.

The basic wild boar population model

Reproduction: Females reproduce only once a year, depending on their age class. There is also a different weekly probability for females to reproduce depending on the season, i.e. the highest probability of reproduction is in April and May (e.g. Boitani et al., 1995). A binomial distribution P_{month} (p = 0.4, x = {1, 12}, N = 12) is used to represent that the maximum reproduction is taking place in spring, whereas reproduction is low or zero in winter. To model the quick reaction of wild boar to changing environmental conditions constraints are imposed for unlimited population growths and a process is included for population regeneration after bad years by allowing a maximum number of females per group to reproduce (breeding capacity). Older females breed first. If, due to any stochastic process, adult females have died, the

subadults can also reproduce. The weekly probability of reproduction P_R per female that is allowed to reproduce is then:

$$P_R = 0.25 * P_{month} \quad (1)$$

The number of piglets per reproducing female $N_{Piglets}$ depends on a Gaussian distribution around the mean and standard deviation.

Table 2. Model input parameters and range. The column 'PI piglets' gives the parameter values systematically changed for assessing the impact of persistently infected offspring on disease persistence.

Parameter	Symbol	Range		PI piglets
		min	max	
Effective infection probability within group	P_{inf_G}	0.005	1	0.05,1
Effective infection probability between group being the fraction of P_{inf_G}	P_{inf_N}	1	20	5
Number of time steps, where piglet is protected by maternal antibodies	TS_{MA}	12	48	12, 48
Time of virus release	*SEASON*	April	October	April
Virulence classification		from low	to high	
Maximum survival time (weeks) of lethally infected boars	T_{MAX}	45	5	5, 30
Exponent, giving the proportion of infected animals that live for a long time	X	1	10	1, 5
Probability of lethal infection (for subadults; values for adults and piglets have to be calculated with the formula described in Materials and Methods)	$1\text{-}P_{Trans}$	0.1	0.6	0.2, 0.6

Group split up: Female groups split up when the carrying capacity has been exceeded and when a neighbouring home range cell is not occupied by yet by another female group. The female subadults, at least 2 in the model, are then randomly moved to the next suitable cell. Moves of up to three cells are allowed, reflecting distances from natal home ranges of about 6 to 9 km. Female groups use their home ranges exclusively, i.e. no other female group can move to that cell. Normally, female groups are very stationary, but group splitting could be an important aspect in population dynamics, when female groups die out due to disease mortality. Group split up is allowed once per year in summer (week 28).

Baseline mortality: Age-dependent mortality is assigned to every time step. The mortality probability per time step $PM_{TimeStep}$ equals the geometric mean of the annual mortality probability PM_{Year} for the corresponding age class, which in turn is determined from survival estimates (SP_{Year}) found in the literature:

$$PM_{TimeStep} = 1 - (1\text{-}PM_{Year})^{1/52} \quad (2)$$

$$PM_{Year} = 1\text{-}\ SP_{Year} \quad . \quad (3)$$

The annual survival SP_{Year} follows a Gaussian distribution with mean and standard deviation as found in literature. The stochastic effect resembles 'good' or 'bad' years for boars. For reasons of symmetry, the mortality probability distribution of adults is cut when the mean is different from 0.5 in the following way: if mean > 0.5 then d = 1–mean, with $adultSP_{Year}$ = (mean–d, 1), resulting in a minimum survival rate of 0.28 (Table 1). To avoid a zero survival probability for piglets, a minimum survival rate of 0.1 is set, coming from mean–1SD.

CSF virus submodel

The CSF virus submodel is based on interventions in the ecological processes of reproduction, group split up and death. The submodel distinguishes between different individual responses to virus strains of low to high virulence, resulting in different mortality probabilities. The epidemiological conditions 'susceptible', 'lethally infected' (including prenatally infected piglets), 'transiently infected' (i.e. virus shedding for 1 week and then latency for 3 further weeks) and 'immune' are identified in the model (Fig. 1).

Infection: The number of infected animals in the group I_G and neighbouring groups I_N is counted, and with an effective infection probability P_{inf_G} (that includes a contact and infection probability) other members of the same group get infected. Infection between groups is modelled with an effective infection probability P_{inf_N}, that is a fraction of the effective infection probability within groups P_{inf_G} (Table 2), as the contact rate between animals of different cohorts is normally lower. The probability P_{SI}, that a susceptible animal becomes infected, is thus:

$$P_{SI} = 1 - ((1 - P_{inf\_G})^{IG} * (1-(P_{inf\_G} / P_{inf\_N}))^{IN}) \quad (4)$$

Response to infection: Mortality probability of infected individuals is dependent on the virulence of the virus, i.e. its ability to kill its host, and other individual traits of the pig, such as age and health condition. For example, the outcome of the disease when pigs in good health condition are infected with a moderately virulent strain, is transient or chronic. On the other hand, pigs under stress and infected with a highly virulent virus strain are lethally infected (case mortality *sensu* (Day, 2002)), and most of them will die within a short time period. To simplify communication, outcomes of low, moderate or high 'virulence' will be referred to in the course of this paper, although it is important to bear in mind that the outcome is not only determined by the virulence of the virus strain, but also by several host factors.

To reflect the different responses of individuals to an infection, a probability that animals get infected only transiently P_{Trans} is determined, depending on the age class of the pig, with

$$P_{Trans}Adult = (P_{Trans})^2,\ P_{Trans}Subadult = P_{Trans}, \text{ and } P_{Trans}Piglet = (P_{Trans})^{0.5} \quad (5)$$

If animals are infected lethally, the proportion of animals living until their individual survival time T_S is modelled with a function depending on a maximum survival time T_{MAX} and an exponent X, with

$$P_{SR} = (1-(T_S / T_{MAX}))^X \quad (6)$$

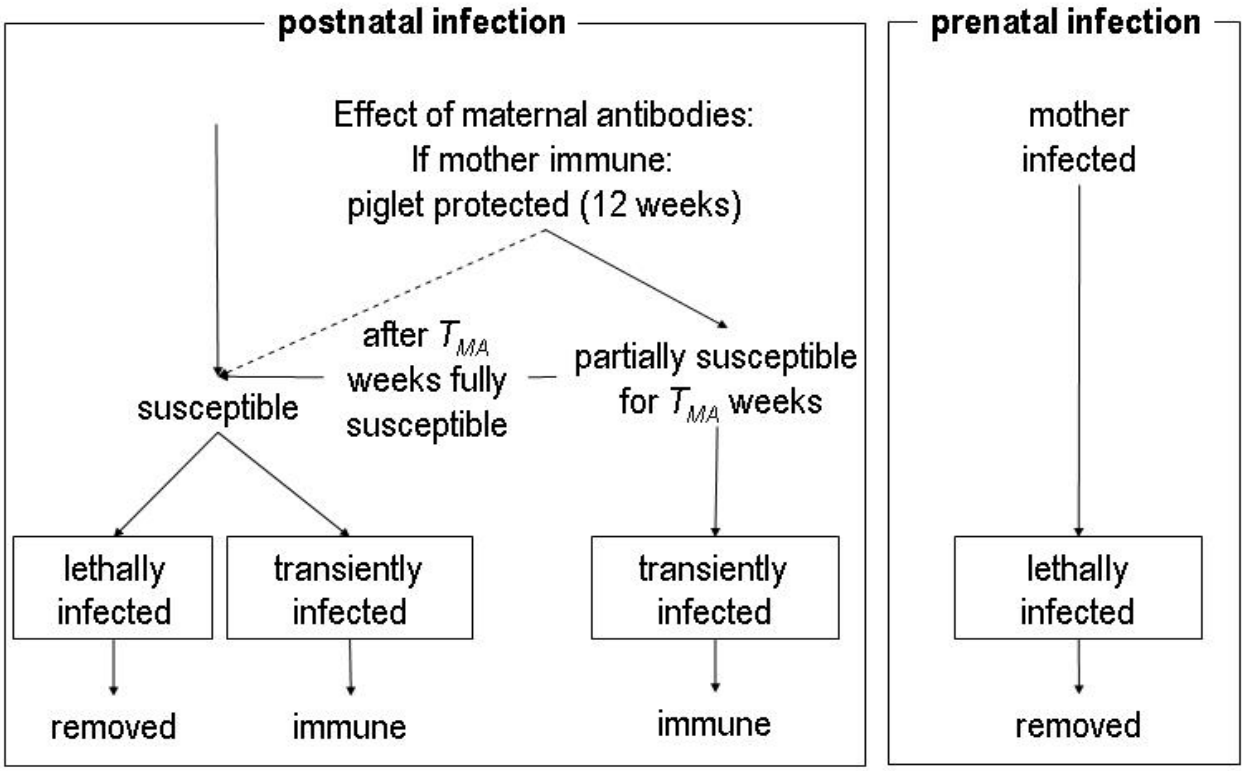


Fig. 1 Flow chart of the disease course. Both lethally and transiently infected individuals shed the virus and can infect other individuals.

The individual survival time T_S for each individual can then be drawn from this distribution. Thus, disease outcome is expressed as a combination of case mortality and the survival time of infected animals. It is assumed that low-virulent strains infect a higher proportion of the animals only transiently, whereas high-virulent strains kill most of the infected animals. It is further assumed that the outcome of an infection with a less virulent virus is chronic rather than acute, i.e. a higher proportion of the animals are shedding the virus for a longer time. The expected lifespan until death is also longer, whereas in a high 'virulence' situation there is a low proportion of transiently and chronically infected animals, with the latter only living for a short time, and a high proportion of animals living only for 4 weeks. Thus, the proportion of acutely and chronically infected pigs is given by the exponent X, with high values of X resulting in many acutely infected, and low values of X in many chronically infected, respectively.

Reproduction: When the female boar is infected, 10/16 of the foeti are aborted, half of the rest are persistently infected (PI) offspring, and the remainder are normal susceptible piglets. The PI piglets are automatically removed (mortality probability = 1) after time step T_S drawn from the distribution described above. We test the effect of PI piglets on disease persistence with a number of parameter sets chosen from the original 7680 parameter combinations (Table 2). If the pregnant sow is already immune, then the piglets are born with maternal antibodies. That means, due to maternal antibodies, piglets do not get infected for the first three months. After that, they have a low antibody titre for TS_{ma} time steps, which makes the outcome of an infection transient. These so-called partially protected piglets are set back to the status 'susceptible' after TS_{ma} time steps. The persistence of the disease with and without this effect of maternal antibodies is analysed and the effect of different duration times is also tested.

Analysis of model results

The effects of the following factors on disease persistence were analysed with generalised linear models (log link and a Poisson error distribution using procedure GENMOD; SAS Institute Inc., Cary, NC): season of virus introduction *SEASON*, effective infection probability within P_{Inf_G} and between groups P_{Inf_N}, the effect of the duration of maternal antibodies TS_{MA}, the three parameters describing the different responses of the host to infection, namely the probability of getting transiently infected P_{trans}, the maximum duration of the infection T_{max} and the proportion of acutely and chronically infected pigs given by the exponent X (Table 2). The disease was defined as persistent if, after 10 years, infected animals were still found.

RESULTS

The model was correctly specified, i.e. deviance was smaller than the degrees of freedom, hence indicating a good agreement between data and the selected link and error distribution. Disease persistence was mostly determined by parameters describing the response of individuals to infection (Table 3). With a high virulence, i.e. high exponent values X and short infection length T_{max}, the disease was more self-limiting, whereas low virulence caused cycling of the disease. Also, the speed of the infection, given by the effective infection probability within P_{Inf_G} and between P_{Inf_N} groups, had a significant effect on disease persistence. That is, the higher the infection probability within and between groups, the higher the probability of persistence. High infection probability guarantees a constant infection chain, even when there are only few susceptibles.

The *season* of virus introduction and the probability of transient infection P_{trans} did not have a significant effect, probably because the most important age class for disease transmission, i.e. piglets, is more prone to lethal infection and thus for a longer infection period determined by T_{Max}. This means that there was always a sufficiently high number of lethally infected susceptibles. Similarly, the duration of maternal antibodies TS_{MA} did not show any significant effect. The presence of maternal antibodies seems to shift the result of an infection to a transient outcome. The results clearly show the significance of a high proportion of chronically infected pigs.

The model was also fixed for the different values for effective infection probability within groups P_{Inf_G} and the duration of the disease T_{max}. For all fixed effective infection probabilities, a high proportion of chronically infected (expressed by low values of exponent X) and a long infection time (T_{max}) was contributed significantly to persistence. For extremely low effective infection probabilities within groups, a high infection probability between groups P_{Inf_N} and many lethally infected 1-P_{trans}, were also significant. With an extremely short survival time of lethally infected T_{max}, i.e. 5 weeks, only a high proportion of chronically infected was significant, indicating that it is crucial that most infected have to survive that time span. With long survival times, the effective infection probabilities within and between groups contributed significantly to persistence.

Persistently infected offspring (PI piglets) had no significant effect on the persistence of the disease (t-test, P=0.5).

Table 3. Analysis of parameter estimates of the full model, testing for disease persistence over 10 years. Parameters with a significant effect on disease persistence are shown in bold.

Model parameter		DF	Estimate	SE	Wald 95% confidence limits		Chi-square	P
Intercept		**1**	**-2.0437**	**0.0879**	**-2.2161**	**-1.8714**	**540.11**	**<.0001**
$\boldsymbol{P_{inf\_G}}$		**1**	**0.6273**	**0.0484**	**0.5325**	**0.7221**	**168.12**	**<.0001**
$\boldsymbol{P_{inf\_N}}$		**1**	**-0.0137**	**0.0029**	**-0.0194**	**-0.0080**	**22.40**	**<.0001**
SEASON	*Autumn*	1	-0.0335	0.04	-0.1118	0.0448	0.70	0.4020
SEASON	*Spring*	0	0	0	0	0	.	.
TS_{MA}		1	-0.0010	0.0013	-0.0037	0.0016	0.60	0.4384
$\boldsymbol{T_{max}}$		**1**	**0.0518**	**0.0015**	**0.0488**	**0.0547**	**1155.79**	**<.0001**
X		**1**	**-0.1725**	**0.0077**	**-0.1876**	**-0.1574**	**501.27**	**<.0001**
$1\text{-}P_{trans}$		1	-0.0073	0.1041	-0.2113	0.1967	0.0	0.9441

DISCUSSION

Most western populations of wild boars have remained free of CSF, and apparently only a limited number of populations in continental Europe have suffered disease outbreaks. It is still not known how the infection can be maintained in a focus at a relatively low rate. Classical Swine Fever foci in wild boars have never been reported to expand into an epidemic wave of infection over large areas, but seem rather limited in size, perhaps due to natural barriers that limit the movement of boars (Artois et al., 2002).

The increased occurrence of endemic situations reported in the literature in recent years is hypothesised to be because of the increasing size and density of the wild boar population and the involvement of viral strains of low virulence (Depner et al., 1998; Fritzemeier et al., 2000; Kern et al., 1999). It is thought that the mechanism of virus spreading through prenatally infected piglets also plays a key role in the survival of the CSF virus in a pig population, as it can lead to persistently infected offspring living for a long time (Liess, 1987; Terpstra, 1988). On the other hand, it is argued that in endemic areas, transplacental infections leading to persistently infected piglets are unlikely to occur at a substantial rate due to the low incidence of new infections in older animals. Thus it appears unlikely that persistently infected animals play a significant role in the maintenance of the virus (Moennig et al., 1999). Another hypothesis is that piglets partially protected by maternal antibodies can maintain the disease, as this process can take up to one year. These transiently infected piglets would spread the disease during dispersal.

In our model, these three hypotheses were investigated. The results clearly support the hypothesis of the involvement of low-virulent strains. Contrary to what would have been expected, persistently infected offspring proved to have no effect. This implies that transplacental infection is not important enough to have a significant effect at the population level. Also, the existence of partially protected piglets did not have any significant effect on persistence. In contrast, the more transiently infected animals that ultimately convert into immunes, the higher the immunisation rate in the population. However, the hypothesis is related to the quicker spread of the disease by movement of these piglets, and this effect was not analysed.

The mechanism behind the low virulence hypothesis is that in times without offspring (winter), chronically infected individuals survive until the next reproductive cycle. However, only the acute course of the disease has been described in the field. This can be due to the difficulty in detecting chronically infected individuals in the field, as they don't show the typical clinical signs. On the other hand, due to winter feeding and an abundant food supply, many populations in Central Europe may experience constant reproduction throughout the year. Another factor could be the size of the population in relation to the speed of the disease spread. If the disease spreads slowly in small foci or the area is large, respectively, then there still exist many susceptibles throughout winter that can be reached by the virus, until the next reproduction cycle begins. Then, the next disease wave can spread while the first outbreak is still ongoing.

Our model shows that there are circumstances allowing wild boars to act as a reservoir for CSF. This has important consequences for the management of the species. For example, the species abundance is favoured in many areas for hunting, and there are reintroduction plans in other areas where wild boars are extinct like in Denmark and England (Goulding et al., 2003). Under both situations, the risks of epidemics associated with wild boar management should be carefully assessed (see Alban et al., (2005) for an example of reintroduction planning in Denmark). This model provides a good means to explore the effect of host density, dispersal and spatial heterogeneity on disease dynamics and to tackle the effect of different management scenarios (increased hunting, selective hunting, vaccination schemes) to limit CSF epidemics.

ACKNOWLEDGEMENTS

The authors are grateful to Dirk Eisinger and Michael Müller for their help in programming and fruitful discussions.

REFERENCES

Alban, L., Andersen, M.M., Asferg, T., Boklund, A., Fernandez, N., Greiner, M., Kramer-Schadt, S., Stockmarr, A., Thulke, H.-H., Uttenthal, Å. and Ydesen, B (2005). Classical swine fever and wild boar in Denmark: A risk analysis. Proceedings the Society for Veterinary Epidemiology and Preventive Medicine, 79-90

Ahmad, E., Brooks, J. E., Hussain, I. and Khan, M. H. (1995). Reproduction in Eurasian wild boar in central Punjab, Pakistan. Acta Theriol. 40, 163-173

Andrzejewski, R. and Jezierski, W. (1978). Management of a wild boar population and its effects on commercial land. Acta Theriol. 23, 309-339

Artois, M., Depner, K. R., Guberti, V., Hars, J., Rossi, S. and Rutili, D. (2002). Classical swine fever (hog cholera) in wild boar in Europe. Revue Scientifique et Technique de l'Office International des Epizooties 21, 287-303

Boitani, L., Trapanese, P., Mattei, L. and Nonis, D. (1995). Demography of a wild boar (*Sus scrofa L.*) population in Tuscany, Italy. Gibier Faune Sauvage 12, 109-132

Day, T. (2002). On the evolution of virulence and the relationship between various measures of mortality. Proceedings of the Royal Society of London B 269, 1317-1323

Depner, K. R., Kern, B. and Liess, B. (1998). Epidemiologische Relevanz der Persistenz von KSP-Virus beim Schwarzwild (Sus scrofa sp.). Amtstierärztlicher Dienst und Lebensmittelkontrolle 5, 244-248

Focardi, S., Toso, S. and Pecchioli, E. (1996). The population modelling of fallow deer and wild boar in a Mediterranean ecosystem. Forest Ecol. Man. 88, 7-14

Fritzemeier, J., Greiser-Wilke, I., Depner, K. and Moennig, V. (1998). Characterization of CSF virus isolates originating from German wild boar. Commission of the European Communities, Perugia, Italy, pp 107-109

Fritzemeier, J., Teuffert, J., Greiser-Wilke, I., Staubach, C., Schluter, H. and Moennig, V. (2000). Epidemiology of classical swine fever in Germany in the 1990s. Vet. Microbiol. 77, 29-41

Gaillard, J. M., Vassant, J. and Klein, F. (1987). Quelques charactéristiques de la dynamique des populations de sangliers (*Sus scrofa scrofa*) en milieu chassé. Gibier Faune Sauvage 4, 31-47

Goulding, M. J., Roper, T. J., Smith, G. C. and Baker, S. J. (2003). Presence of free-living wild boar Sus scrofa in southern England. Wildlife Biol. 9, 15-20

Jezierski, W. (1977). Longevity and mortality rate in a population of wild boar. Acta Theriol. 22, 337-348

Kern, B., Depner, K. R., Letz, W., Rott, M., Thalheim, S., Nitschke, B., Plagemann, R. and Liess, B. (1999). Incidence of classical swine fever (CSF) in wild boar in a densely populated area indicating CSF virus persistence as a mechanism for virus perpetuation. J. Vet. Med. B 46, 63-67

Laddomada, A. (2000). Incidence and control of CSF in wild boar in Europe. Vet. Microbiol. 73, 121-130

Laddomada, A., Patta, C., Oggiano, A., Caccia, A., Ruiu, A., Cossu, P. and Firinu, A. (1994). Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African swine fever. Vet. Microbiol. 134, 183-187

Liess, B. (1987). Pathogenesis and epidemiology of hog cholera. Ann.Rech.Vét. 18, 139-145

McCallum, H., Barlow, N. and Hone, J. (2001). How should pathogen transmission be modelled? Trends Ecol. Evol. 16, 295-300

Moennig, V., Albina, E., Depner, K., Ferrari, G., Guberti, V. and Vassant, J. (1999). Classical Swine Fever in wild boar. European Commission. Report number XXIV/B3/R09/1999

Nahlik, A. and Sandor, G. (2003). Birth rate and offstpring survival in a free-ranging wild boar Sus scrofa population. Wildlife Biol. 9, 37-42

Peck, S. L. (2004). Simulation as experiment: a philosophical reassessment for biological modeling. Trends Ecol. Evol. 19, 530-534

Stubbe, C., Mehlitz, S., Peukert, R., Goretzki, J., Stubbe, W. and Meynhardt, H. (1989). Lebensraumnutzung und Populationsumsatz des Schwarzwildes in der DDR - Ergebnisse der Wildmarkierung. Beiträge zur Jagd- und Wildforschung 16, 212-231

Terpstra, C. (1988). Epizootiology of hog cholera. In Classical Swine Fever and related viral infections, ed. B. Liess, pp. 201-216. Martinus Nijhoff Publishing, Dordrecht, Boston

Teuffert, J., Kramer, M. and Schlüter, H. (1998). Zur Epidemiologie der Schweinepest in Deutschland unter besonderer Berücksichtigung der Aufgaben des praktischen Tierarztes. Der praktische Tierarzt, collegium veterinarium 28, 45-49

Truvé, J. and Lemel, J. (2003). Timing and distance of natal dispersal for wild boar *Sus scrofa* in Sweden. Wildlife Biol. 9, 51-57

Wachendörfer, G., Reinhold, G. E., Dingeldein, W., Berger, J., Lorenz, J. and Frost, J. W. (1978). Analyse der Schweinepest-Epizootie in Hessen in den Jahren 1971-1974. Deutsche Tierarztliche Wochenschrift 85, 113-152

Zanardi, G., Macchi, C., Sacchi, C. and Rutili, D. (2003). Classical swine fever in wild boar in the Lombardy region of Italy from 1997 to 2002. Vet. Rec. 152, 461-465

DEMONSTRATING FREEDOM OF DISEASE AFTER AN EMERGENCY VACCINATION CAMPAIGN WITH AN E2 SUB-UNIT MARKER VACCINE AGAINST CLASSICAL SWINE FEVER: A SIMULATION

J. DEWULF[1], D. VERLOO, F. KOENEN, H. LAEVENS, K. MINTIENS, S. RIBBENS AND A. DE KRUIF

SUMMARY

The aim of this study was to simulate end screening after an emergency vaccination campaign with a marker vaccine against Classical Swine Fever (CSF).

In each run of the model, it is assumed that 500 herds are vaccinated. The number of infected herds and the number of infected animals within an infected herd are modelled as binomial distributions. The animal-level sensitivity and specificity are modelled as beta distributions. At each iteration the number of animals to be sampled in a herd is calculated in order to detect a minimal within herd prevalence (e.g. 1%) with a given level of confidence (e.g. 99%). The model indicates for each herd whether it is categorized as infected or not, based upon a given threshold of positive samples (e.g. 5%). By comparing these results to the simulated proportion of infected herds, it becomes possible to determine the herd sensitivity and specificity.

If a herd prevalence of 5% and a within-herd prevalence of 2% are assumed, the highest combined herd sensitivity (HSe) (77.54%) and specificity (HSp) (74.45%) is obtained at the threshold level of 2%. If a threshold level of 5% is used the HSe decreases to 36.95%, whereas the HSp increases to 98.93%. Varying other parameters (e.g. expected within-herd prevalence, sample size) never results in a situation where an acceptably high HSe and HSp are combined. Therefore, further efforts are needed to improve herd level diagnostics. Also these results should be used to review available simulation models as the choice may influence the conclusions dramatically.

INTRODUCTION

Since 1990, the control of classical swine fever (CSF) in the European Union (EU) has been based on a policy of non-vaccination and stamping-out. Recent outbreaks have shown that in unvaccinated populations, the control of CSF through stamping-out can give rise to considerable economic losses, particularly in areas with high pig densities (Koenen et al., 1996; Meuwissen et al., 1999). This is partially due to the large number of animals that are pre-emptively

[1] Jeroen Dewulf, Department of Reproduction, Obstetrics and Herd Health, Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium. Email: Jeroen.Dewulf@Ugent.be

slaughtered when trying to cope with virus spread in the neighbourhood of infected herds. Ethically, this strategy is being challenged (Torstar, 1998). Therefore, alternative control strategies have been developed and encouraged. Recently the European legislation was modified to facilitate the use of vaccines in the control of epidemics of CSF (Council Directive 2001/89/EC). Ideally in such emergency vaccination scenarios, one should be able to differentiate the vaccinated from the infected animals so that the uninfected pigs remain valuable for consumption.

E2 sub-unit marker vaccines against CSF have existed for a number of years, and recently an enhanced discriminatory serological test was approved (Commission Decision 2003/265/EC). Several simulation studies have been conducted to evaluate the applicability of these marker vaccines in emergency vaccination scenarios (Mange et al., 2001; Mange et al., 2002). These studies concluded that marker vaccination is only beneficial compared with non-vaccination if meat from vaccinated uninfected pigs can be sold on the EU market. Therefore, end screening to determine which herds remained uninfected during the epidemic would be crucial. However none of the above-mentioned simulation models describes the difficulties that one may encounter when end screening is performed to demonstrate freedom of disease in the vaccinated herds.

In the report on the evaluation of a new CSF discriminatory test (Commission Decision 2003/265/EC) it is concluded that, based upon the sensitivity and specificity at animal level, the test needs to be used for diagnosis on a herd basis and not on samples from single animals. However, several authors have advised caution in attempting to extrapolate individual-animal test criteria to the herd level (Carpenter & Gardner 1996; Martin et al., 1992;).

The aim of this study is to simulate an end screening, using the existing data concerning the efficacy of the available marker vaccines (van Earshot, 2003) and the diagnostic characteristics of the recently approved differentiating serological test (Commission Decision 2003/265/EC). This simulation is used to evaluate the difficulties which may be encountered.

MATERIALS AND METHODS

A stochastic simulation model using Monte Carlo simulation was developed in Excel (Microsoft Corporation, Redmond, Washington) and @risk (Palisade Corporation, Newfield, NY).

1. Herds

In each run of the model, it is assumed that 500 herds are vaccinated. The size of each herd is sampled from a lognormal distribution with mean = 1000 and standard deviation =1200 (truncated to the left at 10 and to the right at 8000). This distribution is used since it resembles the frequency distribution of the size of Belgian pig herds.

2. Between herd prevalence

The number of infected herds is modelled as a binomial distribution with n = 500 at a certain herd prevalence (e.g. p = 5%). During the analysis the effect of different herd prevalences is evaluated.

3. Within herd prevalence

For each infected herd, the number of infected animals is again modelled as a binomial distribution with n = herd size and p = a beta distribution with $\alpha 1 = 0.25$ and $\alpha 2 = 12$ (truncated to the left at 0.5% and to the right at 20%). The mean of this distribution is: $\alpha 1 / (\alpha 1 + \alpha 2) = 2\%$. During the analysis the effect of different within-herd prevalences is evaluated.

4. Test sensitivity and specificity

Data on the performance of the serological test were obtained from the evaluation report of a new CSF discriminatory test (Commission Decision 2003/265/EC). In this report the animal-level sensitivity of the serological test in a population of vaccinated and experimentally infected animals at 21 days after infection was estimated to be 98.0%. This is based on 483 samples with 10 false negative results. The animal-level specificity of the test in a population of vaccinated uninfected pigs was estimated to be 98.9%. This is based on 188 samples with 2 doubtful results. In this estimate of the animal-level specificity false positive results due to border disease virus (BDV) or bovine viral diarrhoea virus (BVDV) infections are not taken into account.

In the simulation model, the animal-level sensitivity and specificity are described as posterior beta distributions with a non-informative beta (1,1) prior and a binomial likelihood being beta (s+1,n-s+1) (Bran scum et al., 2004).

5. Sample size

At each iteration the number of animals to be sampled in each herd is calculated in order to detect a given minimal within herd prevalence (e.g. 1%) with a given level of confidence (e.g. 99%) using Eq. (1) (Dodo et al., 2003).

$$n = \left(1 - (\alpha)^{1/D}\right)\left(N - \frac{D-1}{2}\right) \quad (1)$$

Where n = required sample size, α = 1-confidence level (e.g. 1%), D = estimated minimum number of diseased animals in the group (population size*minimum expected prevalence), N = population size.

6. Model outcome

The model returns the simulated proportion of infected herds, and for each herd:

- the herd size
- the simulated within herd prevalence (= 0 for all uninfected herds)
- the number of animals sampled in that herd
- the number of infected pigs that are included in the sample (binomially distributed with n = the sample size and p = the simulated within herd prevalence)
- the within sample prevalence (=the number of infected pigs in the sample divided by the sample size)
- the number of test positive samples (binomially distributed with n = the sample size and p = (within sample prevalence * sensitivity) + (1- within sample prevalence)*(1-specificity)) (Branscum et al., 2004)
- the number of test negative samples (= the sample size – the number of test positive samples)

Additionally following parameters can be calculated:

- the number of correct test positive samples (binomially distributed with n = the number of positive samples in the sample size and p = (within sample prevalence*sensitivity) / ((within sample prevalence* sensitivity) + (1- within sample prevalence)*(1-specificity)))
- The number of false test positive samples (= the number of test positive samples – the number of correct test positive samples)
- The number of correct test negative samples (binomially distributed with n = the number of negative samples in the sample size and p = ((1- within sample prevalence) * specificity) / (((1- within sample prevalence) * specificity) + (within sample prevalence * (1- sensitivity))))
- the number of false test negative samples (= the number of test negative samples – the number of correct test negative samples)

Subsequently the herds are categorized as infected or not if the number of positive samples is above a given threshold (e.g. 5%).

The herd sensitivity (HSe) is calculated as the number of infected herds that have a number of positive samples that is above the threshold, divided by the total number of infected herds. The herd specificity (HSp) is calculated as the number of uninfected herds that have a number of positive samples that is below the threshold, divided by the total number of uninfected herds.

Each simulation comprised of 500 iterations, and the mean and the 95th percentile ranges are reported.

RESULTS

The basic parameter settings used in all the simulations are given in Table 1, unless specific alternative information is given on a certain variable in the results. Whenever one of the parameters is altered to evaluate the effect of this specific parameter, this is clearly indicated.

Table 1. Basic settings of the input variables

Number of infected herds	Binomial n = 500 p = 5 %
Number of infected animals within an infected herd	Binomial n = herd size p = Beta with: • α1= 0.25 • α2 = 12 • left truncated at 0.5% • right truncated at 20%
Sensitivity	Beta α1= 474 α2 = 11
Specificity	Beta α1= 187 α2 = 3
Desired minimal detectable prevalence[a]	1%
Level of confidence[a]	99% (α = 1%)
Threshold[b]	5%

[a] parameters determining the sample size, [b] a herd is classed as infected if the fraction of positive samples is above this threshold.

The distribution of the herd sizes always remained as described in the materials and methods. Throughout the different simulations, the number of animals present in the 500 vaccinated herds is around 481000 (439000-524000). If the sample size is calculated based upon a minimal detectable prevalence of 1% and a level of confidence of 99% the number of animals to be sampled varies around 154000 (150000-158000).

In the most conservative approach, one would not use a minimum number of positive samples ("threshold") but declare a herd infected as soon as one of the samples is positive, independently of herd and sample size. Under these conditions, a HSe of 98.51% (91.66%-100%) is achieved, meaning that of the average 25 infected herds 0 or 1 will be missed. The HSp is only 0.07% (0.05%-0.10%). This results in a situation where, of the average 475 uninfected herds, all or all but one are falsely positive.

When a threshold is used (= a fraction of the sample size that needs to be positive before a herd is categorized as infected) different values of the HSe and HSp are obtained as a function of a varying threshold (Fig. 1).

The most balanced combination of HSe (77.54%) and HSp (74.45%) is reached at a threshold level of 2%. In these circumstances, on average 19 out of the 25 infected herds will be identified correctly as being infected, whereas 6 will be missed. Of the 475 uninfected herds, on average 355 will be correctly diagnosed as uninfected whereas 120 herds will be falsely classed as infected.

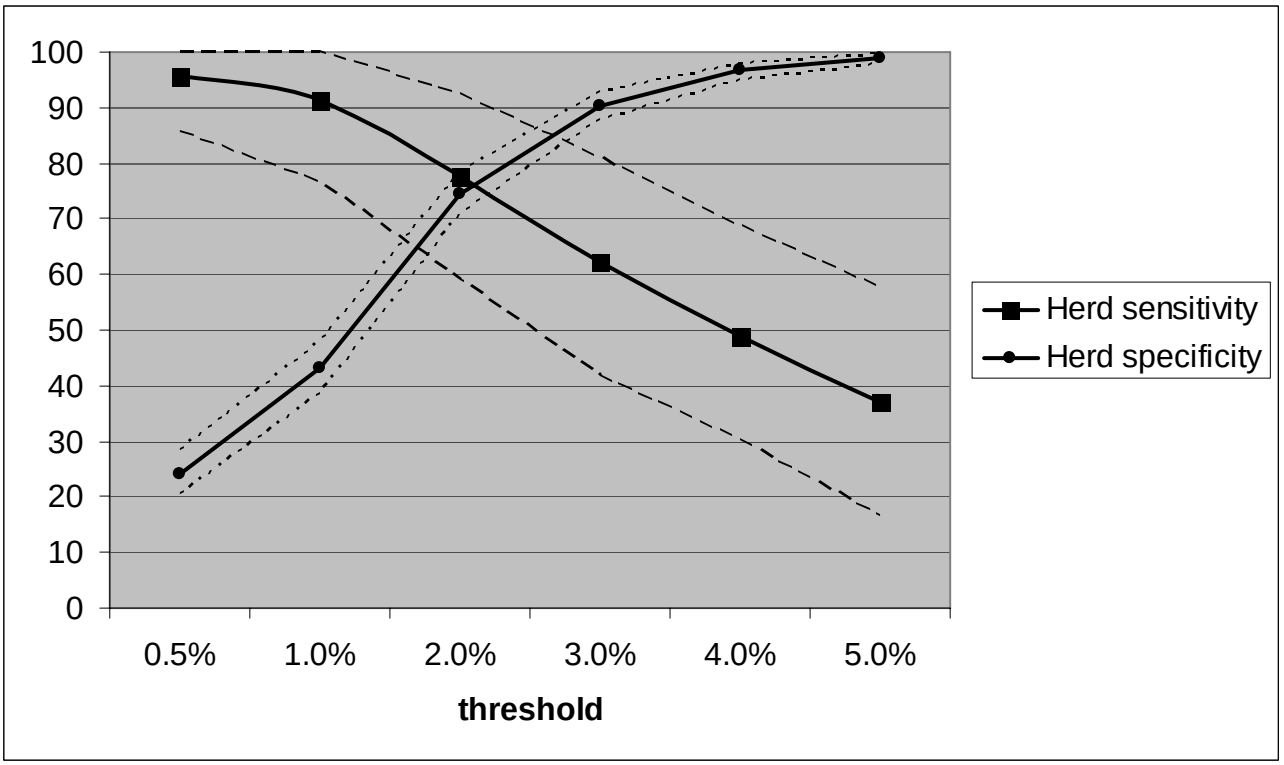


Fig. 1 Varying HSe and HSp (mean, 2.5 and 97.5 percentile) as a function of a varying threshold level

If the threshold level of 2% is maintained but the expected within-herd prevalence is increased to 5% ($\alpha1 = 0.8$; $\alpha2 = 15$; left truncated at 0.5% and right truncated at 20%), the HSe increases to 85.51% (71.42%-96.15%), whereas the HSp remains at a level of 74.41% (70.48%-78.13%). If on the other hand, the expected within-herd prevalence is decreased to 1% ($\alpha1 = 0.15$; $\alpha2 = 15$; left truncated at 0.5% and right truncated at 20%), the HSe decreases to 73.44% (56.25%-90.90%), whereas the HSp remains at a level of 74.57% (70.34%-78.34%).

Increasing the minimum expected prevalence used for the sample size calculations to 2% (equal to the mean within-herd prevalence) results in a discrete increase of the HSp to 74.81% (71.04%-78.90%) whereas a decrease of the HSe is observed to 74.08% (56.25%-90.90%). The number of animals to be sampled decreases to 91000 (89200-92400). If the minimum expected prevalence is further increased to 5%, the HSe decreases to 60.77% (40.74%-80.95%) and the HSp increases to 81.35% (77.91%-84.59%). The total sample size drops further to 40500 (40200-40800).

DISCUSSION

As demonstrated in the results, the outcome of the model is largely influenced by the parameter settings used. In the presented model, an attempt is made to use the most likely settings.

The number of herds that are vaccinated (500) is derived from the already demonstrated and generally accepted fact that emergency vaccination is advisable only when an outbreak occurs in a densely populated pig area (Anon, 1997; Mangen et al., 2002). In such a region, it is expected

that a large number of herds is included in the vaccination area. The distribution of the herd sizes was used to mimic the true distribution of Belgian herd sizes.

As no field experience is available on the use of an E2 marker vaccine in emergency vaccination campaigns in densely populated pig areas, the uncertainty distributions for the within- and between-herd prevalences were estimated from experimental data. In these experiments it has been demonstrated that, in a fully vaccinated population, horizontal transmission is prevented (Dewulf et al., 2004) or largely reduced (Uttenthal et al., 2001) 14 days after single vaccination with a marker vaccine. On the other hand, in experiments which evaluated the protection of vaccinated pigs against infection by natural contact with unvaccinated infected pigs, it was found that, even after double vaccination, pigs were not fully protected, even though no direct contact was possible between the infectious and the vaccinated pigs (Dewulf et al., 2000; Dewulf et al., 2001). As we are simulating an end screening situation, it is assumed that, at the moment of sampling, the epidemic had ended. Furthermore, it is assumed that, during the preceding epidemic all herds (unvaccinated and vaccinated) where CSF was diagnosed through clinical signs and/or virological and serological screening, were already culled. Consequently, it is expected that 3 months after detection of the last infected herd (the time for end screening) only a limited number of infected herds will remain, and that within those infected herds only minor outbreaks had taken place. Herds that experienced a major outbreak are likely to have been detected already during the epidemic.

The animal-level sensitivity and specificity used in the model are based upon information gathered during CSF virus infection and vaccination experiments, in which all samples were of high quality. To determine the sensitivity only those samples were selected that originated from experimentally infected pigs from 21 days after infection onwards and that reacted positively in the virus neutralization test (Commission Decision 2003/265/EC). To determine the specificity, only animals were used that were thoroughly checked for the presence of any pestivirus-specific antibodies before the beginning of the experiment. When the specificity was determined based upon the CSF-Antibody negative field serum samples, the specificity dropped to 97.57% (96.2%-97.6%) (Commission Decision 2003/265/EC). Therefore, it is believed that the values used for animal-level sensitivity and specificity are the upper limits of what is expected under field conditions. Furthermore, it is assumed that the infected animals are randomly distributed throughout the herd. This assumption is probably not entirely valid as it can be expected that minor outbreaks will be localized in certain parts of the herd. On the other hand relocation of the animals occurs during the fattening period as the animals are growing, and this may result in some further mixing.

de Smit (2000) suggested that if discriminatory ELISAs were to be used at a herd level, a CSF infected herd may still be detected if the number of blood samples taken is increased according to the limited sensitivity and the expected reduced prevalence. However, if the number of samples is increased, the number of false positive results will increase accordingly. This is clearly demonstrated in the results where a reasonable high HSe can be obtained if a herd is categorized as being infected as soon as one sample is positive. In these circumstances the HSp is extremely low resulting in almost all uninfected herds becoming falsely positive. Consequently, at the end of the vaccination campaign all vaccinated herds would still be depopulated and the animals destroyed.

To solve this problem, Klinkenberg (2003) suggested that it is possible to achieve a high HSp by declaring a herd to be infected only if the number of positively tested samples is above some “positive threshold”. However, the outcome of our model indicates that a high HSp

inevitably results in a relatively low HSe, and it was not possible to find a threshold value that results in both an acceptably high HSp and HSe, even though large numbers of animals were tested. This is mainly because only limited between- and within-herd prevalences are expected. Additionally, it has to be emphasized that in the simulation model presented, the possibility of false positive results due to the presence of antibodies to other pestiviruses has not been taken into account.

These results indicate that further efforts are needed to improve herd level diagnostics. One way of doing this is by trying to develop an independent confirmatory test which enables truly positive and falsely positive test results to be differentiated. On the other hand it should be mentioned that falsely serologically negative vaccinated pigs do not pose any danger as long as they do not harbour the virus. Therefore, one could try to develop strategies in which absence of the virus is demonstrated rather than absence of antibodies. van Oirschot (2003) suggested that this might be possible by combining clinical inspection, the use of sentinel pigs and testing by means of PCR techniques. Further research is necessary to explore the feasibility of this strategy.

As long as serological diagnosis remains the mandatory test to prove absence of CSF virus in a herd and there is no confirmatory test available, the results of our study indicate that a combined acceptably high HSe and HSp are not achievable. These results should prompt a review of simulation models used to assess the applicability of emergency vaccination using a marker vaccine, as the choice of model may influence the conclusions dramatically.

ACKNOWLEDGEMENTS

This study was supported by grants from the Research Foundation of the Belgian Ministry of Public Health and the Fund for Health and Quality of Animals and Derived Products.

REFERENCES

Anon (1997). The use of marker vaccines in the control of infectious diseases in particular classical swine fever. Report of the Scientific Veterinary Committee of the European Commission

Branscum, A.J., Gardner I.A. and Johnson, W.O. (2004). Bayesian modeling of animal- and herd-level prevalences. Prev. Vet. Med. 66, 101-112

Carpenter, T.E. and Gardner, I.A. (1996). Simulation modelling to determine herd-level predictive values and sensitivity based on individual-animal test sensitivity and specificity and sample size. Prev. Vet. Med. 27, 57-66

Commission Decision 2003/265/EC. Report on the evaluation of a new Classical swine fever discriminatory test. Official Journal of the European Communities

Council Directive 2001/89/EC on community measures for the control of classical swine fever. Official Journal of the European Communities

de Smit, A.J. (2000). Laboratory diagnosis, epizootiology, and efficacy of marker vaccines in classical swine fever: a review. Vet. Q. 22, 182-188

Dewulf J., Laevens H., Koenen F., Mintiens K. and de Kruif A. (2004). A comparative study for emergency vaccination against classical swine fever with an E2 sub-unit marker vaccine and a C-strain vaccine. Prev. Vet. Med. 65, 121-133

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., de Kruif, A. (2001). An E2 sub-unit marker vaccine does not prevent horizontal or vertical transmission of classical swine fever virus. Vaccine 20, 86-91

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H. and de Kruif, A. (2000). An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs. Vaccine 19, 475-482

Dohoo, I., Martin, W. and Stryhn (2003) Sampling. In Veterinary Epidemiological Research. AVC Inc., Charlottetown, Prince Edward Island, Canada, p. 47

Klinkenberg, D. (2003). Towards better control of classical swine fever epidemics: vaccination with an E2 marker vaccine. In Mathematical epidemiology and the control of classical swine fever virus. PhD thesis, Utrecht, Utrecht University. p. 133-142

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J. and Deluyker, H. (1996). Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. Vet. Rec. 139, 367-371

Mangen, M.J., Jalvingh, A.W., Nielen, M., Mourits, M.C., Klinkenberg, D. and Dijkhuizen, A.A., (2001). Spatial and stochastic simulation to compare two emergency-vaccination strategies with a marker vaccine in the 1997/1998 Dutch Classical Swine Fever epidemic. Prev. Vet. Med. 48, 177-200

Mangen, M.J., Nielen, M. and Burrell, A.M. (2002). Simulated effect of pig-population density on epidemic size and choice of control strategy for classical swine fever epidemics in The Netherlands. Prev. Vet. Med. 56, 141-163.

Martin, W.S., Shoukri, M. and Thorburn, M.A. (1992). Evaluating the health status of herds based on tests applied to individuals. Prev. Vet. Med. 14, 33-43

Meuwissen, M.P., Horst, S.H., Huirne, R.B. and Dijkhuizen, A.A. (1999). A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. Prev. Vet. Med. 42, 249-270

Terpstra, C. (1998). Preventive emptying: a compensation for a lack of training. Tijdschr. Diergeneeskd. 123, 324-325

Uttenthal A., Le Potier M., Romero L., De Mia G.M., and Floegel-Niesmann G. (2001). Classical swine fever (CSF) marker vaccine. Trial I. Challenge studies in weaner pigs. Vet. Microbiol., 83, 85-106

Van Oirschot, J.T. (2003). Vaccinology of classical swine fever: from lab to field. Vet. Microbiol. 96, 367-384

WILDLIFE RESERVOIRS

RISK ASSESSMENT FOR INTRODUCTION OF WILD BOAR (*SUS SCROFA*) TO DENMARK

L. ALBAN[1], M.M. ANDERSEN, T. ASFERG, A. BOKLUND, N. FERNÁNDEZ, S.G. GOLDBACH, M. GREINER, A. HØJGAARD, S. KRAMER-SCHADT, A. STOCKMARR, H-H. THULKE, Å. UTTENTHAL AND B. YDESEN

SUMMARY

Danish wildlife organisations have presented the idea that wild boar should be reintroduced in order to preserve nature and broaden national biodiversity. The Danish pig farmers fear that this would lead to the introduction of Classical Swine Fever (CSF), which would have enormous consequences in terms of loss of pork export. A risk assessment was conducted to address the additional risk of introducing and spreading CSF due to reintroduction of wild boar. The OIE guidelines were followed and all available data, as well as expert opinion were utilised. Geographical Information Systems (GIS), InterSpreadPlus, and a programme developed to model wild boar populations and disease transmission were used to model and visualise the differential CSF risk due to wild boar. An active risk communication strategy was put in place from the beginning of the project because reintroduction of wild boar is of interest to the public.

Wild boar use the forest for hiding and reproduction and the surrounding fields for foraging. The habitat demand for one group of sows is around 4km^2 covered with minimum 25% forest or natural vegetation. In total, 9-10% of Denmark consists of suitable or semi-suitable wild boar habitat. This would allow wild boar to establish in several forests, and conflicts are expected between agriculture and wild boar in overlap areas (24% of all Danish pig herds are in these areas). Only a limited migration of wild boar from Germany would be expected, because the wild boar habitat in Southern Jutland is small. Based on information on magnitude and destination of tourism, the risk of exposure to Classical Swine Fever virus (CSFV) by wild boar through infected refuse left by tourists was found to be highest in Western and Northern Jutland compared to the rest of the country.

Outbreaks of CSF within a hypothetical infected wild boar population would last from less than half a year up to one year, depending on the size of the infected wild boar population (the larger the population, the longer the epidemic) and the virulence of the virus (the more virulent virus, the shorter the epidemic). According to the simulations, CSFV would only be transmitted from the domestic population to wild boar if an infected domestic pig herd were located close to an area with wild boar (<5 km). Transmission from wild boar to domestic pigs did not occur in one third of the simulations, whereas in 10% of simulations periodic transmission occurred leading to two or three epidemics. The consequence assessment showed that, on average, the costs related to an epidemic of CSF was only 13% higher if wild boar were present than if not

[1] Lis Alban, Danish Bacon & Meat Council, Vinkelvej 11, Kjellerup, Denmark, DK-8620. Email: lia@danishmeat.dk

present. However, Denmark has not had CSF since 1933, indicating a low base-line probability of introduction, and the presence of wild boar will increase this probability slightly.

INTRODUCTION

Currently there is no established population of free-range wild boar in Denmark. To preserve nature and broaden national biodiversity, Danish wildlife organisations have presented the idea that wild boar should be reintroduced. Historically, wild boar were present in Denmark until around 1800. At that time, only 2% of Denmark was covered with forest.

There is evidence that wild boar play a role in CSF outbreaks in pigs in Europe. Therefore, Danish pig farmers are concerned about the risk associated with the proposed reintroduction. In Europe, certain free-range wild boar populations are infected with CSF virus (CSFV), and the presence of infected wild boar poses a constant risk of transmitting CSFV to domestic pigs (Artois et al., 2002). Examples of this have repeatedly been seen, particularly in Germany (see e.g. ftp://ftp.oie.int/SAM/2003/DEU_A.pdf, accessed November 2, 2004), but also in Slovakia and Luxembourg.

The economic consequences of an outbreak of CSF would be devastating, not only for the individual farmer, but also for Denmark which is the world's largest net exporter of pork. More than 85% of the pork produced is exported. The main markets are other EU member states, in particular Germany, Great Britain, France, and Italy, as well as third countries such as Japan, Russia, and the USA. In 2003, this represented a value of more than 3 billion EURO (corresponding to approximately 6% of the total value of all Danish exports). Therefore, the temporary cessation in exports that would follow an introduction of CSFV would be detrimental for the swine industry.

Thus, it is essential to estimate the additional risk of introduction of CSFV into Denmark posed by the presence of wild boar. To address this, a risk analysis was conducted in collaboration between the Danish Institute for Food and Veterinary Research, the Danish Bacon and Meat Council, the Danish Veterinary and Food Administration, the National Environmental Research Institute and the UFZ Centre for Environmental Research (Leipzig, Germany). The results of the entire risk analysis will be made available to the Veterinary Service as a scientific decision support.

This paper focuses on GIS and the modelling parts of the risk assessment relevant to CSF. The positive aspects related to the presence of wild boar were not covered, nor were questions about other health hazards. Damages to crops were only addressed to a limited extent. A feasibility study should be carried out separately to address population dynamic aspects related to wild boar themselves. The outcome of these investigations will elucidate if and where sustainable populations of wild boar can be established in Denmark.

MATERIALS AND METHODS

In order to estimate the extent of wild boar habitat in Denmark (DK), a map based on an analysis of suitable and semi-suitable areas for wild boar in DK was created. The Area Usage Map (AAK), that is a part of the Area Information System (AIS) (administered by NERI), was used. This consists of different land use types such as forest, natural vegetation, pastures and extensive agriculture, intensive agriculture and urban areas and water bodies. The GIS

programme MapInfo (MapInfo Corporation) was used for this part, whereas ArcGIS (ESRI) was used subsequently to analyse data on tourism. In addition, the software programme InterSpreadPlus as well as a programme specifically developed to model wild boar populations and disease transmission (Kramer-Schadt et al., 2005) were employed.

The risk assessment followed the guidelines of the International Animal Health Organisation (OIE) (http://www.oie.int/eng/publicat/ouvrages/A_IRAvol1.htm, accessed December 31, 2004) (OIE, 2001; Murray, 2002). All available data were included as well as expert opinion. Some parts of the risk assessment were qualitative whilst others were quantitative, depending on the quality and type of available data. This risk assessment contained the following elements: 1) hazard identification, 2) biology and ecology assessment, 3) release assessment, 4) exposure assessment, 5) consequence assessment and 6) risk estimation.

Completion of the risk assessment was followed by a risk management component. An active risk communication strategy was put in place from the beginning of the project because reintroduction of such a large species is of interest to the public. Thus, the entire work constitutes a risk analysis (Murray, 2002).

HAZARD IDENTIFICATION

Only a part of the hazard identification is presented here. Aspects related to the pathogenesis and the clinical course of the disease, as well as vaccination, are not presented.

Classical swine fever virus belongs to the Family *Flaviviridae*, and the Genus *Pestivirus*. It is on OIE List A (http://www.oie.int/eng/maladies/en_classification.htm#ListeA, accessed December 31, 2004). Public health consequences are not relevant for this disease. Classical Swine Fever was last reported in Denmark in 1933. Denmark has the status of 'historically free' from CSF.

Classical swine fever is an infectious contagious disease of pigs. All breeds, including wild boar, are susceptible to the infection. Several virus strains exist and they vary in virulence. Highly virulent strains produce lethal infections, whereas low-virulent strains give rise to mild disease or asymptomatic infections (Mittelholzer et al., 2000). Classical Swine Fever virus is widely distributed across the globe. There have mainly been outbreaks in Asia, South America, and Europe. There is a continuing problem of CSF in Europe, including countries close to Denmark. In particular Germany, Italy, Slovakia, and Luxembourg have ongoing problems, partly due to infection in the wild boar population.

The CSFV is present in body fluids and muscle tissue of infected pigs. It is stable in pH range 5-10, but inactivated at pH 3 or below and above pH 10. Therefore, no destruction of virus will occur as the pH decreases during rigor mortis in muscles. The virus is relatively stable in moist excretions and fresh meat products kept for long-time periods, like ham and dry-cured sausages. However, detergents, lipid solvents and common disinfectants can readily inactivate the virus. Classical Swine Fever virus is highly contagious to pigs through the oral route. One gramme of infected fresh pork could contain 2.2×10^3 oral doses. This demonstrates why feeding untreated swill is very hazardous.

The main source of recent outbreaks of CSF in Europe has been either contact with infected wild boar, illegal swill feeding or contaminated livestock trucks. In Germany, in the period 1990-1998, 59% of the index cases (the first case in a series of related outbreaks) were caused

by infected wild boars, and 23% by swill feeding (Fritzemeier et al., 2000). In countries that have been able to keep their wild boar populations free from CSFV, wild boar does not constitute a problem. Even when domestic pigs are infected, wild boar do not have to become infected, because all domestic pigs with CSFV are culled quickly after diagnosis. This makes awareness and early diagnosis important elements in control of CSF. If wild boar are reintroduced to Denmark, they might get infected with CSFV and hence, pose a risk of transmission to domestic pigs, so CSFV is classified as a potential hazard. The disease introduction pathways considered in this assessment are presented under 'Release Assessment' (Fig. 1).

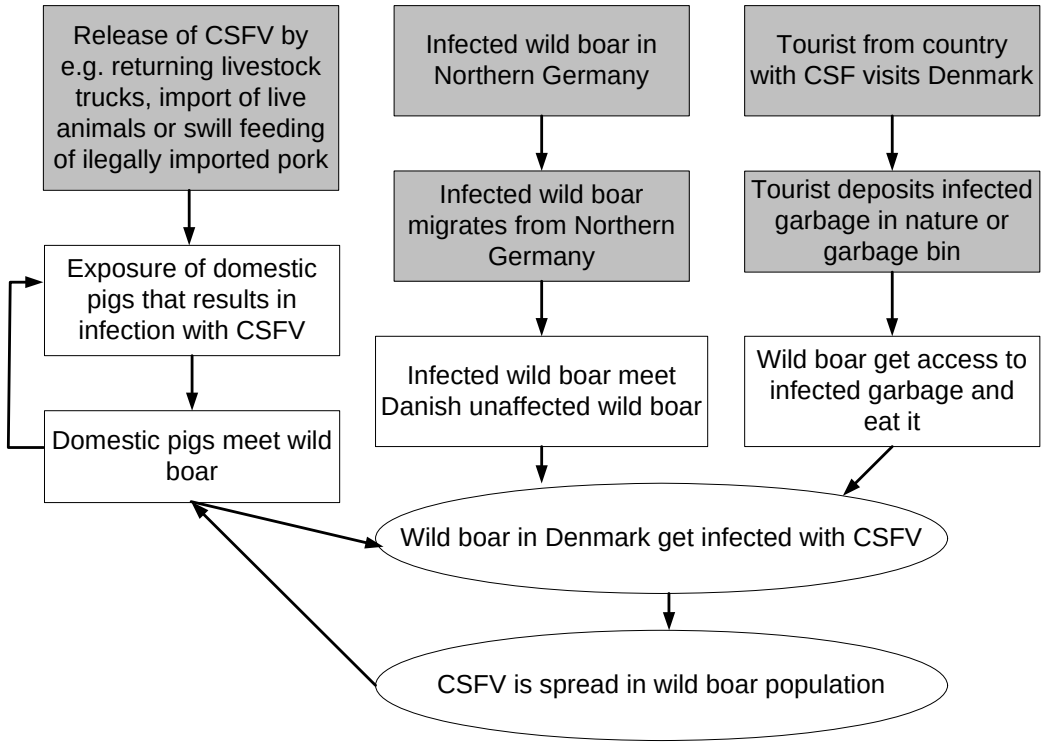


Fig. 1 Disease introductory pathways for CSFV reaching a Danish population of wild boar. Events marked with grey are dealt with in the release assessment, the remaining events in the exposure assessment

BIOLOGY AND ECOLOGY ASSESSMENT

Wild boar use the forest for hiding and reproduction and the surrounding fields (up to 1km from the forest) for foraging. One group of sows needs around 4km^2 covered with minimum 25% forest or natural vegetation.

Suitable areas for wild boar were identified as the following land use types: forest, deciduous forest, coniferous forest, and mixed forest. Natural vegetation was considered to be a semi-suitable habitat, and it was identified by the land use types natural grassland, heath land and peat bog. Pasture and agriculture (intensive as well as extensive) were considered unsuitable land use types. Urban areas and water bodies were considered barriers.

The expected distribution of wild boar was visualised using a buffer of 1km around the suitable habitat and a buffer of 0.5km around the semi-suitable habitat and, based upon these assumptions, Figure 2 was created. It is noted that there are suitable or semi-suitable habitats in several places in Denmark, even though the majority of the country consists of pasture and agriculture, and most forests are small. In total, 9-10% of Denmark consists of suitable or semi-suitable wild boar habitat (calculated without buffers). In conclusion, the wild boar should be able to establish in several parts of the country.

Fig. 2 The Danish wild boar habitat and the pig herds located within the habitat

To display the potential conflict of interest between man and wild boar, a map was created highlighting the wild boar habitat (with buffer) as well as urban areas and location of pig herds. Conflicts are expected in overlap areas. The resulting map showed that 24% of the Danish pig herds are located within areas where the wild boar would reside (Fig. 2). The nature of conflict is likely to concern crop damages and fear of CSF.

RELEASE ASSESSMENT

The scenarios considered for the release assessment were: 1) the migration of wild boar from Germany to Denmark, 2) the transmission of infection to wild boar through infected refuse accidentally left by tourists, and 3) the additional risk of introduction of CSFV to domestic pigs through other external sources, related to wild boar (Fig. 2).

MIGRATION OF WILD BOAR FROM GERMANY

Free-range wild boar populations migrating from northern Germany are assumed to be a possible stepping-stone for the introduction of CSFV into Denmark. Therefore, attempts have been made to shoot any wild boar migrating from Germany into Denmark. Hereby, the natural establishment of wild boar in Southern Jutland - that connects geographically with Germany - has been avoided. The question is what would happen if wild boar were no longer shot when observed trespassing? To assess how large a wild boar population would be established based on migration, a simulation experiment was performed. A spatial model developed by Kramer-Schadt et al. (2005) was used. As a worst case scenario, it was assumed that on the German side of the border, there would be a 20km-wide belt with perfect wild boar habitat allowing 5 animals to breed per $4km^2$. This would result in family sizes of 30-40 animals. The migration of females was simulated, because these are the animals that breed, and the simulation was allowed to run for 25 years.

The results of these simulations showed that only a limited migration of wild boar from Germany is expected if left to itself, because the wild boar habitat in Southern Jutland is small. The wild boar habitat in the southeast part of Southern Jutland would act as a corridor consisting of forest patches only loosely connecting Germany and Denmark. Further north in Southern Jutland there is almost no wild boar habitat. This would limit the spread of wild boar originating from Germany to the most southeast part of Southern Jutland. The model results were supported by the fact that only two free-range male wild boars have been officially observed in Southern Jutland during the previous eight years. One was accidentally hit by a car, and the other was shot when observed close to an outdoor pig production unit. Both animals tested negative for CSFV.

Currently, there is CSFV in wild boar populations further south in Germany but not in the north. It was concluded that as long as CSFV is not present in the wild boar population north of the Kieler Channel, then migrating wild boar *per se* is not associated with a risk of introducing CSFV into Denmark.

THE INFECTED REFUSE ROUTE

In this instance, the scenario is that a tourist brings some kind of contaminated meat and drops the leftovers in a refuse bin or in the environment, and that this meat is subsequently eaten by a wild boar. The probability of this occurring was assessed by estimating the exposure due to tourists originating from countries with a history CSF-outbreaks during the last decade. Unfortunately, data on tourism were only obtainable at county level, so that it was only possible to calculate the relative risk between counties. The risk of bringing in meat was graded according to the type of stay. Stays at hotels (0.01) and holiday centres (0.2) were assumed to have a low risk, whereas stays at camping grounds or in summer cottages were assumed to have a high risk (1.0). The size and quality of the wild boar habitat in a county was also incorporated in the risk by multiplying by the expected wild boar density (animals per km^2).

The relative risk (RR) of exposure was greatest in the two counties that make up the western part of Jutland (Ringkøbing: RR=9.7; Ribe: RR=9.0) and the northern part (Northern Jutland: RR=4)) compared to North and East Zealand (RR=1). This information was incorporated in the further modelling.

INTRODUCTION OF CSFV THROUGH OTHER SOURCES

Recently, a risk assessment was conducted aimed at assessing the risk of introducing CSFV to domestic pigs through sources other than wild boar. The results of this assessment showed that there is only a low probability that CSF virus would enter the country (Bronsvoort et al., 2004). The most risky pathways were livestock trucks passing over the border, imports of live animals and semen, hunters hunting abroad and legal/illegal imports of meat. This is a result of the current trade patterns and control measure already in place. For example, in order to reduce the risk of CSFV introduction, it is a requirement that livestock trucks are washed and disinfected upon arrival into Denmark. If these patterns and actions change, the risk changes. For example, an increased trade in live pigs with the new EU member states might increase the risk. However, these disease introduction pathways do not pose an additional risk of CSF due to wild boar, which is the summary result in the context of the current risk assessment.

EXPOSURE ASSESSMENT

The exposure assessment deals with pathways and associated likelihood of CSFV exposure of domestic pigs or wild boar in Denmark. First, we describe the fate of infection once it has reached a population of wild boar. Subsequently, the interface between wild boar and domestic pigs was studied (Fig. 1).

DISEASE TRANSMISSION WITHIN AN INFECTED HERD OF WILD BOAR

The basis of the further analyses was the assumption that free-range wild boar have been established in Denmark. For the following analysis, hence, we assumed (i) wild boar populations in all suitable areas, and (ii) an introductory infection by any route. The results will therefore allow the analysis of potential epidemics *relative* to each other.

The risk linked with an epidemic in wild boar would accumulate with the time that the infection persists within an area. Indeed, as long as CSFV is spreading within the wild boar population, an infected individual poses a risk of transmitting the disease to domestic pigs in the relevant regions. Therefore, for each group the expected duration of an outbreak starting within that particular group was analysed. For each simulated epidemic, the duration of the epidemic was recorded. This was calculated as the time interval between first infection and removal of the last infected animal. The numerical output of the simulation is an estimate of the time a region is put at risk by the infection in a certain initial wild boar group. Larger values identify wild boar groups which would initiate long-lasting epidemics – hence, releasing wild boar in that area would result in a potentially higher hazard due to increased time at risk compared to a release in areas were epidemics were estimated to be likely to last for only a short time.

The spatial extent, the duration, the intensity, and the probability of being involved in an outbreak were also investigated. For the present, this paper focuses on the duration of an outbreak.

The simulations of CSFV spread within a hypothetical infected wild boar population showed that the outbreaks would last from 7 to 861 days with a median of 112 days. This indicates that in only a small number of instances would the epidemic last for an excessively protracted period. The duration of an outbreak is affected by the size of the wild boar population that gets infected (the larger the population the longer the epidemic) and the virulence of the

virus (the more virulent the virus is, the shorter is the epidemic). However, the landscape would also modify the fate of the epidemic. These results are in accordance with European experience that has shown that the eradication of CSFV in large and dense wild boar populations may last several years.

The forest area around the city of Silkeborg in the centre of Jutland is a key habitat because it is the largest coherent wild boar habitat in Denmark. Furthermore, it is the area that is associated with the highest likelihood of disease spread. In this area it is predicted that the epidemics would last longer, the infected area would be larger, and there would be a higher intensity of disease spread compared with other areas in Denmark. The remainder of Denmark provides less optimal conditions for both wild boar to live and disease spread to occur because all other habitats are fragmented and less coherent compared with Silkeborg.

DISEASE TRANSMISSION BETWEEN DOMESTIC PIGS AND WILD BOAR

The spread of CSFV between domestic pigs and wild boar was simulated by use of the software programme InterSpreadPlus. Seven scenarios were run to elucidate the effect of: (a) presence of wild boar (yes/no), (b) locations for the index case (domestic pig herd/wild boar group), (c) type of control strategy for wild boar (geographical separation and shooting/vaccination).

In order to study the effect of presence of wild boar, it was assumed that in scenarios 1, 3, 5 and 6 free-range wild boar were present in Rold forest (Northern Jutland) and a forest near Silkeborg (Centre of Jutland) (Tab 1). For the scenarios where the CSF epidemic started in a domestic herd, two different sow herds were chosen: one located 0.5km from a wild boar habitat that sold piglets once a week (scenario 1-2), and another located 5km from a wild boar habitat forest, that sold piglets once every second week (scenario 3-4). For each scenario, 100 repetitions were performed, and therefore, the results are probability distributions. For simplicity of interpretation these are presented as summations (median, min, max). An epidemic was defined as the occurrence of a series of related outbreaks among domestic pig herds. If there were more than 100 days between two subsequent outbreaks, then this was defined as two epidemics.

The index case was described as infected on day 1, showing clinical signs on day 7, and detected on day 42. The seven days between infection and clinical signs was based on Uttenthal et al. (2003). The 42 days from infection to detection was chosen as a worst-case scenario based on experience from The Netherlands during their last CSF outbreak (Elbers et al., 1999).

The results show that spreading of infection to wild boar will only occur if the infected domestic pig herd is located in close proximity to an area with wild boar (e.g. 0.5km – scenario 1-2). There was no difference between scenarios 3 and 4 (index case a domestic pig herd located 5km from the forest; with/without wild boar present). When the index case is a domestic herd (scenario 1-4), one epidemic can be expected. The scenarios in which the index case is a group of wild boar have greater variation in the number of epidemics. In around one third of the repetitions, the CSF virus did not reach domestic herds at all, and therefore, no epidemic was started. However, in around 10% of the repetitions the CSF virus in the wild boar population periodically caused outbreaks in domestic herds giving rise to separate epidemics (scenario 5-6). The number of infected domestic herds tended to be lower when the index case was a wild boar

Table 1. The distribution of the simulated number of CSF-epidemics in Danish domestic pig herds. Rows marked with grey are scenarios without wild boar.

Scenario		No. of repetitions that resulted in			
No.	Index case	0 Epidemic	1 Epidemic	2 Epidemics	3 Epidemics
1	Domestic herd located 0.5km from forest		99	1	
2	Domestic herd located 0.5km from forest – No wild boar		100		
3	Domestic herd located 5km from forest		100		
4	Domestic herd located 5km from forest – No wild boar		100		
5	Wild boar	35	58	7	
6	Wild boar –Vaccination[a] used as control strategy	22	64	11	3

[a] In this scenario, vaccination was used to control CSF, whereas in the remaining scenarios geographical separation and shooting was used.

Table 2. Duration and size of simulated CSF-epidemic in Danish domestic pig herds: summation of 100 repetitions per scenario. Rows marked with grey are scenarios without wild boar.

Scenario		No. of herds infected		No. of days in epidemic	
No.	Index case	Median	Min-Max	Median	Min-Max
1	Domestic herd located 0.5km from forest	5	2-17	14	1-127
2	Domestic herd located 0.5km from forest – No wild boar	2	2-6	1	1-19
3	Domestic herd located 5km from forest	5	3-10	14	1-31
4	Domestic herd located 5km from forest – No wild boar	5	3-10	14	1-31
5	Wild boar	2	1-5	1	1-95
6	Wild boar. Vaccination[a] used as control strategy	2	1-10	2	1-166

[a]In this scenario vaccination was used to control CSF in wild boar, whereas in the remaining scenarios geographical separation and shooting was used. For the scenarios where the infection started among wild boar, only repetitions with outbreak among domestic pig herds were included.

compared with a domestic herd. The maximum duration of the epidemic was much higher in cases where wild boar were present in close proximity to domestic herds (Table 2).

CONSEQUENCE ASSESSMENT

To address the economic consequences of the presence of free-range wild boar the costs related to an outbreak of CSF were calculated. Calculations were based on the assumption that the control strategies for domestic pigs and wild boar would be those of today. Four major cost elements were identified: control costs related to domestic pig herds and control costs related to wild boar (both covered by the national budget in some way), the control related costs to the pig sector, and the loss in export of live pigs and pig products following an outbreak of CSF in Denmark. Costs for a general surveillance programme for wild boar in Denmark were not included.

The current paper focuses on scenarios 1 and 2, in which the index case is a domestic herd located 0.5 km from the wild boar habitat (Table 2). Costs related to an outbreak in which wild boar were present (scenario 1) were compared with an outbreak in which wild boar were not present (scenario 2). The calculations showed that the average costs related to an outbreak would be 13% higher if wild boar were present compared to not present. The main reason for this is the increased duration of the epidemic because the virus would circulate in the wild boar population and periodically spread to domestic herds.

RISK ESTIMATION

In this section the results of the release assessment, exposure assessment and consequence assessment will be combined into a final risk estimation

There is a low base-line probability that CSFV will enter Denmark because of the current trade patterns and existing control measures in place to reduce the risk. This has helped to ensure that CSF has not been present in Denmark since 1933. The probability of CSFV entering Denmark will increase slightly if free-range wild boar are reintroduced. A free-range population of wild boar inside Denmark could get infected through contact with infected wild boar migrating from Germany. The risk is considered to be negligible because (a) CSF is not known to exist in wild boar in northern Germany and (b) there is very limited habitat support for wild boar on the Danish side close to the Danish-German border.

Another route of entry is through tourists accidentally feeding CSF-contaminated meat leftovers to wild boar. It was not possible to estimate the probability of this happening, but the relative risk in the counties Ribe, Ringkøbing and Northern Jutland was several times higher than in the remaining part of the country.

If free-range wild boar were present then conflicts would be expected between farmers and wild boar in areas where both reside. However, only in the event of CSFV being introduced into the wild boar population, would the fear of CSF introduction to domestic pigs be justified. If CSFV was introduced into wild boar, then the infection would either die out quickly or an epidemic lasting from half a year to one year would be seen (in extreme cases even longer); this would be dependent upon, among other things, the wild boar habitat in which the virus was released. The larger the wild boar population is, the longer the epidemic will last. The

continuing spread of CSFV in Central Europe has been caused by wild boar populations, which are 100 to 1,000 times larger than that which would be possible in Denmark.

Spread of infection between wild boar and domestic pigs would only be expected to occur if the pig herd is located close to the wild boar habitat. If the index case is a domestic herd, then one epidemic is expected, whereas if wild boar are the index case, then a situation of either zero or more than one epidemic is predicted among domestic pigs.

The economic calculations showed that, on average, the costs related to a CSF outbreak would only be 13% higher if free-range wild boar are present compared with the current situation. However, the probability of an outbreak occurring increases if free-range wild boar are present. Furthermore, the presence of wild boar might result in long-lasting epidemics or more than one epidemic, because of periodic repeated transfer of virus from groups of infected wild boar.

Outdoor production cannot be compared with free-range wild boar because the probability is low of fenced-in animals transmitting CSFV to animals outside the fence before infection is diagnosed among the fenced-in pigs. The main reason for this is that spread of CSFV is not airborne, and direct or indirect contact is needed for transmission to occur.

Risk is a product of probability and consequences. As can be noted, the probability of CSFV entering the country will increase slightly from the present low level. The economic consequences will vary from around 13% extra costs (average) to a much higher level in case of long-lasting epidemics.

REFERENCES

Artois, M., Depner, K.R., Guberti, V., Hars, J., Rossi, S. and Rutili, D. (2002). Classical swine fever (hog cholera) in wild boar in Europe. Rev. sci. tech. Off. Int. Epiz., 2, 287-303

Bronsvoort, B.M. deC, Alban, L. and Greiner, M. (2004). An assessment of the likelihood of the introduction of exotic diseases to the Danish swine population. International EpiLab, Copenhagen, Denmark. 128 p

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H.M., Smak, J.A. and Pluimers, F.H. (1999). The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. Prev. Vet. Med. 42, 157-184

Fritzemeier, J., Teuffert, J., Greiser-Wilke, I., Staubach, C., Schluter, H. and Moennig,V. (2000). Epidemiology of classical swine fever in Germany in the 1990s. Vet. Microbiol. 77, 29-41

Kramer-Schadt, S., Fernández, N. and Thulke, H.-H. (2005). Explaining classical swine fever persistence by combining epidemiological and ecological modelling. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, 57-67

Mittelholzer, C., Moser, C., Tratschin, J.D. and Hofmann, M.A. (2000). Analysis of classical swine fever virus replication kinetics allows differentiation of highly virulent from avirulent strains. Vet. Microbiol. 74, 293-308

Murray, N. (2002). Import risk analysis – Animals and animal products. MAF Biosecurity, New Zealand. 183 p

OIE (2001). International animal health code – Mammals, birds and bees. Tenth edition. Office International des Epizooties. 473 p

Uttenthal, A., Storgaard, T., Oleksiewicz, M.B. and de Stricker, K. (2003). Experimental infection with the Paderborn isolate of classical swine fever virus in 10-week-old pigs: determination of viral replication kinetics by quantitative RT-PCR, virus isolation and antigen ELISA. Vet. Microbiol. 92, 197-212

ESTIMATING THE PROBABILITY OF FREEDOM OF CLASSICAL SWINE FEVER VIRUS OF THE EAST-BELGIUM WILD BOAR POPULATION

K. MINTIENS[1], D. VERLOO, E. VENOT, H. LAEVENS, J. DUFEY, J. DEWULF, F. BOELAERT, P. KERKHOFS AND F. KOENEN

SUMMARY

The Scientific Committee on Animal Health and Animal Welfare of the EU (CEC, 1999) sets recommendations for monitoring Classical Swine Fever (CSF) in wild boar populations, based on the assumption of detecting a minimal expected prevalence of 5%. An alternative method to provide evidence for a wild boar population being free of CSF is proposed and the efficiency of a surveillance programme that was implemented in Belgium is evaluated. The probability of freedom from Classical Swine Fever virus (CSFV) was estimated based on 789 samples collected from wild boars within the surveillance programme and examined by 3 diagnostic methods. Bayesian inference was used, accounting for the diagnostic test characteristics in a no-gold standard framework. The median probability of freedom from CSFV was estimated at 0.970 (95% credibility interval of 0.149 to 1.000). Independent of the choice of the prior information, the posterior distributions for the probability of freedom from CSFV were always skewed close to the upper boundary of 1.

INTRODUCTION

Classical swine fever (CSF) is a highly contagious viral infection in pigs and wild boars and appears on the List A of diseases notifiable to the World Organisation for Animal Health (OIE). The disease usually causes high morbidity and mortality in domestic-pig populations. In the mid-1980s, the European Union implemented an eradication programme combining a non-vaccination policy with a strategy of stamping out infected herds. These control measures stopped CSF being endemic in most of the EU territory – but epidemics of CSF (with severe socio-economic consequences) have occurred in different European Member States ever since (Elbers et al., 1999; Fritzemeier et al., 2000; Koenen et al., 1996; Mintiens et al., 2001; Mintiens et al., 2003; Miry et al., 1991).

Complete and long-term eradication of CSF in the EU is hampered by the incidence of the infection in wild boar populations in different Member States. Wild boars are as susceptible to CSF-infections as domestic pigs (Depner et al., 1995). As a consequence, wild boars represent an important risk (indifferent to geographical borders) for infecting the domestic-pig population. From different studies conducted from 1993 to 1997, Fritzemeier et al. (2000) estimated that 59% of the primary outbreaks of CSF in domestic pigs in Germany were related to direct or indirect contact with infected wild boars. The tradition of keeping domestic pigs free range –

[1] Veterinary and Agrochemical Research Centre, Co-ordination Centre for Veterinary Diagnostics, Groeselenberg 99, B-1180 Brussels, Belgium. Email: koen.mintiens@var.fgov.be

and, consequently, in close contact with the wild boar population – is considered a major cause of CSF-outbreaks in some areas of Sardinia (Laddomada, 2000). Lowings et al. (1994) found evidence for the spread of CSFV from initially infected captive to free-living wild boars and further to domestic pigs in Italy from 1985 to 1991.

In 1998, several CSF-outbreaks in domestic pigs and wild boars were identified in the Rheinland-Palatinate region of Germany at a 1- to 50-km distance from the Belgian eastern border. These new cases alerted the Belgian Veterinary Services, who implemented a surveillance programme in the same year. The objective of the programme was to monitor whether the Belgian wild boar population remained free of CSF and to take effective measures to protect the domestic pig population in the event of CSF infections in wild boar occurring. The treat of CSF introduction into the Belgian wild boar population has not diminished in recent years and the surveillance programme continues.

A report of the Scientific Committee on Animal Health and Animal Welfare of the European Commission (CEC, 1999) includes recommendations for setting up monitoring programmes for CSF-infection in a wild boar population. These recommendations are based on a method described by Cannon & Roe (1982), which allows calculation of the exact probability of detecting infected populations. The method assumes a minimal expected prevalence of infected animals in an infected population. The aforementioned EC report assumes that one would detect at least a 5% prevalence in a CSF-infected wild boar population. However, this assumption is not based on any experimental or epidemiological evidence. Therefore, the proposed methodology could overestimate the probability of a wild boar population being free of CSFV if the true, non-zero, prevalence were < 5%.

The results of the surveillance programme are described over a period from October 1999 to December 2001. Additionally, an alternative method to provide evidence for the East-Belgian wild boar population being free of CSF is proposed and the efficiency of the surveillance programme is evaluated.

MATERIALS AND METHODS

The surveillance programme

The surveillance programme started in 1998 and involved the monitoring of wild boars for CSF-infections within the three provinces (Namur, Liège and Luxembourg, total 13,798 km^2), which mutually include 95% of the Belgian wild boar population (Fig. 1). The programme consisted of the examination on a voluntary basis of a convenience sample of wild boars that were killed or found dead by hunters. It was prescribed that a minimum annual number of 100 animals should be examined per province.

From January 2000 onwards, the programme was extended by dividing the target area into two zones and by implementing additional control measures (Fig. 1).

1. A surveillance zone (~ 125 km^2) bordering the CSF affected area in Germany was defined. This zone was assumed to have higher risk for introduction of CSFV. Therefore, all wild boars that were killed or found dead were required to be examined for the presence of CSF infection and movement restrictions for domestic pigs were implemented.

2. In the screening zone (the remaining parts of the three provinces and with lower risk for CSFV introduction) in which the monitoring of wild boars killed or found dead continued on a voluntary basis. For Liège and Luxembourg provinces only, an additional 50 animals were to be examined every year in the communities bordering Germany and the Grand Duchy of Luxembourg.

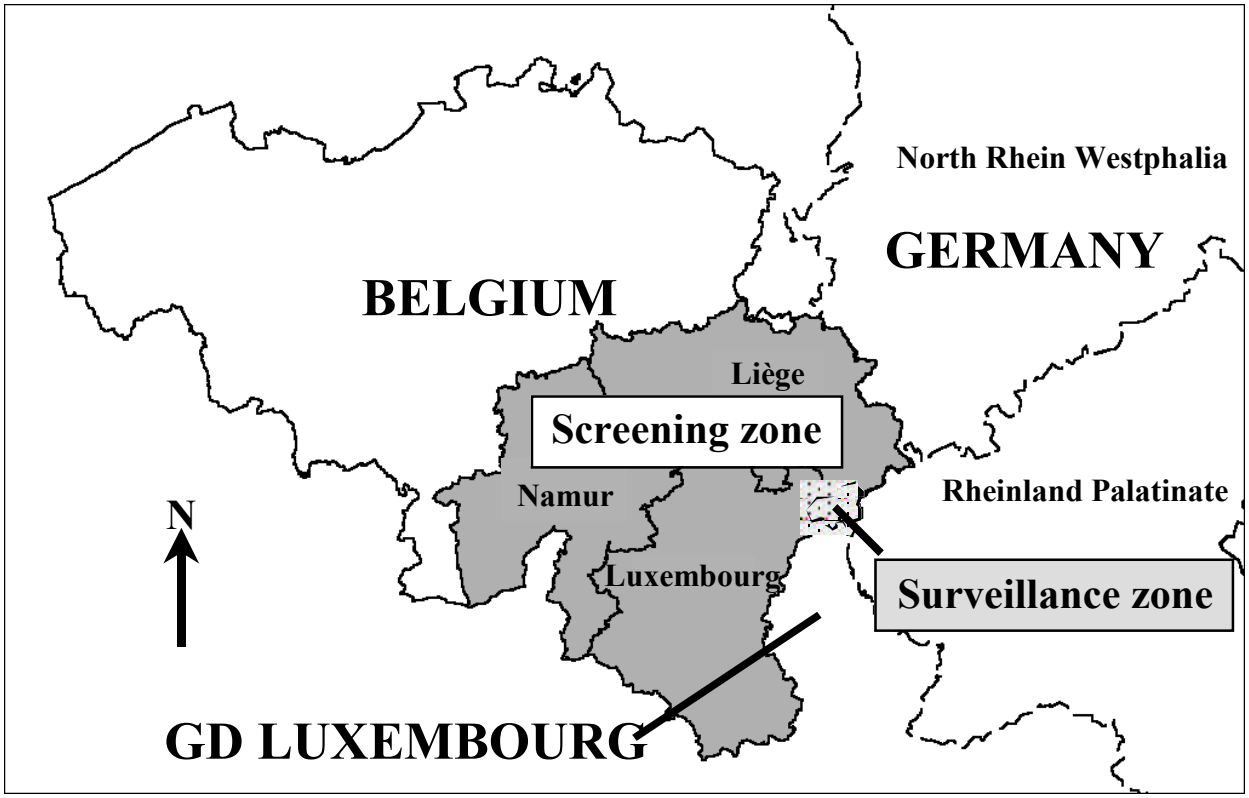


Fig. 1 Map of Belgium indicating the screening and surveillance zone for CSF in wild boar

Sample collection

Blood samples and lymph nodes were collected from each wild boar that was examined within the surveillance programme. If sampling from lymph nodes was not possible, kidneys were used. All samples were collected at the Control Centre of Loncin and dispatched to the Veterinary and Agrochemical Research Centre in Brussels for further analysis.

For each animal that was sampled, additional information was recorded (e.g. sampling date, postal code of the municipality in which the animal was shot or found dead, sex, age group (young, subadult, adult) and estimated weight).

Diagnostic methods

Three different diagnostic methods were employed for the samples collected:

1. Antibody detection: A sequential testing procedure was performed to detect antibodies against CSFV in serum samples. As a first step in this procedure, a competitive enzyme-linked immunoassay (ELISA) test (HerdChek CSFV antibody ELISA, IDEXX) was used. Samples that tested positive by the ELISA test were then simultaneously examined by a CSFV neutralisation test and a Bovine Viral Diarrhoea (BVD) virus neutralisation test, based on the neutralisation assay (NPLA) as described by Holm Jensen (1981). Only samples that tested positive on the ELISA and the CSFV neutralisation test and negative for the BVD virus neutralisation test were reported to contain antibodies against CSFV. For all other samples, it was concluded that antibodies against CSFV were absent. According to these decision rules, the dichotomised results of this combined sequential testing procedure was interpreted as a single diagnostic test for further analysis.

2. Virus detection: Organ-tissue suspensions were inoculated on a monolayer of sub-confluent PK15 cells and cultivated on multi-cup plates to isolate CSFV. The virus was identified by an anti-CSF immunoglobulin conjugated with fluorescein isothiocianate (Koenen et al., 1996).

3. Virus RNA detection: A single-tube RT-nPCR test was performed to detect CSFV RNA in tissue samples (McGoldrick et al., 1999).

To the authors' knowledge, no information was available on the sensitivity and specificity of these three diagnostic methods.

Freedom of CSFV

Due to a lack of knowledge of the dynamics of CSFV in a wild boar population, a minimal expected prevalence resulting from an introduction of CSFV could not be selected and justified. Hence, the commonly used methods described by Cannon & Roe (1982) or by Cameron & Baldock (1998) for calculating the probability of freedom of disease in a population were not applicable. However, estimating the prevalence of CSF infected wild boars in the Belgian population would provide evidence on the absence of the virus in the population and would provide input for a surveillance programme, suitable for the Belgian situation.

Using Bayes theorem, the posterior probability of freedom of CSFV (F), given the observed test results (T), can be derived:

$$P(F|T) = \frac{P(T|F)P(F)}{P(T)} \quad (1)$$

When the observed test results are obtained by applying a diagnostic test to a survey sample, one can estimate the probability of freedom of CSFV (F), the prevalence given the population is not free ($prev$), and the sensitivity (Se) and specificity (Sp) of the diagnostic test given the data. Equation (1) can than be extended:

$$P(F, prev, Se, Sp|T) = \frac{P(T|F, prev, Se, Sp)P(F, prev, Se, Sp)}{P(T)} \quad (2)$$

Using Bayesian inference, the posterior probability distributions for parameters *F, prev, Se* and *Sp* are estimated by combining the binomial likelihood and the prior distributions of the four parameters. This multinomial likelihood for a positive test result can be written as:

$$P(T^+|F, prev, Se, Sp) = (1-F)(prev \times Se + (1-prev)(1-Sp)) + F(1-Sp) \quad (3)$$

The likelihood function, Eq. (3), takes into account that the observed prevalence only occurs when the population is not free of CSFV and can, under the following assumptions, be extended to the multinomial likelihood based on 3 test methods (Table 1).

1. The results of all diagnostic methods that were used in this study are independent, conditional on the infection status of the tested animals (all three methods detect different biological phenomena).

2. The probability of a negative test result given that an animal is non-diseased is the same in a CSF free or non-CSF free population.

3. The probability of having a non-CSF free population given that an animal is infected is equal to 1.

In this study, *F*, *prev*, and Se_i and Sp_i of the three diagnostic methods were calculated based on the samples that were collected from wild boars within the surveillance programme from October 1999 to December 2001 (and analysed by all three diagnostic methods). Any qualitative superiority of one of the diagnostic methods over the two others was ignored (no-gold standard method). Using the three diagnostic methods on all samples, 8 different combinations of test results could be obtained. For each of 8 test result combinations, the likelihood function to observe a given test result combination was formulated under the assumption of conditional independence. This likelihood described the probability of observing the data given the sensitivity (Se_i) and specificity (Sp_i) of each of the three testing methods, the probability of freedom of CSFV (*F*) and the CSF-prevalence (*prev*) in the population when not free of the virus (Table 1). This resulted in 8 equations with 8 unknown parameters. Posterior densities for the 8 unknown parameters (Se_i and Sp_i for i = 1 to 3, *F*, and *prev*), were obtained applying Bayesian inference using Gibbs sampling (Gelfand & Smith, 1990) in the WinBugs software, version 1.4 (Gilks et al., 1994). Three parallel sequences with different starting values were run for each model and convergence was monitored with the Gelman-Rubin test (Gelman et al., 1995). To assure stable posterior density outcomes, all models ran for another 10,000 iterations after the Gelman-Rubin test converged to 1 (which determined the burn-in period).

The model had 7 degrees of freedom while a total of 8 parameters had to be estimated. This made it unidentifiable without prior information on at least one parameter. No clear prior information on the parameters could be obtained from literature or reliable sources. Therefore it was decided to include different options for the prior information and to evaluate their influence on the posterior probability distribution of the parameters. No prior information was available on the probability of the Belgian wild boar population being free of CSFV. Since *F* was our main parameter of interest, we only used a non-informative prior distribution (Beta(1,1)) for *F*.

Table 1. Likelihood contributions to observe a given combination of test results when using 3 diagnostic methods in parallel

Test-result combination			**Likelihood contribution**		
T_1	**T_2**	**T_3**			
+	+	+	$(1-F)*(prev*Se_1*Se_2*Se_3+(1-prev)*(1-Sp_1)*(1-Sp_2)*(1-Sp_3))$	+	$F*(1-Sp_1)*(1-Sp_2)*(1-Sp_3)$
+	+	-	$(1-F)*(prev*Se_1*Se_2*(1-Se_3)+(1-prev)*(1-Sp_1)*(1-Sp_2)*Sp_3)$	+	$F*(1-Sp_1)*(1-Sp_2)*Sp_3$
+	-	+	$(1-F)*(prev*Se_1*(1-Se_2)*Se_3+(1-prev)*(1-Sp_1)*Sp_2*(1-Sp_3))$	+	$F*(1-Sp_1)*Sp_2*(1-Sp_3)$
+	-	-	$(1-F)*(prev*Se_1*(1-Se_2)*(1-Se_3)+(1-prev)*(1-Sp_1)*Sp_2*Sp_3)$	+	$F*(1-Sp_1)*Sp_2*Sp_3$
-	+	+	$(1-F)*(prev*(1-Se_1)*Se_2*Se_3+(1-prev)*Sp_1*(1-Sp_2)*(1-Sp_3))$	+	$F*Sp_1*(1-Sp_2)*(1-Sp_3)$
-	+	-	$(1-F)*(prev*(1-Se_1)*Se_2*(1-Se_3)+(1-prev)*Sp_1*(1-Sp_2)*Sp_3)$	+	$F*Sp_1*(1-Sp_2)*Sp_3$
-	-	+	$(1-F)*(prev*(1-Se_1)*(1-Se_2)*Se_3+(1-prev)*Sp_1*Sp_2*(1-Sp_3))$	+	$F*Sp_1*Sp_2*(1-Sp_3)$
-	-	-	$(1-F)*(prev*(1-Se_1)*(1-Se_2)*(1-Se_3)+(1-prev)*Sp_1*Sp_2*Sp_3)$	+	$F*Sp_1*Sp_2*Sp_3$

with :

+ , -	Test result for the i-th diagnostic method
F	Probability of freedom of CSFV
prev	Prevalence of the infection in the study population, given that the population is not free of CSFV
Se_i	Diagnostic sensitivity of the i-th diagnostic method
Sp_i	Diagnostic specificity of the i-th diagnostic method

RESULTS

Descriptive statistics

A total of 1,282 animals were sampled from October 1999 to December 2001 (1,201 from the screening zone and 81 from the surveillance zone). Most were collected during consecutive annual hunting campaigns (mid-October to end-December). The sampled animals were nearly equally distributed over age groups and sex categories across the 4 years.

Blood samples from 889 animals were analysed using the described serological methods. Nine samples were positive for antibodies to CSFV, 850 samples were negative (for 30 samples, no distinct test result could be derived). Tissue samples from 1,183 animals were examined by the inoculation method and all were CSFV negative. Tissue samples from all 1,282 animals were examined by the single tube RT-nPCR test and all were negative. Samples of 789 animals were examined by all 3 diagnostic methods. Nine (of the 789) were sero-positive but all other diagnostic results were negative.

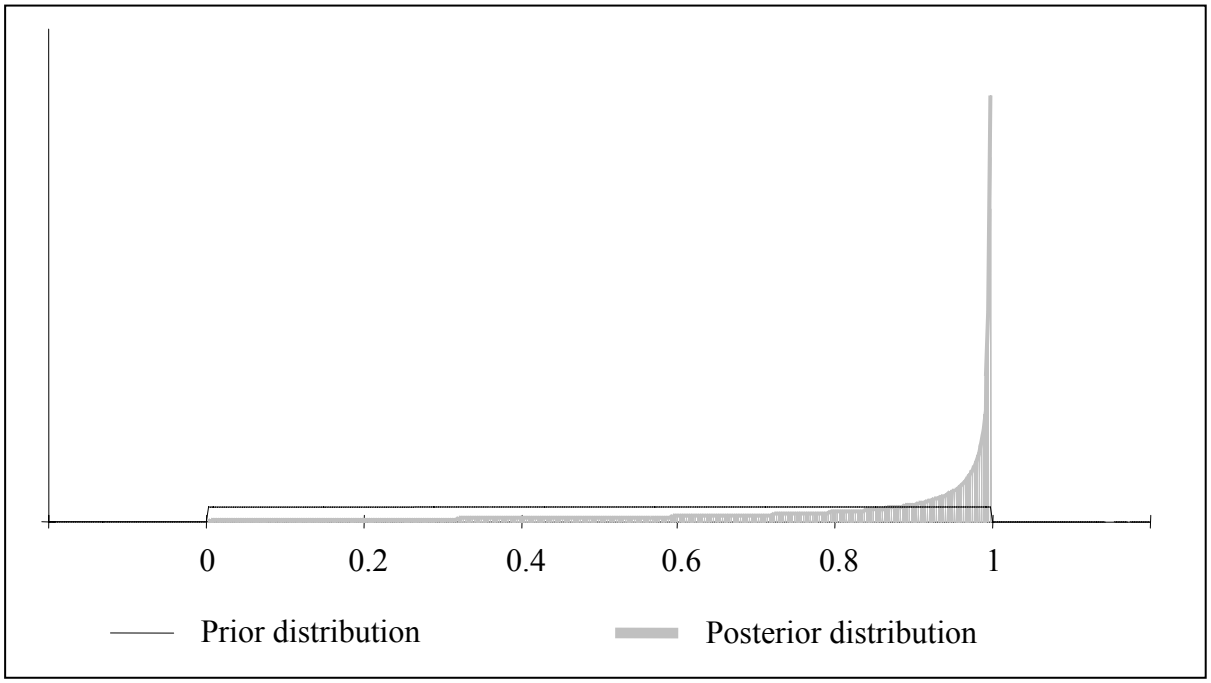


Fig. 2 Prior and posterior distribution for the probability of freedom from CSFV when choosing the least informative prior information (Se & $Sp \geq 0.5$; $prev$ = Beta(1, 1); F=Beta(1,1))

Parameter estimations

The diagnostic results for the 789 animals that were examined by all 3 diagnostic methods were used for estimating the 8 parameters. The prior and the posterior probability distributions obtained when choosing the least informative prior information (Se_i & $Sp_i \geq 0.5$; non-informative prior for $prev$) are displayed in Fig. 2. For this option, the posterior median probability of freedom from disease was estimated at 0.970 with a 95% credibility interval of 0.149 to 1.000.

DISCUSSION

To obtain unbiased parameter estimates, individuals in a study population should have equal probability of being selected for a survey sample (simple random sample). But, selecting a simple random sample from a wildlife population is almost impossible since it requires the identification of each individual in the population. In our study, parameter estimates are based on a convenience sample of wild boars that were killed or found dead by hunters. This may have led to an overestimation of *prev* and an underestimation of *F*, since CSF infected animals may be ill and weak, which may give them a higher probability of being found dead or shot by hunters. However, the estimate obtained for *F* is extremely high. Therefore the number of CSF infected animals is expected to be very low and the effect of a possible selection bias is negligible.

The sampling probability was also higher for animals in areas with higher risk of introduction of CSFV (surveillance zone and the communities bordering Germany and the Grand Duchy of Luxembourg). Moreover, the samples, which had a positive serological result, were obtained from animals from these high-risk areas. This means that the probability of freedom of CSFV in the population of the whole study area (surveillance zone and screening zone) is expected to be higher than the estimate obtained. Separate estimations per area were not possible, because of a lack of detailed information on the size of the wild boar population in the different areas.

The method that was used to estimate *F* did not assume that the information provided by one diagnostic method was superior to the others (absence of gold standard) and the probability was calculated considering the quality of the three diagnostic methods for the situation of very low disease prevalence. However, this situation required prior knowledge to be included for the model to convert. The posterior distributions of most parameter were not sensitive to the choice of prior information on the sensitivity and specificity of the diagnostic methods. Only the posterior distributions of the sensitivity of the diagnostic methods depended highly on the prior distributions. This was expected, since almost all test results in the survey sample were negative. It shows that it is impossible to obtain a reliable estimate of the diagnostic sensitivity in a population with low or zero disease prevalence. The choice of the prior distribution for *prev* had an influence on its posterior distribution. Here again it is obvious that the data could not give a precise likelihood for *prev*, since hardly any positive results were available. The choice of the prior distribution for *prev* also had an influence on the posterior distribution of *F*. Choosing a more informative prior for *prev* narrowed the 95% credible interval of the posterior distribution of *F* and pushed its median towards 1. This can be explained by the fact that the CSF prevalence in the population equals the prevalence given that the population is not free of CSFV (*prev*) times the probability that the population is not free of CSFV (*F*). Any increase of *prev* would result in an increase of *F*, since the CSF prevalence in the population is constant.

The aim of the surveillance programme is to provide evidence for the East-Belgian wild boar population being free of CSFV. As an alternative to the methodology proposed in the report of the European Commission concerning CSF in wild boar (CEC, 1999), the probability of freedom of CSFV was calculated, without assuming a minimal expected prevalence if CSF were present in the population. All posterior distributions for the probability of freedom of CSFV are lying close to the upper boundary of 1, irrespective of the choice of prior information. This represents a large gain in knowledge, since no prior information was used for the probability of freedom of CSFV and the uncertainty about the accuracy of the diagnostic methods was taken into account.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of the Veterinary Services, the 'Centre de Lutte' of Loncin and the 'Centres de Prévention et de Guidance Vétérinaire' of Liège, Luxembourg and NamurProvinces for their co-operation to this study.

REFERENCES

Cameron, A.R. and Baldock, F.C. (1998). A new probability formula for surveys to substantiate freedom from disease. Prev. Vet. Med. 34, 1-17

Cannon, R.M. and Roe, R.T. (1982). Livestock disease surveys: a field manual for veterinarians. Australian government publishing service, Canberra

CEC (1999). Classical swine fever in wild boar. Report XXIV/B3/R09/1999. European Commission, Brussels, Belgium

Depner, K.R., Muller, A., Gruber, A., Rodriguez, A., Bickhardt, K. and Liess, B. (1995). Classical swine fever in wild boar (Sus scrofa)--experimental infections and viral persistence. Dtsch. Tierarztl. Wochenschr. 102, 381-384

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H.M., Smak, J.A. and Pluimers, F.H. (1999). The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. Prev. Vet. Med. 42, 157-184

Fritzemeier, J., Teuffert, J., Greiser-Wilke, I., Staubach, C., Schluter, H. and Moennig, V. (2000). Epidemiology of classical swine fever in Germany in the 1990s. Vet. Microbiol. 77, 29-41

Gelfand, A.E. and Smith, A.F.M. (1990). Sampling-based approaches to calculating marginal densities. J. Amer. Statist. Assoc. 85, 398-409

Gelman, A., Carlin, J.B., Stern, H.S. and Rubin, D.B. (1995). Bayesian Data Analysis. Chapman and Hall, London, 488 p

Gilks, W.R., Thomas, A. and Spiegelhalter, D.J. (1994). A language and program for complex Bayesian modelling. Statistician 43, 169-178

Holm Jensen, M. (1981). Detection of antibodies against hog cholera virus and bovine viral diarrhea virus in porcine serum. Acta Vet. Scand. 22, 85-98

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J. and Deluyker, H. (1996). Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. Vet. Rec. 139, 367-371

Laddomada, A. (2000). Incidence and control of CSF in wild boar in Europe. Vet. Microbiol. 73, 121-130

Lowings, J.P., Paton, D.J., Sands, J.J., De Mia, G.M. and Rutili, D. (1994). Classical swine fever: genetic detection and analysis of differences between virus isolates. J. Gen. Virol. 75 (Pt 12), 3461-3468

McGoldrick, A., Bensaude, E. and Paton, D.J. (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestivirial RNA with fluerescent probes. J. Virol. Methods 79, 85-95

Mintiens, K., Deluyker, H., Laevens, H., Koenen, F., Dewulf, J. and de Kruif, A. (2001). Descriptive epidemiology of a classical swine fever outbreak in the Limburg Province of Belgium in 1997. J. Vet. Med. B 48, 143-149

Mintiens, K., Laevens, H., Dewulf, J., Boelaert, F., Verloo, D. and Koenen, F. (2003). Risk analysis of the spread of classical swine fever virus through "neighbourhood infections" for different regions in Belgium. Prev. Vet. Med. 60, 27-36

Miry, C., Castryck, F., Koenen, F., Broes, A. and Segers, E. (1991). Quelques aspects de l'epizootie de peste procine classique in Belgique en 1990. Epidémiol. Santé Animal 20, 23-32

SAVING BUDGET IN RABIES CONTROL – REVISITING A CLASSIC EPIDEMIOLOGICAL THRESHOLD

D. EISINGER[1] AND H.-H. THULKE

SUMMARY

In large-scale, long-term vaccination programmes against fox rabies, managers aim for a population immunisation of over 70%, a figure motivated by Anderson et al.'s population model of 1981 (Anderson et al., 1981). With two models available, one of them reproducing the classically accepted figure of 70%, and the other giving a lower prediction, model assumptions that mediated the different prediction of the figure were investigated. By adapting these assumptions, the models became structurally equivalent and predicted the same level of population immunity required to eradicate the disease. These findings suggest that the more general representations used in earlier models are apparently inappropriate to provide an absolute estimate for the control threshold of the rabies-fox system. Thus, when adapted strategies are sought to cope with modern resource limitations in rabies contingency planning, there is a need to revisit the classical threshold value of Anderson et al. (1981).

INTRODUCTION

In the 1980s, Anderson and colleagues proved the practicability of oral vaccination as control measure against rabies in foxes utilising a model from population ecology (Anderson et al., 1981). Ever since this seminal study, the figure of 70% as the population immunity level required to eradicate rabies has been used as a benchmark for successful management (European Commission, 2002) and a huge amount of resources have been devoted to achieving this figure in the field. The success that oral vaccination programmes have had in eradicating rabies in Central Europe appears to confirm the utility of this threshold. Moreover, although Anderson's model is rather general (i.e. non-spatial, time-continuous, population-based) compared to modern spatially-explicit, time-discrete, individual-based simulation models (Deal et al., 2000; Smith and Harris, 1991; Tischendorf et al., 1998), many modelling studies that followed the original have found a similar value for the required immunisation level (Artois et al., 1997; Suppo et al., 2000; Tischendorf et al., 1998). However, some vaccination programmes did not achieve the level of 70% immune foxes, but nevertheless managed to eradicate rabies from the target region (Artois et al., 1997; Schlüter & Müller unpublished data). Thus, doubt has arisen recently as to whether the previously accepted target level of population immunity in rabies control could be somewhat overestimated. If this is the case, resources may have already been wasted in over-baiting whole countries, and this situation is likely to continue bearing in mind that rabies control has now to move to the large regions in Eastern Europe. Hence, after nearly

[1] Dirk Eisinger, Department of Ecological Modelling, UFZ Centre for Environmental Research, Permoser Str. 15, D-04318 Leipzig, Germany. Email: dirk.eisinger@ufz.de

25 years, it is necessary to address directly the value of the immunity level required to eradicate the disease, i.e. to explore revision of the cardinal rule of rabies control in foxes.

For this reason the required immunisation level was estimated with two existing models. The first one successfully supported decision making during large-scale and long-term oral vaccination programmes (Thulke et al., 1999; Thulke et al., 2000; Tischendorf et al., 1998) and the second, a refined version, was built to test emergency management strategies in rabies free fox populations, thus tailored to the local dynamics (Eisinger, 2005). Surprisingly, these structurally similar models identified quite different immunisation levels required to eradicate rabies. The difference between the two models was observed consistently throughout the whole range of infection probabilities tested (Fig. 1). Consequently, this study was designed to identify model features that are responsible for the different predictions. First, coarse-scale epidemiological characteristics of rabies were re-measured, such as the speed of the advancing front wave and the number of infected fox groups. If these were found to be different, the respective model rules that were responsible for the difference were identified and adjusted accordingly to bring the two models into line. After each step, any divergence of predicted values of immunisation level required for eradication was examined. At the end of the procedure, it was possible to identify which model assumptions determined the difference in prediction.

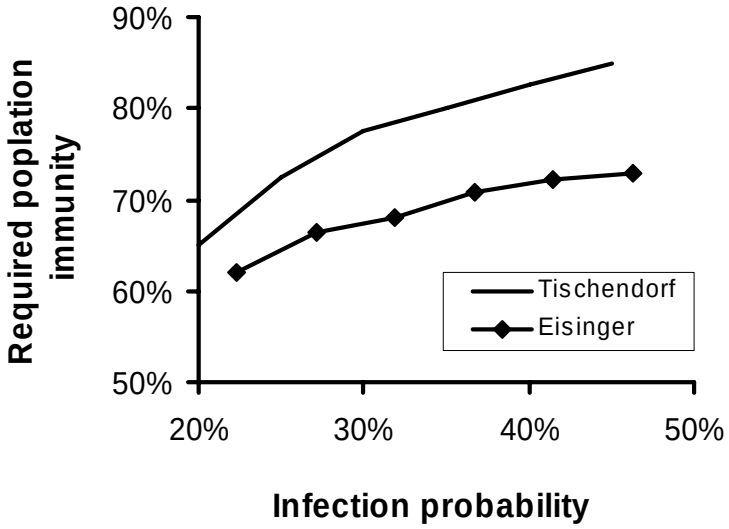


Fig. 1 The population immunity level required to give 80% probability of eradicating rabies within 4 years in relation to the bimonthly probability that an infected fox group infects a neighbouring group. In the Eisinger model the probability was measured from simulation runs, as the model performs weekly time-steps. The Tischendorf model requires for all assumed infection dynamics a noteworthy higher level of population immunity to eradicate the disease when compared to the Eisinger model.

MATERIALS AND METHODS

This analysis was based on two existing models. The first was described by Tischendorf et al. (1998) and the second by Eisinger et al. (2005) and full details can be found in these reports. Only the basic outline of the models is described here and, in the results section, the adjustments made to bring their predictions in line are outlined. Both models used an area of 280*140 cells. A standardised rabies epidemic was started by external infection of the first line of cells along the small side of the simulation area.

The model of Tischendorf et al. (1998)

This grid-based model uses fox groups as the smallest unit, i.e. one cell is either occupied by a fox group or it is empty. One group is either susceptible or infected, i.e. might become infected by, or infect neighbouring groups. Each time step lasts 2 months. Thus, after the primary infection of one fox of a group the whole cycle of incubation, infection of all other group members, their incubation period and potential infection of neighbouring groups can fit within this period. An infected group dies out (i.e. cell is set empty) with a probability of 80%. The remaining 20% of infected fox groups are infectious for another period. This model rule compensates for fox groups whose infection cycle is not in line with the bimonthly trigger. The main feature of an epidemic model is the transmission. Thus, the model incorporates as a parameter, the probability of an infectious group infecting a neighbouring group within a bimonthly period, which is adjusted during the mating season (PSI: 20%-45%). There is no mortality of foxes other than by rabies because it is assumed that removed individuals will be replaced by non-residential foxes immediately. In autumn, 3-4 juveniles disperse from each susceptible group, and 2-3 infected juveniles disperse from currently infected groups. The dispersal follows a correlated random walk with the final distance drawn from a distribution of dispersal distances observed in the field. Each empty cell colonized by at least one susceptible fox comprises a new fox group. Non-empty cells in which at least one infected fox settles become infected. Vaccination is modelled by lowering the infection probabilities by the level of assumed population immunity, i.e. PSI_{eff}= PSI*(1-ImmunisationLevel).

The model of Eisinger et al. (2005)

The spatial unit of this model is also the area covered by one fox group, represented by a grid cell. However, the model uses a time scale of one week and traces the foxes of a group individually. After a fox gets infected, there is an average incubation period of 2 weeks (drawn from a negative exponential distribution). In the following infectious week, at the end of which the fox dies, the fox infects all other group members. The newly infected group members pass through their own incubation period after which each might infect neighbouring cells; as also the primary infected fox must also have done. Hence, the effective infection probability after 2 months depends on the average number of foxes in the group, the length of the incubation period and the weekly infection probability. The effective bimonthly PSI was measured in simulations for each version of the Eisinger model to link to the value to the infection probability of the Tischendorf model. The individual-based model results in varying fox density by inclusion of a monthly adult mortality of 6.1% without rabies. Together with an assumed maximum of 5 adults per group, this gives an average density of about 3 foxes per cell without rabies. Three or four juveniles are born in spring and might become infected by in-group contacts. Juveniles do not experience any mortality other than from rabies, so that 3-4 juveniles disperse in autumn from

all non-infected groups (identical to the Tischendorf model). However, in contrast to the Tischendorf model, the number of juveniles dispersing from an infected group depends on the time of infection, the incubation period and the mortality due to rabies evaluated for each fox individually. During this study, the same dispersal kernel was applied as in the Tischendorf model. Vaccination was modelled to mimic aerial baiting in the field and assumes the distribution of 20 baits/km^2. Locally, baits are assigned according to distributions found for heterogeneous home range sizes (Thulke et al., 2004). After adjusting for bait losses (i.e. taken by competitors or not found), the respective actual number of baits is assigned to individual foxes of the group. Then, the resulting immunisation level is measured at the population level. This level can be adjusted by changing the number of bait losses. Thus, immunisation is modelled on an individual basis and each fox is either immunised 100% or not at all, in contrast to the lowered group susceptibility in the Tischendorf model.

Comparison of epidemiological features

In order to compare both models, it is important to ensure that they are similar with respect to epidemiological dynamics, because any difference would be likely to result in different levels of population immunity required for eradication. Ensuring the same bimonthly infection probability between neighbouring fox groups guaranteed the same transmission dynamics. Further the congruence of both models was examined with respect to the speed of the epidemic front, the number of infected dispersing juveniles and the number of infected fox groups. These characteristics were estimated before vaccination started as well before the epidemic wave had crossed the 280 cells of the naïve simulation area. In detail:

1) The speed of the epidemic wave was calculated as the time elapsed between the occurrences of the first infection behind the 40th row and the first infection beyond the 240th row (i.e. MaxX-40).

2) The number of infected dispersing juveniles in any 5 years in the Tischendorf model is simply 2.5 times the number of infected cells in the two-month period of dispersal. In the Eisinger model, the number of dispersing juveniles which are within the incubation period is counted explicitly.

3) The number of infected fox groups in the first 5 years equals the number of cells which have been infected at least once within that time.

If these three estimates are similar in both models, both are expected to predict the same level of population immunity required for eradication.

RESULTS

1) The wave speed of both models was found to be similar (Fig. 2a). This is encouraging because the origin of the Tischendorf model was expressly built to decode the wave pattern of the rabies epidemic (and successfully did this (Jeltsch et al., 1997)). In consequence, other characteristics had to be sought to explain the difference in the predicted minimum sufficient immunization level (Fig. 1).

2) Figure 2b shows the striking difference between the number of infected dispersing juveniles in the Tischendorf and the Eisinger models. Although we expected the numbers to be

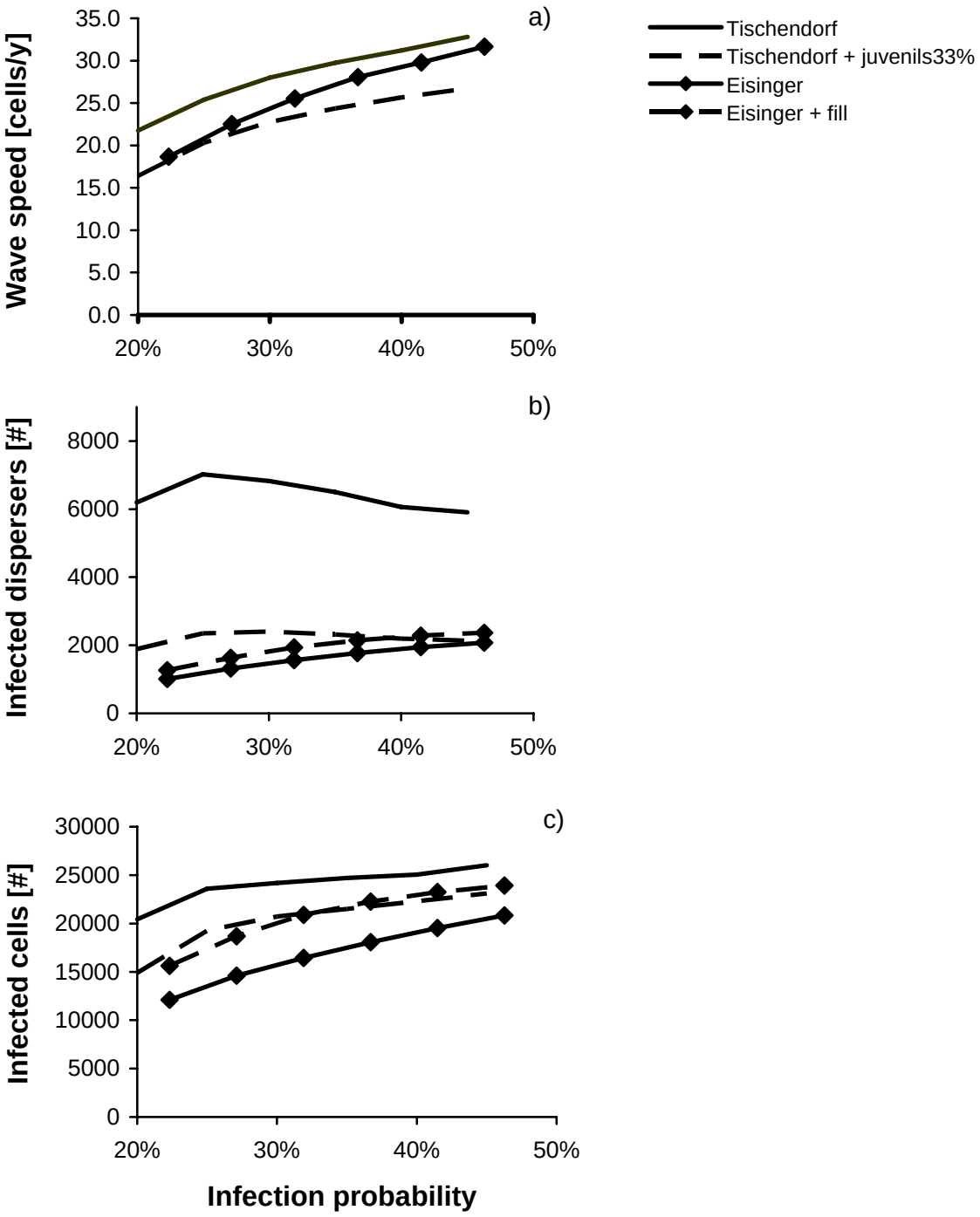


Fig. 2 Comparison of epidemiological features. Tischendorf + juvenils33% is the Tischendorf model with infected dispersers reduced to 33%. Eisinger + fill is the Eisinger model with fox groups filled up to mean group size if originally smaller than this. a) Wave speed. Eisinger + fill model is not shown as it is identical to the Eisinger model. b) Number of infected dispersing juveniles summed for the first 5 years of an epidemic. c) Number of fox groups which have been infected at least once in the first 5 years of an epidemic.

similar, it appears that many infected juveniles in the Eisinger model die of rabies before dispersal, and hence reduce the effective value. To adjust for this, two thirds of dispersing juveniles were removed from the Tischendorf model. This modification reduced the number of infected dispersing juveniles to a level equivalent to that in the Eisinger model (see Tischendorf + juvenils33% vs. Eisinger model, Fig. 2b). As a consequence, the wave speed in the Tischendorf model was also reduced, but remained qualitatively in the range of that of the Eisinger model (Fig. 2a). However, the predicted level of population immunity required for eradication was not reduced adequately (Fig. 3a, hashed line). Apparently, a further epidemiological feature was still represented differently in the two candidate models.

3) The number of infected cells was examined and this was also found to vary between both models (Tischendorf + juvenils33% vs. Eisinger model, Fig. 2c). Suspicion arose that partially-filled cells, following dispersal in the Eisinger model, were responsible for the observed difference since, in the Tischendorf model, all cells are treated as inhabited by an entire fox group after at least one juvenile has settled in an empty cell. Indeed, rabies might be expected to survive more easily if cells are filled immediately. Hence, a rule was added to the Eisinger model that all cells with a fox group size below the overall mean are filled to the mean number of foxes per cell after dispersal (i.e. in this case, three foxes). This modification successfully removes the difference in the number of infected cells between the Tischendorf + juvenils33% model and the Eisinger + fill model (Fig. 2c). However, surprisingly, the immunisation levels predicted as required for successful eradication are still not identical (Fig. 3b).

4) Finally, the different implementation of the vaccination in the two models was addressed. The Tischendorf model simply reduces the infection probability between fox groups by the level of population immunisation. The vaccination procedure in the Eisinger model is more complex: Baits are distributed non-uniformly between fox groups, and within groups, to individual foxes. In contrast to the Tischendorf model, in the Eisinger model there might be some fox groups 100% immunised and some not immunised at all. Only the assumed overall immunisation level is identical in the two models. In the final experiment, the homogeneous vaccination coverage used in the Tischendorf model was implemented in the Eisinger model. Specifically, baits were no longer explicitly distributed, and the neighbourhood infection probabilities were reduced from PSI to PSI-PopulationImmunisation. This adjustment finally removed the difference between the two last candidate models (i.e. Tischendorf + juvenils33% and Eisinger + fill + homo_vac, Fig. 3c).

DISCUSSION

Vaccination programmes against fox rabies need a target figure of population immunity (the percentage of immunised foxes in the host population) to aim for and this can be used to measure the success of ongoing vaccination campaigns (Brochier et al., 1988; European Commission, 2002; Vos, 2003). Ever since Anderson et al. (1981) demonstrated the feasibility of the eradication of rabies by vaccination with an analytical population model, this figure has been set to 70% and the performance of vaccination campaigns has been judged by the realisation or surpassing of this threshold (Barrat & Aubert, 1993; European Commission, 2002; Fleming, 1997; Marks & Bloomfield, 1999). Moreover, several models built as management support tools in relation to rabies control, used the confirmation of this figure as a validation criterion (Artois et al., 1997; Suppo et al., 2000; Tischendorf et al., 1998).

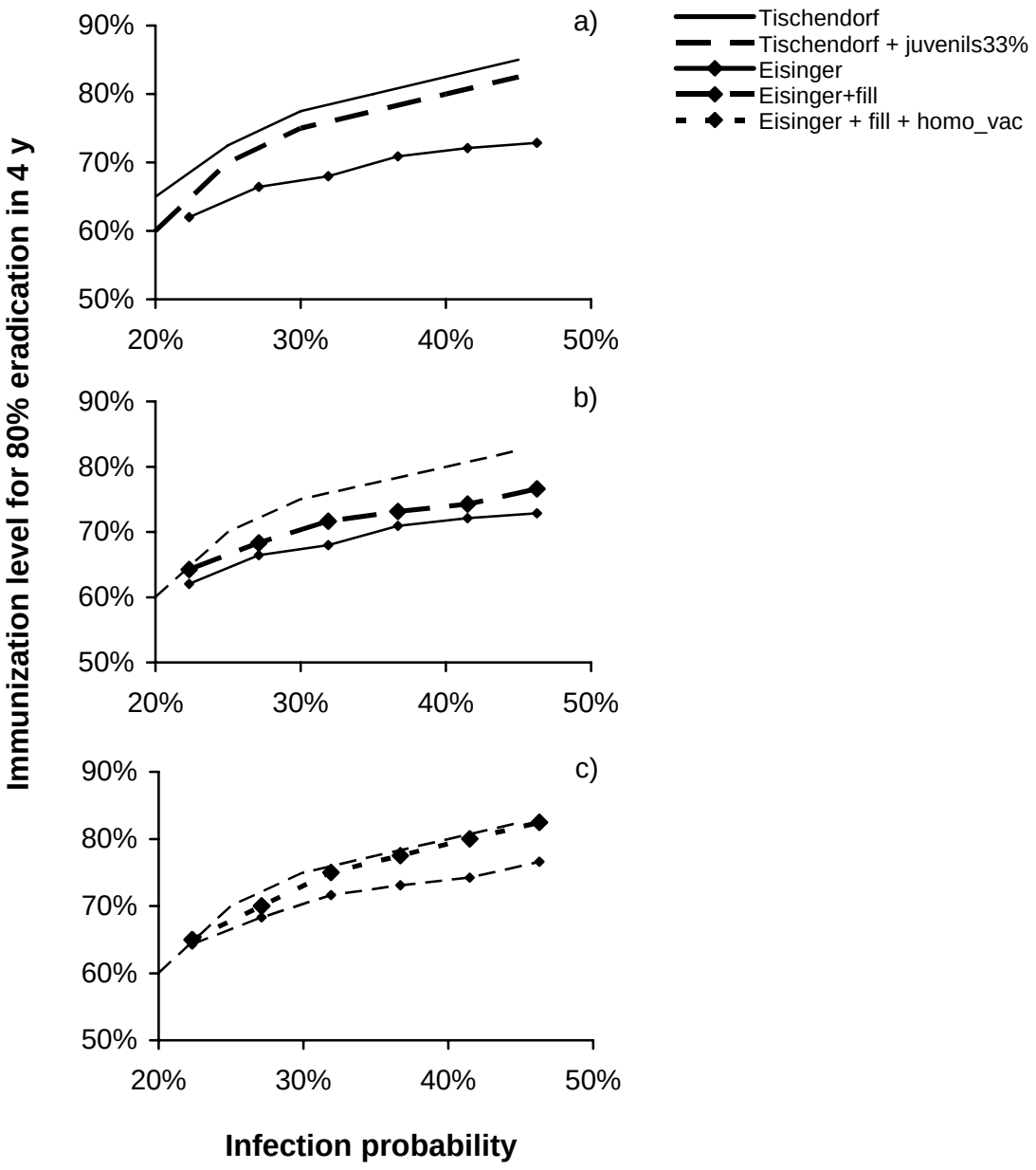


Fig. 3 The population immunization level required to eradicate rabies: Comparison of the models. a) In the Tischendorf model, if the number of dispersing juveniles is reduced to one third (Tischendorf + juvenils33%), the predicted minimum immunisation level necessary for eradication is lowered, but still noticeably different from the prediction of the Eisinger model. b) After mimicking the yes-or-no rule for the occupancy of cells (i.e. fox group home-ranges, see text) of the Tischendorf model in the Eisinger model (Eisinger + fill), the predicted levels of required immunity come closer but there is still noticeable disagreement in the pattern. c) If the technically implicit assumption of homogeneous vaccination coverage of the Tischendorf model is transcribed into the adjusted Eisinger model syntax (Eisinger + fill + homo_vac), both model structures predict the same level of required population immunity.

Recently a simulation model was developed to support emergency control planning in the event of new disease outbreaks in completely susceptible populations (Eisinger, 2005). As part of the model testing, the level of population immunity required to eradicate the disease was analysed. Surprisingly, a large difference from the estimates obtained from previous models was observed. Since both models (Anderson and Eisinger) were shown to be successful in the settings for which they were designed, it was decided to investigate whether model differences could be reconciled by exploring and adjusting their specific structures. As a first step, the approach to the model developed by Tischendorf et al. (1998) was applied. The Tischendorf model, although structural similar to the Eisinger model, nevertheless reproduced the threshold value for required population immunity of the original Anderson et al. (1981) model.

Although both models (Tischendorf and Eisinger) are similar, i.e. both are fox group based, use the same dispersal kernel and the same effective transmission probability between neighbouring fox groups, they differ with respect to large scale characteristics of the simulated rabies epidemic (i.e. the number of affected fox groups and infectious dispersers). Without the comparison of patterns that are not related to the original purpose of both models it would have been impossible to reveal the intrinsic differences. Following this study, reasons were identified that explain why models concerning the same epidemiological system often differ in their prediction when utilised to answer questions they were not specifically designed for (Starfield et al., 1990). However, the process also served to validate the robustness of some components of the existing models, e.g. the explanation of the wave pattern of a rabies epidemic by the Tischendorf model in the area it was originally designed for (Jeltsch et al., 1997). Irrespective of the greatly reduced number of juveniles dispersing from infected cells (i.e. Tischendorf + juveniles33%), the wave characteristic did not change substantially (Fig.3a).

Three differences were found in the models' structural design, which were responsible for the variation in the predicted threshold of population immunity required for eradication.

1. The number of infected juveniles dispersing from an infected fox group in autumn.
2. The mechanism governing how an empty territory is re-colonized.
3. Whether immunisation status of fox groups is assumed to be applied uniformly across all groups or explicitly modelled for individual foxes within groups.

All of these three processes were originally modelled in more detail in the Eisinger model, as the question of emergency treatment requires a local management compared to the long-term and large-scale vaccination programme which the Tischendorf model was concerned with. It is noteworthy that all three issues taken together result in a higher degree of heterogeneity in the representation of the modelled system:

1. Although the number of offspring was exactly the same in both models, following individual life/death traits of foxes in the Eisinger model removed several juveniles that could effectively have transferred the disease during dispersal. This resulted in a more heterogeneous distribution of potential transmission events throughout the epidemic area.
2. Behind the wave front, fox groups have a lower density than in the susceptible area and hence re-colonisation occurs at a slower rate. This effect is again incorporated into the Eisinger model by following individual foxes. Consequently, the spatial memory of the fox population reflects the heterogeneous host population structure arising from the passage of the lethal epidemic, for a longer period.

3. The individual-based bait delivery links the uniform bait distribution with non-uniform fox habitat on the ground and produces distinctly heterogeneous immunisation coverage within the Eisinger model.

Comparing both possible representations of the ecological system the more plausible realization of the processes in the Eisinger model argues for the lower predicted immunisation threshold than previously accepted. However, this means that the standard figure of 70% might need a systematic revision as it is, although conservative, probably too high for further use in large areas, for example in Eastern Europe. For this purpose, models tailored to the estimation of the threshold have to replace the ones prepared to analyse the dynamics of the controlled rabies-fox system in general.

REFERENCES

Anderson, R.M., Jackson, H.C., May, R.M. and Smith, A.D.M. (1981). Population dynamics of fox rabies in Europe. Nature 289, 765-770

Artois, M., Langlais, M. and Suppo, C. (1997). Simulation of rabies control within an increasing fox population. Ecol. Modell. 97, 23-34

Barrat, J. and Aubert, M.F. (1993). Current status of fox rabies in Europe. Onderstepoort J. Vet. Res. 60, 357-363

Brochier, B., Thomas, I., Iokem, A., Ginter, A., Kalpers, J., Paquot, A., Costy, F. and Pastoret, P.P. (1988). A field trial in Belgium to control fox rabies by oral immunisation. Vet. Rec. 123, 618-621

Deal, B., Farello, C., Lancaster, M., Kompare, T. and Hannon, B. (2000). A dynamic model of the spatial spread of an infectious disease: the case of fox rabies in Illinois. Environmental Modeling & Assessment 5, 47-62

Eisinger D., Thulke H.-H., Müller T. and Selhorst T. (2005). Emergency vaccination of rabies under limited resources – combating or containing? BMC Infect Dis 5.

European Commission (2002). Report of the Scientific Committee on Animal Health and Animal Welfare: The oral vaccination of foxes against rabies. http://www.europa.eu.int/comm/food/fs/sc/scah/out80_en.pdf

Fleming, P.J.S. (1997). Uptake of baits by red foxes (Vulpes vulpes): Implications for rabies contingency planning in Australia. Wildl. Res. 24, 335-346

Jeltsch, F., Müller, M.S., Grimm, V., Wissel, C. and Brandl, R. (1997). Pattern formation triggered by rare events: lessons from the spread of rabies. Proc. R. Soc. Lond. B 264, 495-503

Marks, C.A. and Bloomfield, T.E. (1999). Bait uptake by foxes (Vulpes vulpes) in urban Melbourne: the potential of oral vaccination for rabies control. Wildl. Res. 26, 777-787

Smith, G.C. and Harris, S. (1991). Rabies in urban foxes *(Vulpes vulpes)* in Britain: the use of a spatial stochastic simulation model to examine the pattern of spread and evaluate the efficacy of different control regimes. Phil. Trans. R. Soc. Lond. B 334, 459-479

Starfield, A.M., Smith, K.A. and Bleloch, A.L. (1990). How to model it: problem solving for the computer age. McGraw-Hill, New York. 206p

Suppo, C., Naulin, J.M., Langlais, M. and Artois, M. (2000). A modelling approach to vaccination and contraception programmes for rabies control in fox populations. Proc. R. Soc. Lond. B 267, 1575-1582

Thulke, H.-H., Tischendorf, L., Staubach, C., Jeltsch, F., Müller, T., Selhorst, T. and Schlüter, H. (1999). Controlling rabies in foxes by oral vaccination - learning from a simulation model. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine: 24-26 March 1999; Bristol. 140-151

Thulke, H.-H., Tischendorf, L., Staubach, C., Selhorst, T., Jeltsch, F., Schlüter, H. and Wissel, C. (2000). The spatio-temporal dynamics of a post vaccination resurgence of rabies in foxes and emergency control planning. Prev. Vet. Med. 47, 1-21

Thulke, H.-H., Selhorst, T., Müller, T., Wyszomirski, T., Müller, U. and Breitenmoser, U. (2004). Assessing anti-rabies baiting - what happens on the ground? BMC Infect Dis 4, 9

Tischendorf, L., Thulke, H.-H., Staubach, C., Müller, M.S., Jeltsch, F., Goretzki, J., Selhorst, T., Müller, T., Schlüter, H. and Wissel, C. (1998). Chance and risk of controlling rabies in large-scale and long-term immunized fox populations. Proc. R. Soc. Lond. B 265, 839-846

Vos, A. (2003). Oral vaccination against rabies and the behavioural ecology of the red fox (Vulpes vulpes). J. Vet. Med B 50, 477-483

AQUACULTURE

INFECTIOUS PANCREATIC NECROSIS (IPN) RISK FACTORS IN SEA-CULTURED ATLANTIC SALMON (*SALMO SALAR*) IN SCOTLAND

R.S. RAYNARD[1], A.G.MURRAY, R. KILBURN AND W. A. LESCHEN

SUMMARY

Infectious pancreatic necrosis (IPN) is a disease that is causing increasing problems for farmed salmon production. To identify factors behind outbreaks of this disease a case-control study was developed. This consisted of a questionnaire sent to 67 Scottish marine salmon farms identified as having had clinical IPN in 2002 or 2003 (cases) and 112 other marine salmon farms (controls). Response rates were nearly 60% and the results were analysed by calculating odds ratios and by multiple regression modelling. Key factors in a multiple regression model of probability of clinical IPN outbreaks were smolt stocks (S1 or S ½), number of sources of smolts and distance from neighbouring sites. There was also a positive association with proximity of processing plants and negative associations with frequent removal of dead fish and road delivery of smolts. An association with low temperature appeared to be mostly coincidental, due to an association between IPN and S1 stock that are put to sea in spring when the water is cold.

INTRODUCTION

Infectious pancreatic necrosis (IPN) is a viral disease affecting a variety of fish species, but notably salmonids, under aquaculture conditions (OIE, 2003). The effect on farmed Atlantic salmon is particularly serious because not only are fry affected, as with other species, but smolts are also affected when they are first put to sea. Their loss is of considerable economic significance, and IPN is considered to be the most damaging viral disease of salmon production in the EU (Ariel & Olesen, 2002; Anon, 2003).

The pathogen causing IPN is the aquabirnavirus IPNV (OIE, 2003; Anon., 2003). This virus is very robust, being essentially immune to solar UV (Kitamura et al., 2004), capable of surviving in the guts of pisciverous birds (McAllister & Owen, 1992) or persisting in symptom less carrier fish for years (Yamamoto, 1975). Infection with IPNV is now very widespread, being found in over 80% of Scottish marine salmon farms (Murray et al., 2003).

Although prevalence of IPNV is >80% in marine salmon farms the number of clinical IPN cases reported to the Fisheries Research Service is lower at 12.5% (Bruno, 2004). Therefore, about 84% of infected sites do not have clinical disease (100x[0.8-0.125]/0.8). Disease is a multifactorial conditional involving, pathogen, host and environment (Arkoosh et al., 1998). Stress is also a strong contributor in clinical IPN outbreak in covertly infected fish (Taksdal et al., 1998). In order to reduce the impact of disease, it is necessary to understand factors behind

[1] Rob Raynard, Fisheries Research Services (FRS) Marine Laboratory, Aberdeen, AB11 9DB UK. Email: raynardr@marlab.ac.uk

both pathogen spread and actual disease activation. Here, a case-control study is described, aimed at identifying factors that distinguish salmon farms that have cases of clinical IPN disease from control sites, which may have IPNV, but have not reported clinical disease.

Case-control methods have been extensively used in Norwegian salmon aquaculture to investigate factors behind outbreaks of furunculosis (Jarp et al., 1993, 1994), IPN (Jarp et al., 1994), infectious salmon anaemia (Jarp & Karlsen, 1997) and the non-infectious disease cataracts (Ersdal et al., 2001) in both fresh and marine waters. Similar methods have been used to identify risk factors for White Spot Syndrome virus in Vietnamese shrimp (Corsin et al., 2001). Thus, case-control methods have been applied to identify risk factors for IPN in farmed Scottish salmon. In addition, a questionnaire was developed (Leschen, 2003) to obtain information on key environmental and management factors behind cases of IPN and used the results to identify key risk factors associated with IPN disease. Further details of the study can be found in Murray et al., (2004).

MATERIALS AND METHODS

Application of case control methods requires the definition of 'case' and 'control' sites or samples. In the case of clinical IPN in Scotland, a list of reported clinical cases is maintained at the Fisheries Research Services (FRS) Marine Laboratory (Bruno, 2004). Material from fish farms was collected by FRS fish health inspectors and clinical IPN disease confirmed by microscopic examination (Bruno, 2004) and IPNV identified by standard virological methods (OIE, 2003). Case sites were defined as sites listed in 2002 or 2003. Control sites were selected from sites that are not on this list (this may include unreported cases).

Having defined case and control, it was necessary to obtain data on these sites to determine if any systematic differences existed between the two groups. The method applied was a questionnaire survey (Kelsey et al., 1996). This was developed using experience gained from earlier questionnaires developed by the Shetland Salmon Farmers' Association (Leschen, 2003). This questionnaire consisted of questions designed to determine key environmental and management factors that might be associated with cases of IPN.

The questionnaire was sent to 67 case sites (33 from 2002 and 31 from 2003) and 112 valid control sites (21 inactive sites and 7 duplicate questionnaires being excluded). A follow up reminder postcard and, if considered necessary, telephone reminders were used to obtain returns of nearly 60% (59.7% cases, 56.3% control sites). This is considered a good return for a postal questionnaire and thus suitable for analysis (Kelsey et al., 1996).

The key outcome measure used for this case-control study was the odds ratio (Kelsey et al., 1996). This is the ratio of the odds of a factor applying to case sites with respect to the odds of the factor applying to the control sites:

$$odds\_ratio = \frac{\Pr(factor \mid case) \ / [1 - \Pr(factor \mid case)]}{\Pr(factor \mid control) \ / [1 - \Pr(factor \mid control)]} \quad (1)$$

The use of odds ratio discounts for the degree to which a factor applies generally to the whole population of cases and controls. This means that factors that contribute little to current IPN caseload could have large odds ratios indicating that if these factors became more prevalent

IPN caseload could significantly increase. Confidence intervals can be added (Kelsey et al., 1996) and these do reflect the amount of available data:

$$\ln(confidence) = \ln(odds\_ratio) \pm z_c \sqrt{1/a + 1/b + 1/c + 1/d} \quad (2)$$

The terms a = number of cases positive for the factor, b = number of controls negative for the factor, c = number of cases negative for the factor, d = number of controls negative for the factor. The parameter z_c is the confidence coefficient, 1.96 for 95% confidence. Exponentiation of the upper and lower log limits gives confidence limits about the odds ratio itself.

For some factors, quantitative data were obtained from the questionnaire, e.g. distance to the nearest neighbouring site, rather than whether the nearest neighbour was more than a given distance from the site. With such data it is possible to determine a regression of the relative probability of a site being a case rather estimating the effect-size of a factor if the data were categorically coded. Note that the probability is relative because case sites have been selectively targeted and so contribute a disproportionate number of sampled sites. For basic odds ratios, the median value of all case and controls was used, e.g. the odds ratio that a case site was >3 km from its nearest neighbour relative to odds that a control site was >3 km from its nearest neighbour.

RESULTS AND DISCUSSION

Odds ratios

Odds ratios were generated for all the factors listed in the questionnaire. Odds ratios indicate a strong negative effect of the factor if <<1 (constrained to be ≥ 0) or a strong positive effect if >>1. Odds ratios around 1 indicate no detectable effect, although this may be due to lack of data rather than lack of effect. These odds ratios are sorted by the magnitude of the effect of the factor (either the odds ratio or the inverse odds ratio of this was <1).

It must be emphasised that all the findings of this case control analysis are statistical associations. All findings of the study must be considered as useful guidance for further work rather than conclusive. For example, a coincidental association between IPN and temperature will be addressed later.

One issue that may distort results is the regional distribution of IPN cases. Clinical IPN has historically been associated with Shetland (Smail et al., 1992) and so environmental associations could be coincidental associations with regional factors caused by variation in IPN prevalence between regions (the ecological fallacy). However, recently IPNV has become much more widely distributed (Murray et al., 2003). When the IPN cases used in this survey were assigned to regions they appeared to have been fairly evenly distributed throughout Scotland in 2002 and 2003 (as a proportion of local salmon farms) and hence there should be no particular regional bias (Fig. 1)

The complete list of factors for which odds ratios have been produced is discussed by Murray et al. (2004). In this paper, only selected factors that are of interest for the management of IPN are described (in some cases due to their lack of effect) (Table 1).

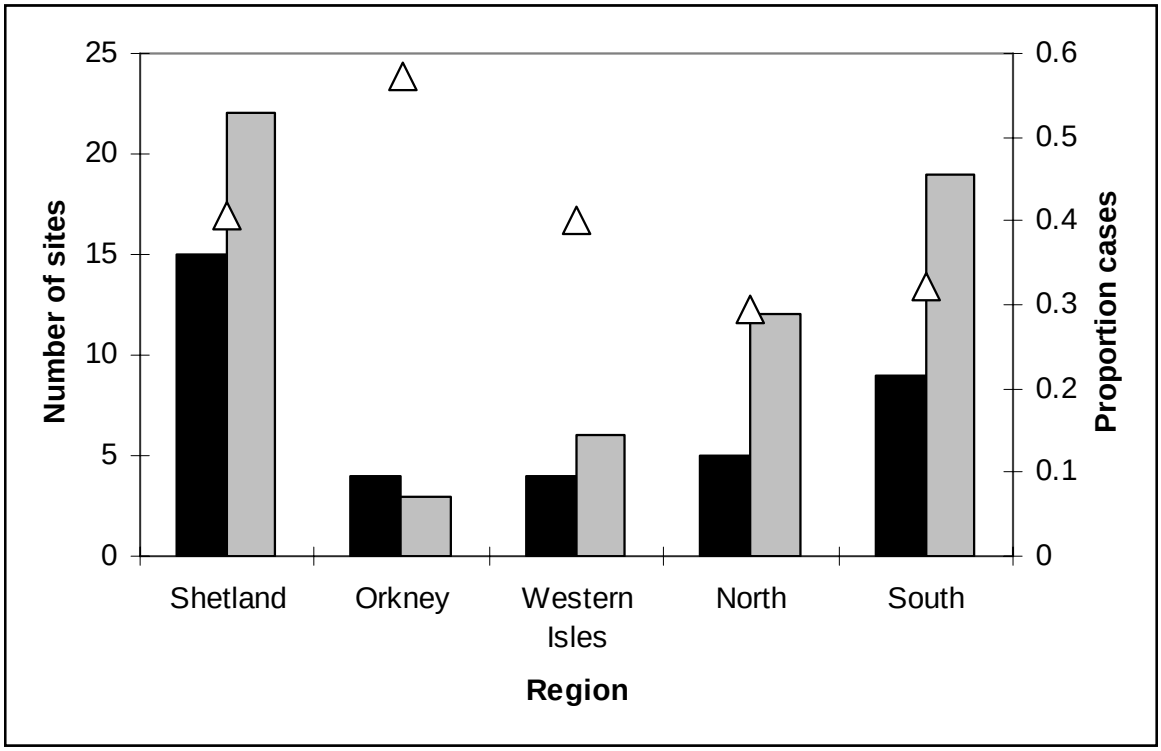


Fig. 1 Regional distribution of case (black bars) and control (grey bars) sites throughout Scotland and the ratio of case to control (triangles). The ratio of case to control is similar in all regions (30-40%) except Orkney (57%) from which only 7 sites were sampled.

Table 1 shows odds ratios for factors with upper and lower confidence intervals that are asymmetrical in order to show the range of likely values. Relatively few factors are significant at 95% and other factors significant at 90% are shown as this indicates that they are potentially important.

The largest effect listed in Table 1 is the effect of smolt stock type: S ½ smolts are strongly negatively associated with clinical IPN, while S1 smolts are strongly positively associated within. This agrees with the results of Bruno (2004).

There is a strong likelihood that higher than average losses are associated with clinical IPN. This is almost certainly not causal, but rather an effect of clinical IPN. Were it not so, clinical IPN would not be an economic problem.

Low temperatures are associated with clinical IPN; however, S1 smolts are put to sea in spring when temperatures are at their lowest. These data will shortly be analysed for the temperature effect showing smolt stock type to be a confounding variable.

The final positive association for IPN cases, significant at the 95%level, is with the use of multiple sources of smolts. Jarp et al. (1994) also found an association between multiple sources and IPN disease.

At the lower significance level of 90%, there are three further factors: road delivery of smolts, the presence of a processing plant within 5 km and whether the site received >400,000 smolts. The road delivery may reflect use of local smolts (there is no association, positive or negative, with other methods of delivery (data not shown). In a similar analysis, processing

plants were identified as a risk factor for ISA infection in Norway (Jarp & Karlsen, 1997). The receipt of large numbers of smolts may be partly confounded by the fact that such sites may receive smolts from many sources.

Table 1. Selected case-control results for management factors with 90% confidence interval (significant at 95%). Those factors significant at 95% are shown in bold and 90% (80% confidence range) in bold italics.

Factor	Odds Ratio	Lower	Upper
S ½ stock	**0.229**	**0.094**	**0.559**
S1 stock	**4.263**	**1.972**	**9.213**
>5% losses	**2.800**	**1.205**	**6.506**
Temperature <8 Celsius	**2.761**	**1.603**	**4.755**
>1 source of smolts	**2.189**	**1.014**	**4.722**
Road delivery of smolts	***0.461***	***0.183***	***1.161***
Processing plant within 5 km	***2.107***	***0.908***	***4.892***
400k+ smolts	***1.921***	***0.950***	***3.883***
Removal of morts >3 days	1.729	0.843	3.549
Problem with transfer	1.719	0.572	5.161
Neighbour 3-15 km	0.600	0.285	1.265
Fallowing	0.865	0.311	2.412
IPN on site previously	0.876	0.353	2.173
Moving staff/equipment	0.876	0.430	1.787
Transfer diet	0.923	0.449	1.899
Area Management Agreement	0.959	0.482	1.911

Certain factors, while not significantly associated with IPN at the 90% level, are worthy of further investigation. Frequent removal of mortalities is complicated because some sites with no clinical problem rarely remove mortalities, while sites with a clinical problem are likely to remove dead smolts, thus obscuring any beneficial effects. Frequent removal of dead smolts in summer was shown to reduce risk of ISA (Jarp & Karlsen, 1997), but this effect did not occur in winter. Problems with the transfer of smolts were rarely reported so that, even if risk was high per event, with only a few events, their significance may have been obscured. Conversely, recall bias may also be important in that events may be more likely to be recalled if there were subsequent problems.

The presence of a neighbouring site within <3 km is only weakly associated with IPN, although this factor will be further analysed to show that distance is a major factor associated with IPN outbreaks at greater distances.

Several management factors that might have been expected to help control IPN outbreaks do not appear to do so. These are fallowing, moving staff or equipment between sites, belonging to an Area Management Agreement or the use of special transfer diets. Very few sites were not fallowed, so lack of a detectable effect could be due to lack of data. However, the other factors do apply to large numbers of both positive and negative sites in both case and controls and therefore the lack of effect appears to be real. However, it is possible that Area Management

Agreements designed in the light of better data on IPN epidemiology could be effective at controlling cases.

A history of IPNV on site prior to 2001 does not appear to be associated with IPN outbreaks in 2002 or 2003. Interestingly, unpublished analysis of IPNV inspection records appears to show that IPNV infection of sites does not persist over longer time scales.

Key factors and their interactions

Among the most significant IPN risk factors identified by the study are smolt stock type and temperature. However, as mentioned earlier, S1 smolts are put to sea in spring when temperatures are lower. It was found that, when temperature is plotted against the frequency distribution of the proportions of smolts put to sea, the patterns are almost identical for S1 smolts, i.e. there is no detectable effect of temperature on IPN cases (Fig. 2). Median temperatures are 7.8 and 8 ºC, nearly 75% of deployments occur between 7.1 and 8.5 ºC, in both cases and controls. It is only outside this range, in the lower part (i.e. the lower $1/8^{th}$ of temperatures) that there is deviation, with temperature at case sites at the time of deployment tending to be about a degree cooler than control sites. With S½ smolts there are differences between the case and control frequency distributions, but this is probably because there are very few cases of IPN in sites stocked with S½ smolts. Within the S½ stocked control sites, median temperature was 10 ºC at the time the smolts were put to sea, which is warmer than 90% of S½ smolt deployments. The range of temperatures over which S½ smolts were put to sea does appear broader than for S1 with 75% occurring between 8 and 12ºC, and overlap does occur, but the difference in the frequency distributions is clear (Fig. 2). Therefore, it is concluded that low temperature at the time sites are stocked is not, or is only marginally, associated with clinical IPN, except by coincidental association with S1 smolts stocking.

The odds ratios show that the use of multiple sources of smolts is strongly associated with IPN. This relationship strengthens with each additional source (Fig. 3), so about 45% of control sites use one source, but only 25% of case sites do, similar proportions of both case and control sites receive smolts from two (30%) or three (18%) sources. Overwhelmingly greater proportions of case sites compared to control sites receive smolts from four (22% to 7%) or five (5% to 2%) suppliers. Clearly, the more sources of smolts that a farm uses, the greater its risk of clinical IPN.

Sites receiving >400,000 smolts had significantly higher odds ratios for clinical IPN. However, this may be confounded in that such sites are more likely to receive smolts from multiple sources. There is a significant, if weak, relationship: thousands of smolts received = 238 + 90 × number of smolt sources ($p = 0.000087$, $r^2 = 0.15$). Alternatively, it may be that large sites are inherently more likely to develop clinical IPN.

The proportion of sites whose nearest neighbour is within a certain distance has been plotted for distances of up to 15 km (Fig. 4). In addition, the risk ratio has been plotted (this is the simple ratio of cases to controls with increasing distance from nearest neighbour). Data for sites separated by more than 15 km from their nearest neighbour were excluded as some of these data points appeared unreliable, e.g. an entry of 1,000 km was almost certainly 1,000 m.

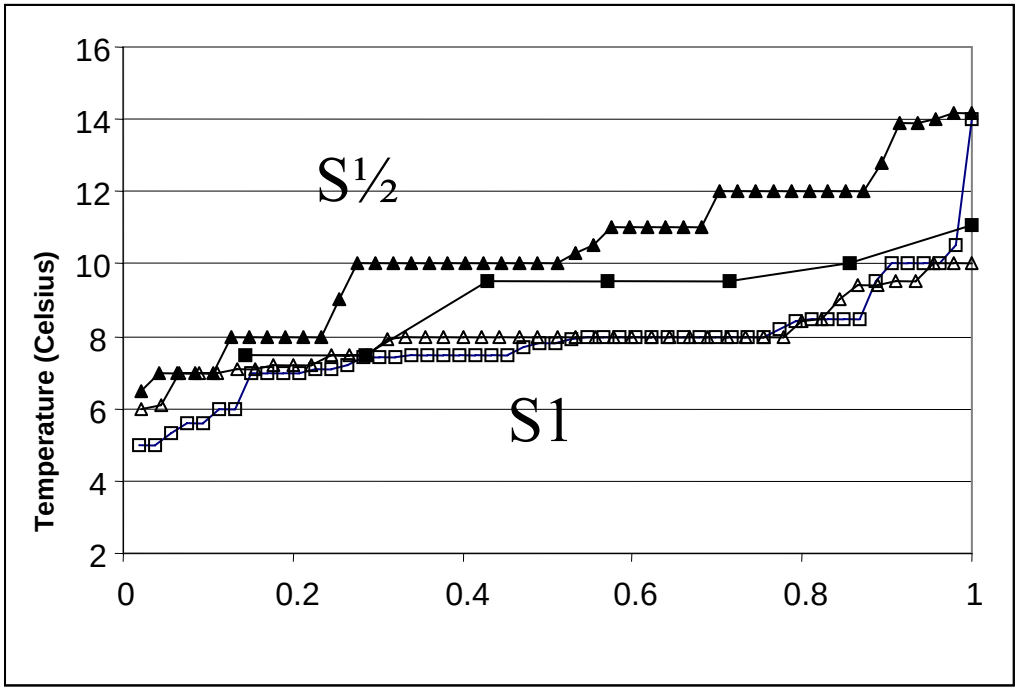


Fig. 2 Frequency distributions for temperature at the time when smolts are put to sea for S1 cases (solid square) and controls (solid triangle) and S½ cases (hollow squares) and controls (hollow triangles)

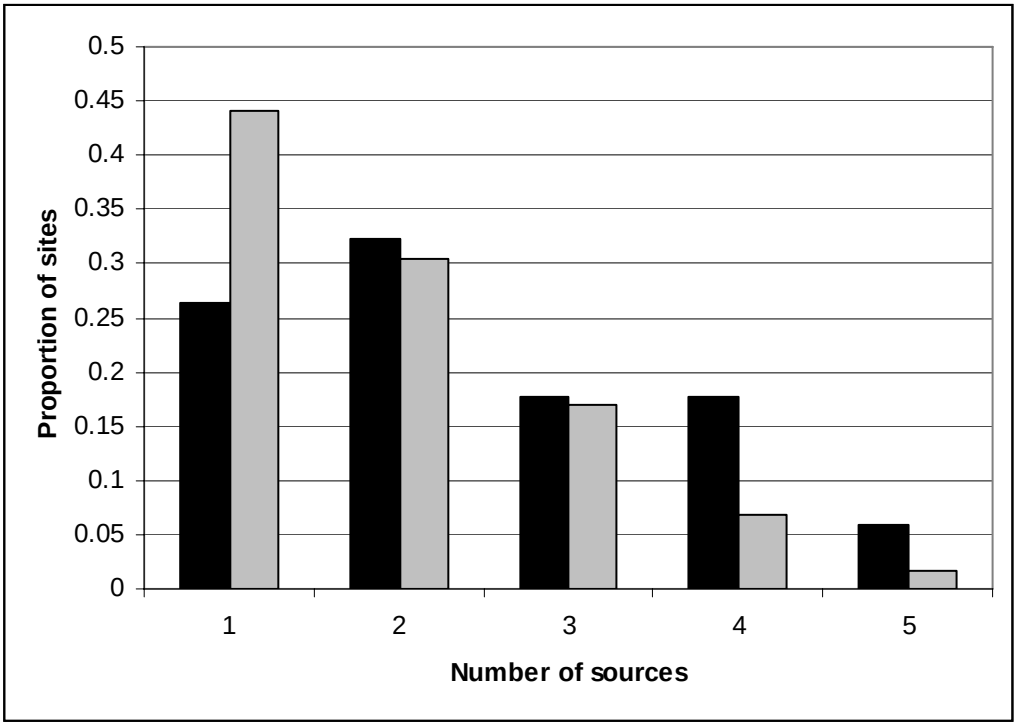


Fig. 3 Histogram of case (black bars) and control (grey bars) sites receiving smolts from different numbers of sources

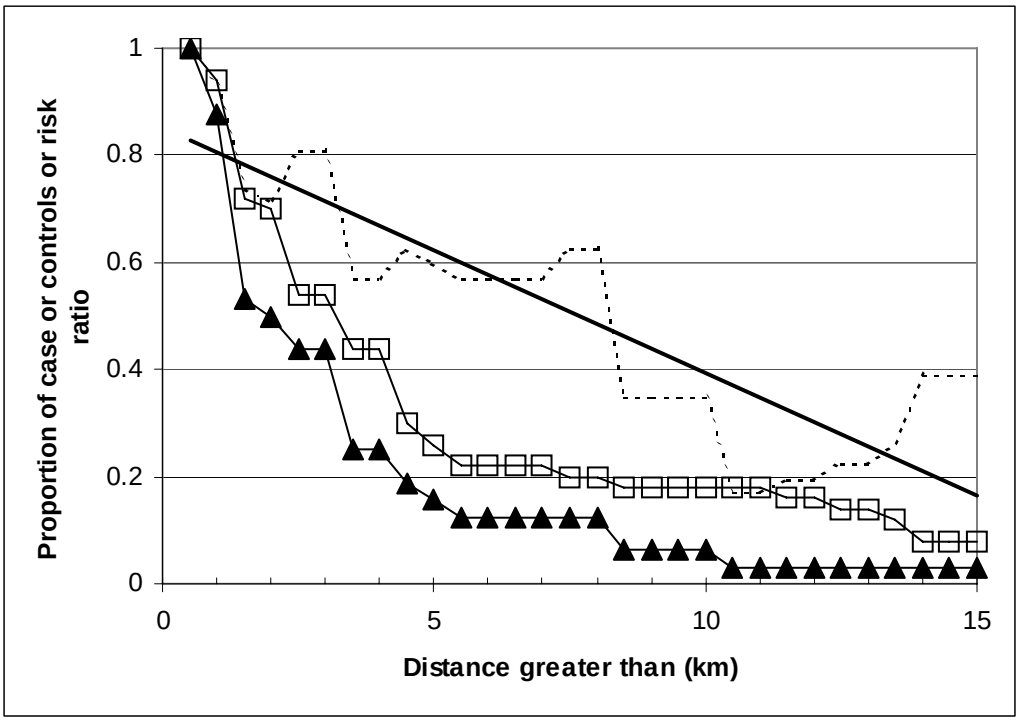


Fig. 4 Proportions of sites whose nearest neighbour is within x km for control (hollow squares) and case (solid triangles) sites. Also shown is the risk ratio (dotted line) the ratio between case and control, as this falls (regression = 4.75% km^{-1}) the relative risk falls.

All case and control sites have a nearest neighbour at ≥ 0.5 km and, therefore, the risk ratio (Kelsey et al., 1996) would be 1 (odds ratio incalculable). However, 26% of control sites but only 15.6% of case sites are 5 km or more from their nearest neighbour. This gives a risk ratio of 0.6 and odds ratio of 0.527. This means that the proportion of cases to controls falls as distance to the nearest neighbour increases. This protective effect strengthens with distance up to about 8-12 km, when the risk ratio shows signs of saturating at 0.3. This implies that separation of >10 km would have little extra protective effect. The rate of fall of the risk ratio with distance implies about 4.75% reduction in relative risk with each km of separation (as a proportion of the risk of transmission between immediate neighbours). Very few sites are separated by such distances from their nearest neighbour, so more data are required to ascertain the minimum distance between neighbours at which protection is maximised.

There may be a positive feedback effect if disease risks are reduced on a significant number of sites (Halloran & Struchiner 1995). This might mean risk factors are further reduced. This occurs because reduced vulnerability of individual sites to clinical disease will lead to a reduction in clinical disease, in a straightforward manner. However, this reduction in clinical outbreaks will itself lead to a reduction in infection pressure which will lead to a secondary reduction in exposure to IPNV and hence a further drop in clinical IPN. Thus, reduction of risk factors has the potential to be of greater benefit than the direct association of these risk factors with disease implies.

A regression model

Key risk factors have been identified for IPN outbreaks: whether there is S1 or S½ stock on site, the number of sources of smolts a site uses and distance to nearest neighbour. Multiple

regression approaches can be used to model such data (Revie et al., 2003). A multiple regression model has been fitted to the IPN case control data to incorporate the key factors:

Pr(IPN case) = 2.67% +28.8% if S1 + 10.8% per source of smolts – 1.75% per km from nearest neighbour.

The model fits observations with $r^2 = 0.178$, but observations are 0 or 1, while the model generates continuous probabilities, therefore r^2 cannot be large. If the modelled probabilities are used to fit stochastically generated 1 or 0 results, the resultant r^2s are similar, so it can be concluded that the model describes the data well. The probabilities of the individual factors are 0.009 for S1, 0.025 for the number of smolts and 0.168 for distance from neighbour. Exclusion of distance from the model reduces r^2 to 0.148, substantially reducing the model's fit. Inclusion of the other factors with significant odds ratios: road delivery of smolts, total number of smolts and a processing plant within 5 km, increases r^2 to 0.190, showing that significant complication of the model does not improve output significantly.

The model describes the probability of a site in the sampled dataset being an IPN case. Because case sites were selected and disproportionately sampled the ratio of cases: controls in the observations are higher than in the population. The ratio of cases to controls is less exaggerated than normalised values for which the numbers of case and controls are the same as in Fig. 4. This is partly why the risk ratio for distance alone is 4.75% km^{-1}, while the multiple regression model risk declines 1.75% km^{-1}. There is also a relationship between the distance to the nearest neighbour and number of sources of smolts (0.05 km $sources^{-1}$, $p = 0.08$) because farms using many sources of smolts tend to be in more crowded areas. This is equivalent to 0.5% km^{-1}.

Sites with a processing plant within 5 km are likely to be closer to their neighbours (mean distance 2.7 km as opposed to 4.3 km). This may be why the addition of processing plant data does not improve the multiple regression model, in spite of the odds ratio being significant: distance to neighbour is reflecting both neighbour and processing plant information.

CONCLUSION

This case-control study has identified potentially important risk factors associated with outbreaks of clinical IPN in Scotland in 2002 and 2003. It must be emphasised that the outcomes of this study are statistical associations, which may be confounded (as temperature largely appears to be) or for which clinical IPN may be causal rather that caused by (vaccination). Therefore, all the associations found require verification with further study.

The questionnaire approach has proven a very useful and efficient means of obtaining data from a large number of sources. Results depend on honest reporting from a large proportion of selected sites and, with collaboration of industry bodies, a good return has been achieved. One problem with the design of the questionnaire became apparent during analysis in that negative answers were not always recorded differently from a failure or a lack of knowledge. Redesign of the questionnaire to include explicit negative answers (yes/no/don't know) would improve this. However, the application of a similar questionnaire to study future trends in IPN, or other disease, would be likely to be productive in many situations.

The key factors emerging from the study are use of S1 versus S½ smolts, the number of sources of smolts used by a site and its distance from neighbouring sites. Other factors that may

play a role are the presence of a processing plant in the vicinity, frequent removal of dead fish, problems with smolt transfer and road delivery of smolts (possibly because this implies local sourcing).

Most management actions appeared to have very little role or effect: fallowing, belonging to an Area Management Agreement (AMA), sharing staff or equipment with other sites and the use of special transfer diets. In the case of fallowing, this may be due to lack of data as almost all sites do fallow. The other factors do not appear important, but perhaps this is because they do not go far enough, for example if all sites in an AMA sourced from the same restricted number of suppliers perhaps this would reduce the risk of IPN at the area level. However, it does imply that management activities need to be reconsidered in the light of improved understanding of IPN epidemiology.

ACKNOWLEDGEMENTS

The time and effort taken by all those involved in marine salmon farming who responded to the questionnaire and their associations, the Shetland Salmon Farmers Association and Scottish Quality Salmon, who helped make the study possible, is gratefully acknowledged. The study was funded by the Crown Estate.

REFERENCES

Anon (2003). Final report of the aquaculture health joint working group on infectious pancreatic necrosis in Scotland. Fisheries Research Service, Aberdeen, 90p

Ariel, E. and Olesen, N.J. (2002). Finfish in aquaculture and their diseases – a retrospective view on the European community. Bull. Eur. Assoc. Fish Pathol. 22, 72-83

Arkoosh, M.R., Casillas, E., Clemons, E., Kagley, A.N., Olson, R., Reno, P. and Stein, J.E. (1998). Effect of pollution on fish disease: potential impacts on salmonids populations. J. Aquat. Animal Health. 10, 182-190

Bruno, D. (2004). Changes in prevalence of clinical infectious pancreatic necrosis among farmed Scottish Atlantic salmon, *Salmo salar* L. between 1990 and 2002. Aquaculture. 235, 13-26

Corsin, F., Turnbull, J.F., Hao, N.V., Mohan, C.V., Phi, T.T., Phuoc, L.H., Tinh, N.T.N. and Morgan, K.L. (2001). Risk factors associated with white spot syndrome virus infection in a Vietnamese rice-shrimp farming system. Dis. Aquat. Org. 47, 1-12

Erdsal, C., Midtlyng, P.J. and Jarp, J. (2001). An epidemiological study of cataracts in seawater farmed Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 45, 229-236

Halloran, M.E. and Struchiner, C.J. (1995). Causal inference in infectious disease. Epidemiology. 6, 142-151

Jarp, J. and Karlsen, E. (1997). Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 28, 79-86

Jarp, J., Tangen, K., Willumsen, F.V., Djupvik, H.O. and Tveit, A.M. (1993). Risk factors for infection with *Aeoromas salmonicida* subsp. *Salmonicida* in Norwegian freshwater hatcheries. Dis. Aquat. Org. 176, 81-86

Jarp J., Gjevre, A.G., Olsen, A.B. and Bruheim T. (1994). Risk factors for furunculosis, infectious pancreatic necrosis and mortality in post-smolt Atlantic salmon *Salmo salar* L. J. Fish Dis. 18, 67-78

Kelsey, J.L., Whittemore, A.S., Evans, A.S. and Tompson, W.D. (1996). Methods in observational epidemiology 2nd Edition. Oxford University Press, New York

Kitamura, S.-I., Kamata, S.-I., Nakano, S.-I. and Suzuki, S. (2004). Solar UV does not inactivate marine birnavirus in coastal seawater. Dis. Aquat. Org. 58, 251-254

Leschen, W.A. (2003). Development of a case control study (for the identification of risk factors) for infectious pancreatic necrosis (IPN) in Scottish salmon farms. MSc Thesis, Stirling University

McAlister, P.E. and Owen, W.J. (1992). Recovery of infectious pancreatic necrosis virus from the faeces of wild pisciverous birds. Aquaculture. 106, 227-232

Murray, A.G., Busby, C.D. and Bruno, D. (2003). Infectious pancreatic necrosis virus in Scottish Atlantic salmon farms, 1996-2001. Emerg. Infect. Dis. 9, 455-460

Murray, A.G., Leschen, W.A., Kilburn, R. and Raynard, R.S. (2004). A case-control study for the identification of risk factors behind clinical outbreaks of infectious pancreatic necrosis (IPN). FRS Marine Laboratory Report 06/04, Aberdeen

Office International des Epizooties (OIE) (2003). *Diagnostic manual for Aquatic Animal Diseases, Chapter 2.2.3 Infectious Pancreatic Necrosis* pp74-81 6th Edition, Office International des Epizooties, Paris

Revie, C.W., Gettinby, G., Treasurer, J.W. and Wallace, C. (2003). Identifying epidemiological factors affecting sea lice *Lepeophtheirus salmonids* abundance on Scottish salmon farms using general linear models. Dis. Aquat. Org. 57, 85-95

Smail, D.A., Bruno, D.W., Dear, G., McFarlane, L.A. and Ross, K. (1992). Infectious pancreatic necrosis (IPN) virus subtype sp serotype in farmed Atlantic salmon, *Salmo salar* L., post smolts in the Shetland Isles, Scotland. J. Fish Dis. 18, 631-638

Taksdal T., Ramstad, A., Stangeland, K. and Dannevig, B.H. (1998). Induction of infectious pancreatic necrosis (IPN) in covertly infected Atlantic salmon, *Salmo salar* L., post smolts by stress exposure, by injection of IPN virus (IPNV) and by cohabitation J. Fish Dis. 21, 193-204

Yamamoto T. (1975). Infectious pancreatic necrosis (IPN) virus carriers and antibody production in a population of rainbow trout *Salmo gairdneri* Can. J. Microbiol. 21, 1342-1347

EVIDENCE OF INTER-SPECIES INTERACTION BETWEEN SEA LICE IN SCOTTISH SALMON FARMS?

C.W. REVIE[1], G. GETTINBY, E. MCKENZIE, L. KELLY, C. WALLACE AND J.W. TREASURER

SUMMARY

Despite novel medicines for the control of sea lice on farmed Atlantic salmon, infections remain a threat to the health of fish and the commercial viability of farms throughout European, North American and South American waters. In Scotland, the dynamics of sea lice populations, and in particular the species *Lepeophtheirus salmonis* and *Caligus elongatus*, have been of interest since the inception of fish farming.

In this paper, evidence is assimilated from epidemiological trends, time series analysis, odds ratios and regression models of risk factors that suggests, contrary to received wisdom and observations from other countries, that the two major lice species found on Scottish salmon farms do not act independently. Indeed, subject to certain density-dependent criteria there would appear to be evidence of asymmetrical competition between the species. These findings have implications for the sustainable control of sea lice infections, as any integrated sea lice management strategy should take account of the ecological interaction between the species.

INTRODUCTION

The study of sea lice populations goes back to the 1800s, when early descriptions of the copepodites discussed in this paper were recorded by von Nordmann, 1832 – in the case of *C. elongatus* and Krøyer, 1837 – in the case of *L. salmonis*. Although it was recognised that such species were common parasites of marine fish, much of the interest was academic. Interest grew when salmon farming became established as a viable prospect during the second half of the 20th century. It was quickly recognised that intensively managed fish populations were susceptible to sea lice and yet little was known about the life-cycle, epidemiology and effects of these species. Some of the early observational work on sea lice populations on salmon raised in cages was undertaken in Scotland by Rae (1979). From this and other work (Bron et al., 1993; Grant & Treasurer, 1993; Treasurer & Grant, 1994; Wootten et al., 1982) it became clear that sea lice were not only a production constraint but a major threat to the welfare of both farmed and wild salmon populations, and similar problems were identified in other salmon-producing countries (Bristow & Berland, 1991; Hogans & Trudeau, 1989; Johnson & Albright, 1991 Tully, 1989).

[1] Crawford W Revie. University of Strathclyde, Livingstone Tower, Richmond Street, Glasgow, G1 1XH, UK. Email: crawford.revie@cis.strath.ac.uk

Following a decade of careful study, considerably more is known about sea lice populations (Heuch et al., 2003; Pike & Wadsworth, 1999). In particular, it is recognised that in the major salmon producing countries, Norway, Chile, Scotland, Canada, Ireland and the USA, some form of sea lice control is unavoidable if salmon are to achieve their growth potential and remain healthy. In response to this, the aquaculture industry has relied on the application of a range of veterinary medicines against a background of changing farming practices and the need to conserve effective treatments in the face of the threat of resistant populations to what is currently a limited number of safe and efficacious treatments.

One of the major challenges is to understand the dynamics of sea lice populations on farmed salmon and, in particular, how such populations respond to control regimens. In the UK, it has been known for some time that the majority of sea lice infections are *L. salmonis*. This species is widespread throughout the world and exceptional in that it only infects salmonids. However, in the UK, salmon may also become infected with the less prominent lice species *Caligus elongatus*, which parasitises a wide range of fish hosts (Kabata, 1979). To date, this species has attracted less attention and most use of veterinary medicines is targeted at controlling the more prevalent *L. salmonis* species. There is concern that *C. elongatus* may become prominent following more successful control of *L. salmonis* as it is suspected that the two species may interact in a competitive fashion. This communication draws on new and reported findings to examine the evidence for such competition using a selection of epidemiological approaches.

MATERIALS AND METHODS

Data

Use has been made of the SULLepsiS data set (Revie et al., 2003). With the support of Defra and Scottish Quality Salmon, this data set was established as a research resource in collaboration with one of the major salmon producers in Scotland (Marine Harvest). Data on sea lice measurements arising from routine monitoring of salmon production cycles at over 30 farms located in Scottish lochs and coastal sites between 1996 and 2000 are stored in an Access database (Revie et al., 2002a). The database provides a record of counts of chalimus and adult stages for the two abundant species *L. salmonis* and *C. elongatus* found on samples of fish taken from cages throughout the two-year production cycles. Such information is routinely collected, according to an established protocol (Treasurer & Pope, 2000), in order to monitor fish health and to assess the need for the application of treatment and its impact. Concurrent data have also been established on site attributes (Revie et al., 2002b). These include farm management practices such as fallowing period, stocking policies, administration of treatments; and also key environmental factors such as water temperature, loch flushing times and salinity. During the five-year observational period, marketing authorisations were available for various veterinary treatments including the administration of hydrogen peroxide, dichlorvos and cypermethrin as bath treatments and, latterly, the in-feed administration of emamectin benzoate (Grant, 2002).

Analyses

Findings have been drawn from a selection of sources where detailed analyses have been reported on the epidemiological patterns of infection. These analyses have included plots of abundances over the two-year production cycles (Revie et al., 2002a), correlation analysis between species, time series plotting and modelling of counts observed in the years 1997 to 2000 (McKenzie et al., 2004), and regression models for the identification of abundance risk

factors (Revie et al., 2003). Further analyses are reported on the odds ratio of *L. salmonis* to *C. elongatus* counts per fish and the associated 95% confidence intervals in order to understand further the relationship between the co-existence of the two species.

RESULTS

Over the period 1996-2000, samples of around 90,000 salmon farmed in locations along the west coast of Scotland were examined for lice counts. These provided results on the abundance of both species, defined as the number of sea lice per fish (Bush et al., 1997).

Epidemiological Signatures

One of the most compelling arguments for different ecological behaviour of the *L. salmonis* and *C. elongatus* lice species and their interaction is the pattern of abundances across all sites and two-year production cycles. Figure 1 shows this pattern of infection for the two species on an 'average' farm over the 104 week period. In the case of *L. salmonis*, it can be seen that after limited activity over the first nine months (weeks 1 to 35), there is a steady, upwards climb in infection levels throughout the remainder of the cycle. This upward trend is variable due to a series of peaks and troughs in the presence of treatment interventions. However, in the case of *C. elongatus*, the pattern of abundance is similar and consistent in both the first and second years, with a distinctive rise in numbers around week 26 and a steady decline after week 37. Although the pattern of infection is similar for *C. elongatus* in both years, the numbers of *C. elongatus* observed per fish in the first year are higher. Clearly, the two species display very different infection patterns, providing distinctive epidemiological signatures for the two-year production cycle. It must be recognised that these are the overall national patterns and that there

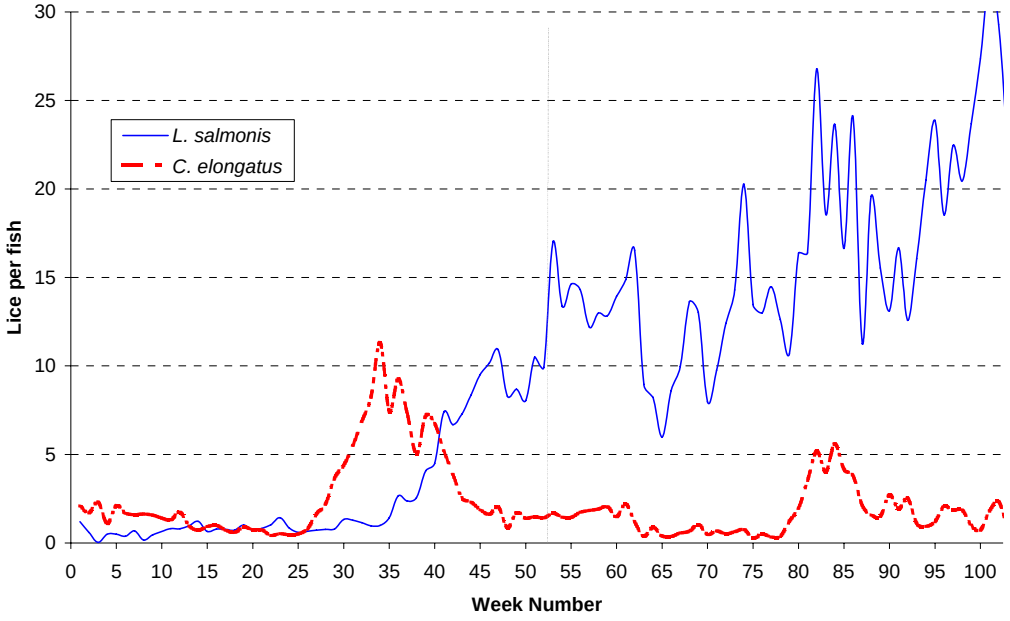


Fig. 1 Mean weekly abundance of mobile *C. elongatus* and *L. salmonis* over a typical two-year production cycle, based on 33 sites from 1996 to 2000

is considerable variation from one site to another due to local spatial and temporal effects (Revie et al., 2002c).

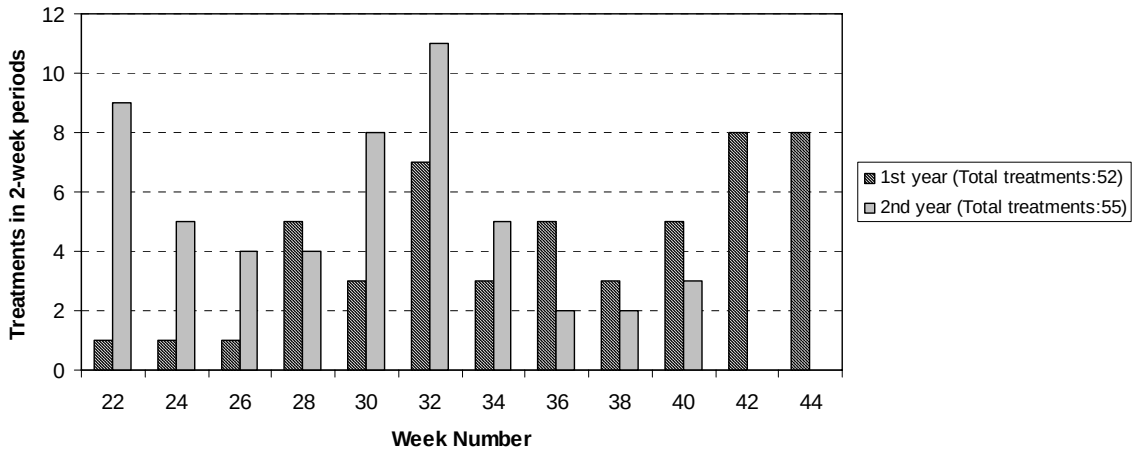


Fig. 2a Timing of treatment on 21 and 23 sites in their first and second year of production respectively between 1997 and 2000

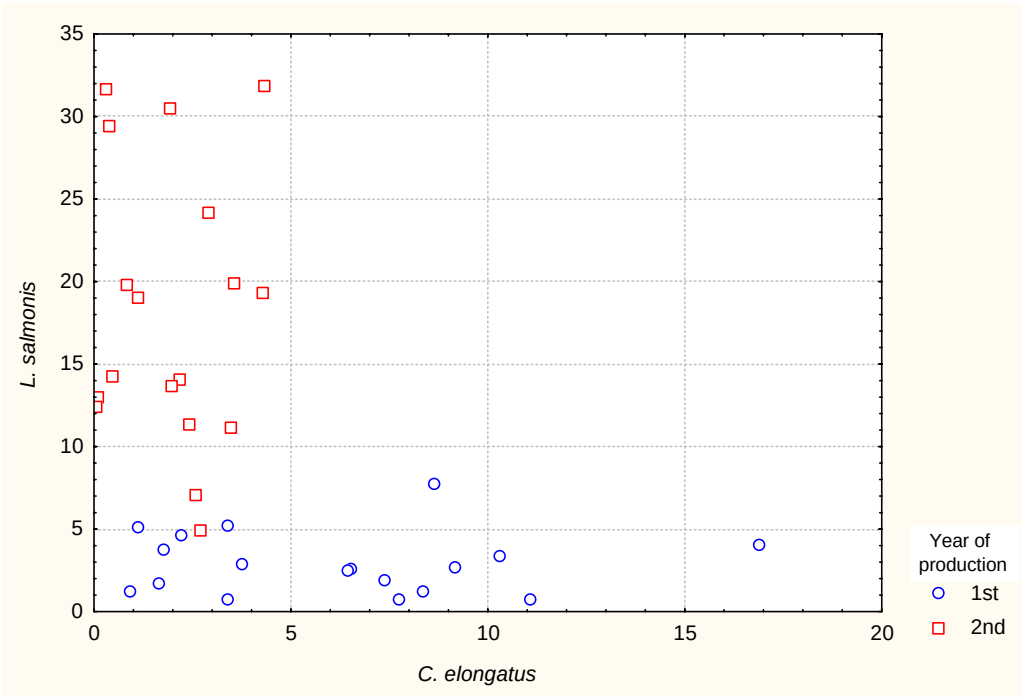


Fig. 2b Scatter plot of pairs of *L. salmonis* and *C. elongatus* mobile mean abundances averaged over the period from weeks 25 to 40 for those site-years receiving between 3 and 5 treatments in either the first or second year of production

Treatment Effects

Pike and Wadsworth (1999) suggested there was "*little evidence for the possibility*" that the two species were directly in competition for fish. The assumption has been that the increased

treatment levels in the second year of production for *L. salmonis*, the species which is predominantly targeted for control, also act to control *C. elongatus* more effectively, and that this is a sufficient explanation of the reduced level of infection in the second year.

This assumption was explored in a study which directly compared abundance levels of the two species in sites with roughly equivalent levels of treatment over a four-year period (Revie et al, 2002c). Specifically, any site receiving between three and five treatments in either the first or second years of production between 1997 and 2000 were compared. However, in response to a comment on the article in question, the timing of these treatments was investigated and exhibited the 'treatment profiles' for the first and second years as shown in Fig. 2a. Given that the abundance values reported were the mean levels over the weeks 25 to 40, when *C. elongatus* is known to be primarily present in Scotland, it was interesting to note that a fair proportion of the treatment in the first year fell after week 40, whilst this was not the case in the second year. This led to the question of whether the apparent inverse correlation reported based on 44 sites, as shown in Fig. 2b, would still hold when better matching of treatment took place.

To address the question of matched treatments more carefully, only the period of maximum *C. elongatus* infection was considered. Thus, treatment interventions in weeks 25 to 40 were reviewed and only sites having either 2 or 3 treatments within this Caligus window were included in the analysis. This led to treatment profiles which were much more similar across the two years (Fig. 3a). When looking at the abundance levels of the two species over the 47 sites which met these criteria, the scatter plot showed a very similar profile to that reported earlier as seen in Fig. 3b. Indeed, the correlation value (R=0.62) was higher than had previously been found, indicating that there is, indeed, evidence that the two species are inversely related.

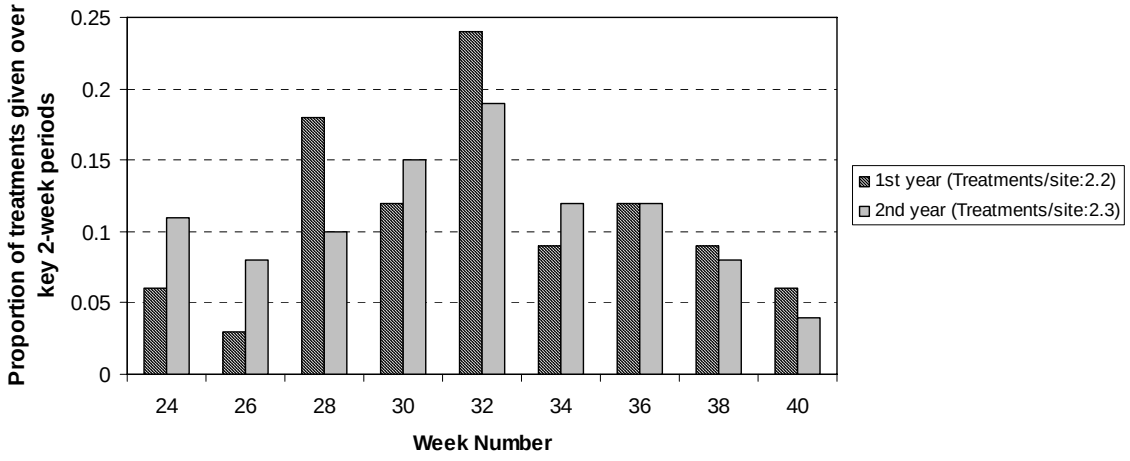


Fig. 3a Selection of 16 and 31 sites in their first and second year of production respectively, with similarly timed treatment profiles between 1997 to 2000

Time Series Analysis

Figure 4a shows the mean number of adult sea lice counts per fish, from fish routinely sampled between 1997 and 2000 across three sites in Loch Sunart on the west coast of Scotland.

On each site and at each of the sampling points, samples of 5 fish were typically inspected from each of a random selection of cages. The distinctive signature of the two species in response to the two-year production cycle, as illustrated in Fig. 1, can be seen. There are high counts for *L. salmonis* in each of the second years of the production cycle, 1998 and 2000; and low counts for the first years of the production cycle 1997 and 1999. The reverse is true for *C. elongatus*, as counts are low in 1998 and 2000 and high in 1997 and 1999.

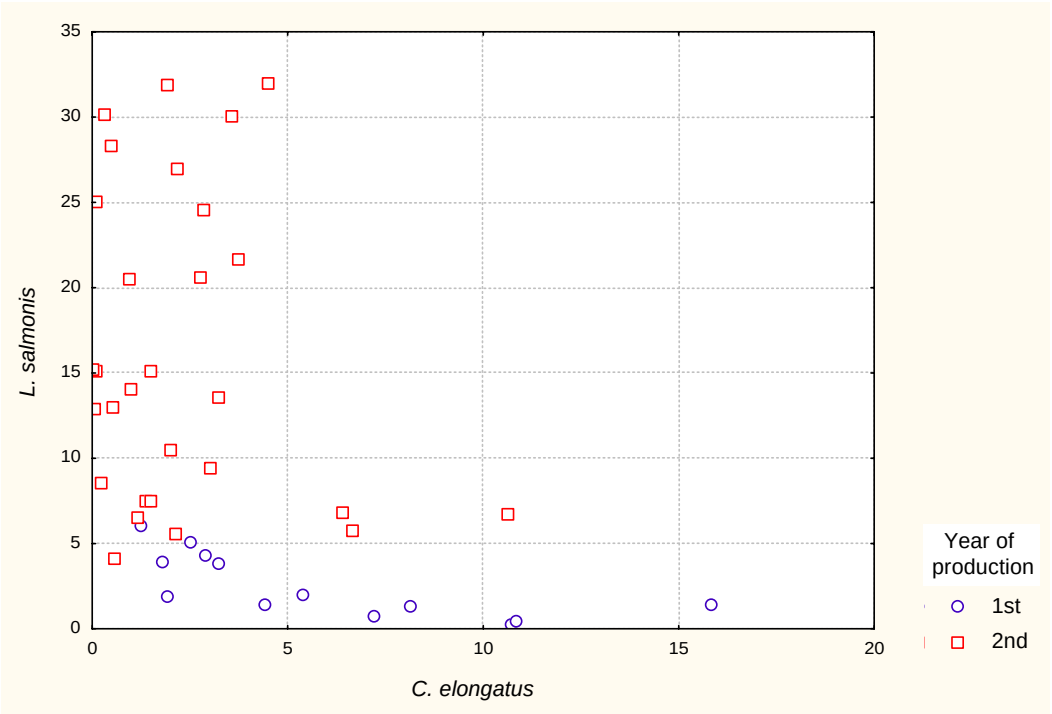


Fig. 3b Scatterplot of pairs of *L. salmonis* and *C. elongatus* mobile mean abundances averaged over the period from weeks 25 to 40 for those site-years receiving either 2 or 3 treatments over that period in the first or second year of production

Both species illustrate time series phenomena associated with secular trends, cyclical production variation and seasonal variation. A rigorous time series analysis has been undertaken for *C. elongatus* (McKenzie et al., 2004), as it exhibited less irregular variation, and it was found that counts could be satisfactorily described in mathematical terms using a combination of sine and cosine periodic functions. Furthermore, the inclusion of treatment as a covariate in the model did not explain any of the variation, suggesting that *C. elongatus* populations were not significantly influenced by the treatment regimens. The patterns for *L. salmonis* are much more irregular than those for *C. elongatus*, and, as yet, no satisfactory time series model has been discovered.

The asymmetrical presence of the two species can be seen more clearly from Fig. 4b, where the logarithm of the odds ratios of the *L. salmonis* to *C. elongatus* counts at each point in time and their associated 95% confidence intervals have been plotted. This plot clearly illustrates that *L. salmonis* is by far the dominant species in the second year of the production cycle, whereas *C. elongatus* is dominant in the first year. The plot reaffirms the observation that the two species are inversely related and do not easily co-exist, as illustrated in Figs. 2b and 3b. There is also

evidence of *C. elongatus* challenge fish each year, but the species is only able to remain in greater numbers in the first year of production when *L. salmonis* counts are low, and not in the second year of production when most fish are already infected by *L. salmonis*.

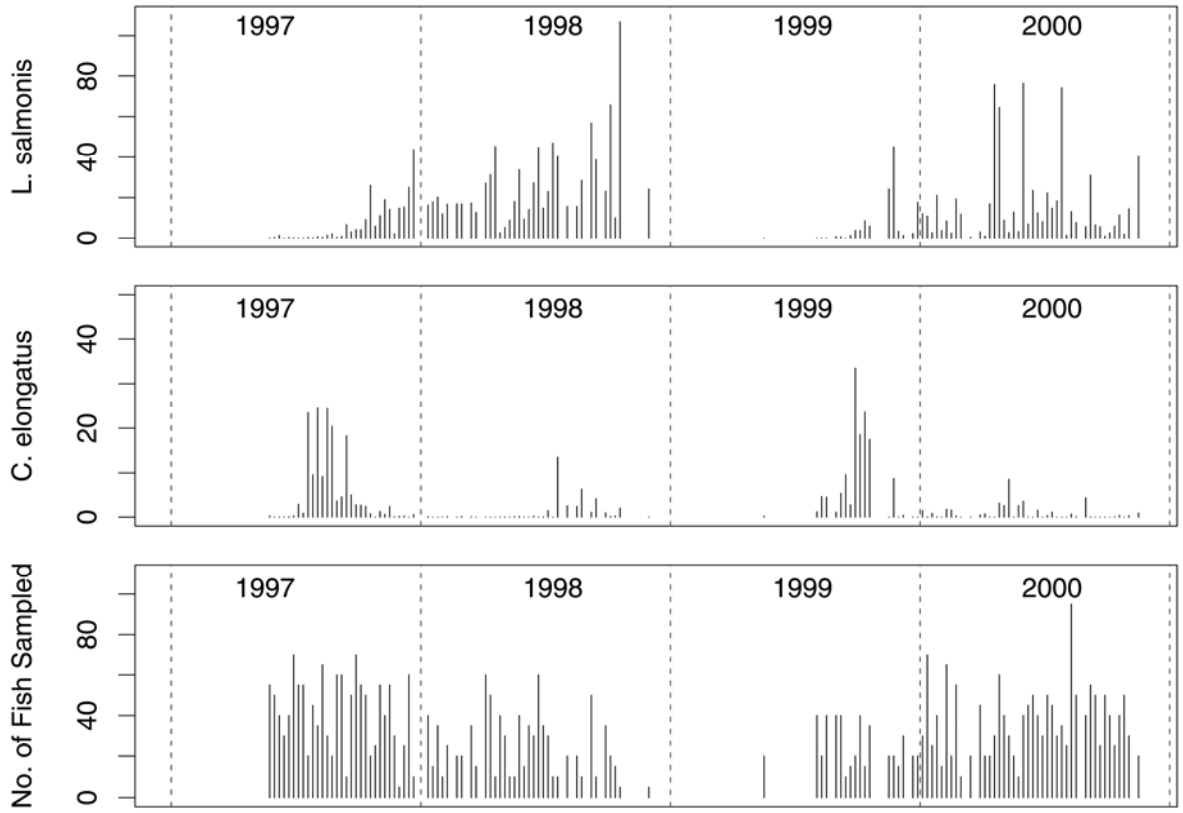
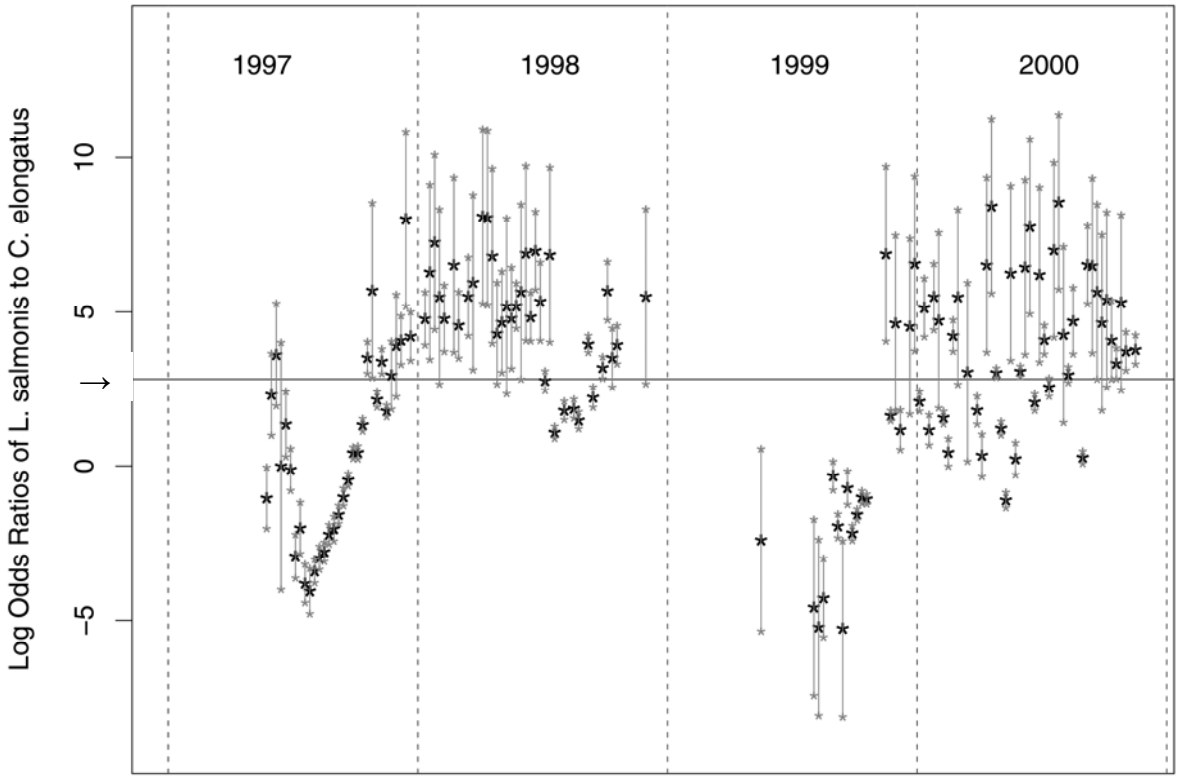


Fig. 4a Adult *L. salmonis* and *C. elongatus* counts per fish for Atlantic salmon routinely sampled in the Loch Sunart production cycles 1997-1998 and 1999-2000

Risk factors

A large number of factors have been considered to influence lice counts during the production cycle. Seawater temperature, salinity, wild fish, the proximity of neighbouring farms and other loch attributes have long been suspected of being key environmental risk factors, whereas stocking density, fallowing and net height are regarded as key management risk factors. The extent to which these factors individually and jointly contribute to the variation in lice infections is difficult to quantify. Revie et al. (2003) adopted a multiple linear regression approach, whereby logarithmically transformed adult *L. salmonis* lice counts, averaged over six-month periods on up to sixty production cycles between 1996 and 2000, were modelled for association. Although the best model could only explain around 70% of the total variation in abundance for the important first half of the second year of the production cycle, many suspected factors were ruled out.



→ Horizontal line at 2.8 represents the mean value of the Log Odds Ratios

Fig. 4b The logarithm of the odds ratio of the adult *L. salmonis* to *C. elongatus* counts at each point in time and their associated 95% confidence intervals for Atlantic salmon in the Loch Sunart production cycles 1997-1998 and 1999-2000.

Table 1. Key risk factors required in stepwise linear regression models to explain the differences in levels of *L. salmonis* and *C. elongatus* on sites in Scotland between 1996 and 2000

	L. salmonis	*C. elongatus*
Influences	Treatment level (+) Treatment type Loch flushing time (+) Current speed (-)	Biomass (-) Cage volume (-) Current speed (-) [Treatment type]
Does not influence	Biomass Increased fallowing Presence of neighbours Stocking density Strategic density	Treatment level Increased fallowing Presence of neighbours Strategic density

Those factors found to be significant correlates of risk ($p<0.05$) and those found not to influence levels ($p>0.05$) are listed in Table 1. This table also shows that when the analysis is applied to *C. elongatus* counts on the same production cycles, the set of risk factors obtained is not identical. In particular, the abundance of *C. elongatus* was best explained by density dependent factors biomass and cage volume, along with current speed all of which, when increased, decreased the abundance. In the case of *L. salmonis*, biomass and cage volume were not risk factors, although treatment level and type were. The appearance of treatment as a risk factor for *L. salmonis* is not surprising since treatment is applied to reduce levels of this species and is not principally targeted towards *C. elongatus*.

DISCUSSION

Several hypotheses can be proposed from the results obtained.

First, *L. salmonis* and *C. elongatus* have distinctive epidemiological signatures as evidenced by the pattern of abundance during the two year production cycles. These patterns show that the presence of one species typically diminishes the presence of the other with *C. elongatus* being the more prevalent species in the first year when fish are small and *L. salmonis* in the second year when fish are approaching their adult weight.

Secondly, taking care to use sites matched on a comparable number of treatments and over the period of *C. elongatus* challenge, lice abundances of the two species on fish sampled contemporaneously during the course of the production cycle are found to be strongly inversely correlated.

Thirdly, time series analysis and odds ratios of the abundance of one species to another suggest that *L. salmonis* is the dominant species, and its presence modulates the presence of *C. elongatus*.

Fourthly, the identification of risk factors suggests that *C. elongatus* is influenced by density-dependent factors, whereas *L. salmonis* is not, and that variation in *L. salmonis* abundance is not influenced by the abundance of *C. elongatus*.

These findings are consistent with *C. elongatus* being an annual species which takes its opportunity to parasitise salmon in the absence of *L. salmonis*, but less so when *L. salmonis* is abundant. Once *L. salmonis* becomes established in sufficient numbers on fish, the interaction between the two species is such that *C. elongatus* is unable to compete.

The relationship between the two species could be made clearer if data were available for sites which either excluded the presence of *L. salmonis* or *C. elongatus*. In particular, it would be interesting to know if in the absence of *L. salmonis*, the abundance of *C. elongatus* diminishes in the second year of the production cycle. This, and the observed regularity of the time of challenge of the *C. elongatus* i.e. weeks 24 to 40, would be consistent with annual challenges arising from seasonal migrant wild fish populations. Alternatively, it may be possible to conduct tank-based experiments to study the potential species interaction. This has been carried out for two parasitic copepods which parasitise turbot, with some evidence of asymmetrical competition (Dawson et al., 2000). However, if there is a ‘density’ effect, the numbers of lice required for infection may raise fish welfare issues. In the case of the study involving turbot, it was found that the generalist parasite (in the present case *C. elongatus*)

demonstrated a greater sensitivity to competition than did the specialist copepod (here *L. salmonis*).

It is recognised that the results presented here are open to other interpretations but, as they stand, they are consistent with there being evidence of inter-species interaction between sea lice in Scottish salmon farms. Such an inter-species interaction could have a bearing on selection for resistance. If *C. elongatus* species are not directly targeted, contact with chemical treatments that are not totally effective could select for resistance. Moreover, any treatment regimens which can be highly effective in controlling *L. salmonis*, such as the increasing use of emamectin, may provide an opportunity for *C. elongatus* to be become more prominent. It is important that future Scottish strategies recognise that these species do interact and that sea lice control should focus on both *L. salmonis* and *C. elongatus*.

ACKNOWLEDGEMENTS

The work which forms the basis of this paper has been supported by Defra research grants ENV12 and VM02134, and the collaboration of the industrial partner Marine Harvest (Scotland) and Scottish Quality Salmon is gratefully acknowledged.

REFERENCES

Bristow, G.A. and Berland, B. (1991). A report on some metazoan parasites of wild marine salmon (*Salmo salar* L.) From the west coast of Norway with comments on their interactions with farmed salmon. Aquaculture. 98, 311-318

Bron, J.E., Sommerville, C., Wootten, R. and Rae, G.H. (1993). Influence of treatment with dichlorvos on the epidemiology of *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* (Nordmann, 1832) on Scottish salmon farms. In: Boxshall GA, Defaye D (eds) Pathogens of wild and farmed fish: sea lice. Ellis Horwood, Chichester, UK, pp 263-274

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. J. Parasitol. 83, 575-583

Dawson, L.H.J., Renaud, F., Guégan, J.F. and de Meeûs, T. (2000). Experimental evidence of asymmetrical competition between two species of parasitic copepods. Proc. R. Soc. Lond. B. 267, 1973-1978

Grant, A.N. (2002). Medicines for sea lice. Pest. Man. Sci. 58, 521-527

Grant, A.N. and Treasurer, J.W. (1993). The effects of fallowing on caligid infestations on farmed Atlantic salmon (*Salmo salar* L.) in Scotland. In: Boxshall GA, Defaye D (eds) Pathogens of wild and farmed fish: sea lice. Ellis Horwood, Chichester, UK, pp 255-260

Heuch, P.A., Revie, C. and Gettinby, G. (2003). A comparison of epidemiological patterns of salmon lice (*Lepeophtheirus salmonis*) infections in Norway and Scotland. J. of Fish Dis. 26, 539-551

Hogans, W.E. and Trudeau, D.J. (1989). Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtus* and *Lepeophtheirus salmonis* (Copepoda: Caligoida) parasitic on cage-cultured salmonids in the lower Bay of Fundy. Canadian Technical Report of Fisheries and Aquatic Sciences 1715, 14pp

Johnson, S.C. and Albright, L.J. (1991). Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda:Caligidae) under laboratory conditions. J. Mar. Biol. Assoc. UK. 71, 425-436

Kabata, Z. (1979). Parasitic Copepoda of British Fishes. The Ray Society, London, UK. 468p

McKenzie, E., Gettinby, G., McCart, K. and Revie, C.W. (2004). Time series models of sea lice *Caligus elongatus* (Nordmann) abundance on Atlantic salmon *Salmo salar* L. in Loch Sunart, Scotland. Aqua. Res. 35, 764-772

Pike, A.W. and Wadsworth, S.L. (1999). Sealice on salmonids: their biology and control. Adv, Parasitol, 44, 233-337

Rae, G.H. (1979) On the trail of the sea lice. Fish Farmer 2:6, 22-25

Revie, C.W., Gettinby, G., Treasurer, J.W., Grant, A.N. and Reid, S.W.J. (2002a). Sea lice infestation on Atlantic salmon and the use of ectoparasitic treatments on commercial farms in Scotland. Vet. Rec. 151, 753-757

Revie, C.W., Gettinby, G., Treasurer, J.W., Rae, G.H. and Clark, N. (2002b). Temporal, environmental and management factors influencing the epidemiological patterns of sea lice (*Lepeophtheirus salmonis*) infestations on farmed Atlantic salmon (*Salmo salar* L.) in Scotland. Pest. Man. Sci. 58, 576-584

Revie, C.W., Gettinby, G., Treasurer, J.W., Grant, A.N. and Rae, G.H. (2002c). The epidemiology of the sea lice, *Caligus elongatus* Nordmann, in marine aquaculture of Atlantic salmon, *Salmo salar* L., in Scotland. J. of Fish Dis. 25, 391-399

Revie, C.W., Gettinby, G., Treasurer, J.W. and Wallace C. (2003). Identifying epidemiological factors affecting sea lice (*Lepeophtheirus salmonis*) abundance on Scottish salmon farms using general linear models. Dis. of Aqua. Org., 57, 85-95

Treasurer, J.W. and Grant, A.N. (1994). 'Second' louse species must not be ignored. Fish Farmer 17:4, 46-47

Treasurer, J.W. and Pope, J.A. (2000). Selection of host sample number and design of a monitoring programme for ectoparasitic sea lice (Copepoda: Caligidae) on farmed Atlantic Salmon. Aquaculture 187, 247-260

Tully, O. (1989). The succession of generations and growth of the caligid copepods *Caligus elongatus* and *Lepeophtheirus salmonis* parasitising farmed Atlantic salmon smolts (*Salmo salar* L.). J. Mar. Biol. Assoc. UK. 69, 279-287

Wootten, R., Smith, J.W. and Needham, E.A. (1982). Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. Proc. Roy. Soc. Edin. 81b, 185-197

STOCHASTIC SIMULATION OF LIVE FISH MOVEMENT IN ENGLAND AND WALES TO PREDICT POTENTIAL SPREAD OF EXOTIC PATHOGENS

M. THRUSH[1] AND E. PEELER

SUMMARY

To assist in contingency planning for exotic salmonid disease outbreaks, a stochastic model was developed to assess the role of live fish movement in the potential geographical distribution of an introduced pathogen with time to first detection. The CEFAS-Environment Agency Live Fish Movement Database (LFMD) was used to provide details of live salmonid movements that occurred throughout England and Wales in 2002. Probability functions were used to model the timing and destination of movements between fish farms and fisheries based on their previous trading activities. Monte Carlo simulations were run to track the progression of potential disease transmission through river catchments with time. In 5% of simulations, 63 or more catchments (of a total 198) were contacted and, in 1% of simulations, 75 or more catchments were contacted after 12 months. In future, this model will contribute to the development of risk-based disease surveillance by identifying farm sites and river catchments most at risk of infection.

INTRODUCTION

The UK is free of a number of the most serious salmonid diseases that are widespread in Europe, notably viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and the monogenean parasite, *Gyrodactylus salaris*. The protection of farmed and wild salmonids depends on effective contingency plans for the elimination of exotic diseases should an outbreak occur. The movement of live animals is frequently the most important route of spread, especially long distance spread, for an introduced disease (Anderson, 2002; Davies, 2002). A qualitative risk analysis has identified the anthropogenic movement of live Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) as the most important route for the transmission of *G. salaris* between river catchments (Peeler et al., 2004). The main aquaculture species in England and Wales are rainbow trout and brown trout (*Salmo trutta*), which are farmed on over 260 freshwater sites in 64 of a total 198 river catchments (Fig. 1). Considerable numbers of live fish movements occur between farm sites (mainly juvenile fish sold for on-growing) and from farm sites to recreational fisheries or open waters (non-farm sites) for restocking. Data on live fish movements provide a vital resource for forward and backward contact tracing in the event of a disease outbreak and also make a valuable resource for a predictive model of live fish movement.

[1] Mark Thrush, Centre for Environment, Fisheries & Aquaculture Science (CEFAS), Barrack Road, The Nothe, Weymouth DT4 8UB, UK. Email: m.a.thrush@cefas.co.uk

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) is the competent authority for the control of notifiable fish diseases in England and Wales. All fish farming businesses are registered with CEFAS and farmers have a legal obligation to keep records of all movements of live fish and gametes on and off their sites and to make this information available to CEFAS Fish Health Inspectors on mandatory visits. Movements to recreational fisheries or open waters for restocking require consent from the Environment Agency (EA) under Section 30 of the Salmon and Freshwater Fisheries Act, 1975. The Live Fish Movement Database (LFMD) is maintained by CEFAS to manage all data relating to fish farm registration, fish imports and exports, and rearing of non-native fish species. The details of farm to farm and farm to non-farm fish movements are logged on the LFMD by CEFAS and the EA, respectively.

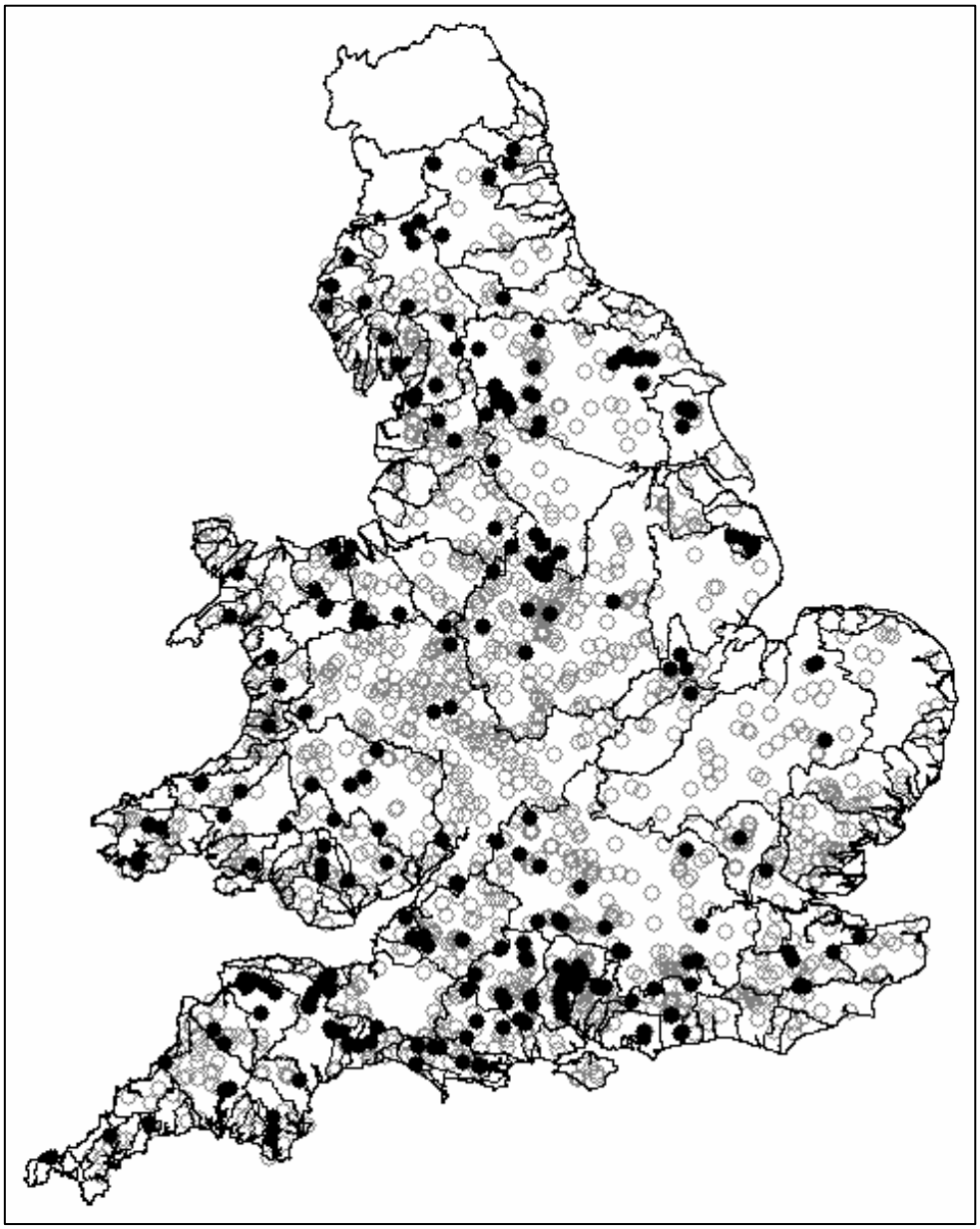


Fig. 1 Distribution of salmonid fish farm sites (filled circles) and non-fish farm sites (fisheries and open waters, open circles) among 198 river catchments in England and Wales

Mathematical disease modelling is a rapidly expanding area of human and veterinary epidemiology and examples of its use include: assessment of the global impact of AIDS (May & Anderson, 1987); the design of childhood mass immunisation programmes (Nokes & Anderson, 1988) and assessment of control strategies for outbreaks of classical swine fever (CSF) in the Netherlands (Jalvingh et al., 1999; Klinkenberg et al., 2003). Animal disease modelling was extensively used to assess competing strategies for the control of foot-and-mouth disease (FMD) during and after the 2001 epidemic in the UK (Green & Medley, 2002; Keeling et al., 2001; Woolhouse et al., 2001). The application of mathematical modelling to aquatic animal health, although historically limited to theoretical population studies (Clers, 1993; Levy & Wood, 1992), has more recently been used to quantify the impact of bacterial kidney disease (BKD) on chinook salmon (*Oncorhynchus tschawytscha*) in North America (Hamel, 2002). No published papers have attempted to model live fish movements as a route of disease spread in aquatic animals.

In this study, the movement relationships between the farm sites registered for salmonid production (rainbow trout, brown trout and Atlantic salmon) in England and Wales were established. Stochastic modelling techniques were used to provide input to an ongoing epidemiological assessment of the likely spread of introduced pathogens through the movement of live fish.

MATERIALS AND METHODS

The LFMD was interrogated by standard structured query language (SQL) reports to determine all destinations of live fish movement in 2002 from each of the 261 farms registered on the database in April 2004 for holding stocks of salmonid fish. The results were exported to Excel® spreadsheets (Microsoft® Corporation, Seattle, WA), transformed into standardised arrays and combined to provide a movement relationship matrix.

A stochastic model was developed to project a single site-to-site contact scenario over a 52-week period within a MS-Excel spreadsheet. For the purpose of the simulation, binomial distribution functions were used to determine when each site made a fish movement (for example, the sale of fish to another site) and destinations for movements were predicted using site-unique discrete-probability distribution equations generated directly from the movement relationship matrix.

Simulations were achieved using @Risk® (Palisade Corporation, Newfield, NY), a risk analysis software add-on for MS-Excel. The sampling method was Latin hypercube and a total of 522,000 iterations were run using individual exporting sites (index sites) seeded with a positive status (generating 2610 scenarios for each of 200 exporting sites). Positive status was passed to naïve sites by forward movement contact from the index site through successive weeks of the scenario. Additional contacts were generated by movements from any farm subsequently acquiring positive status, either directly from the index site, or via one or more intermediaries. This process was automated by a Visual Basic® (Microsoft® Corporation, Seattle, WA) control macro, which substituted a different farm as the index site at the start of each new iteration. On completion of each iteration, a separate macro was used to append the contact status of all farm sites, non-farm destinations and their corresponding river catchments at 3, 6, 9 and 12 months to output files.

RESULTS

Live fish were transported from 200 farm sites registered for the production of salmonid fish to a total of 1653 freshwater destinations (farm and non-farm) in England and Wales in 2002. The distribution of river catchments contacted during simulation after 3 and 12 months are shown in Figures 2 and 3 respectively. The median number of catchments contacted after 3 and 12 months were 16 and 53, respectively. In 5% of simulations, 63 or more catchments were contacted and, in 1% of simulations, 75 or more catchments were contacted after 12 months. The worst-case scenarios, predicted by the model, were 31 and 104 catchments contacted after 3 and 12 months respectively.

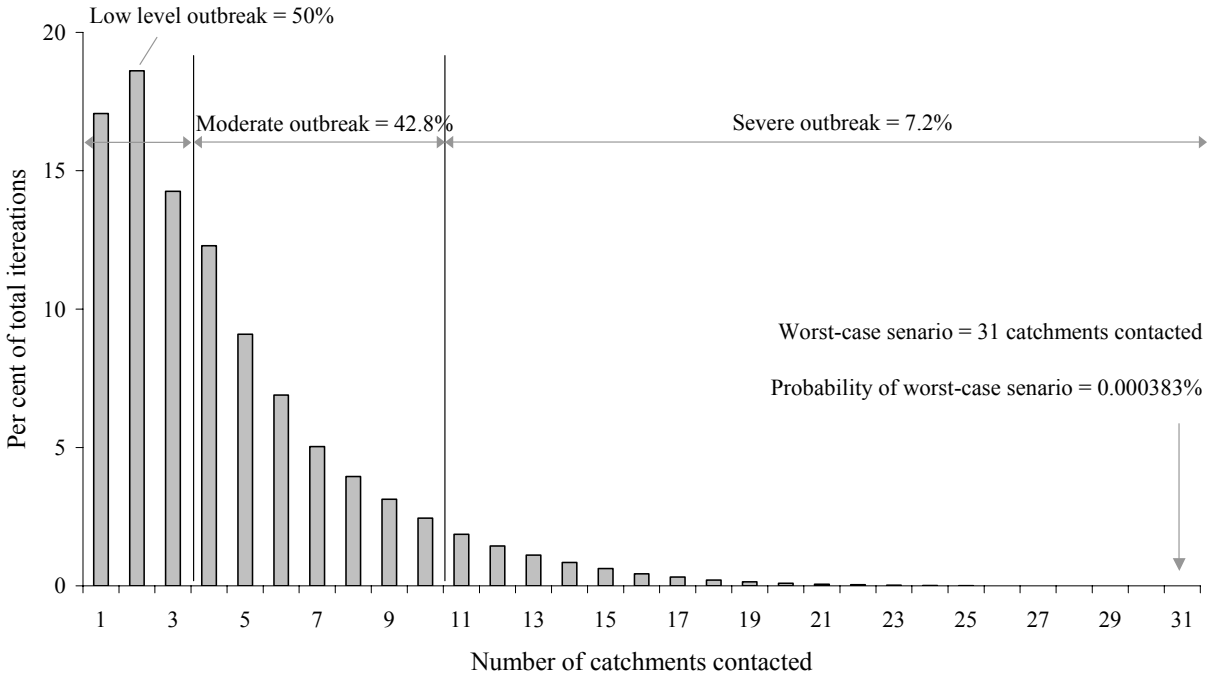


Fig. 2 Distribution of river catchments contacted after 3 months from single index farm inputs

A framework of outbreak categories has been overlaid on the catchment contact distributions in Figures 2 and 3. Degrees of severity were assigned to outbreak scenarios as low, moderate and severe for the involvement of up to 3, 4-10 and 11 or more catchments respectively. Assuming an introduced disease remains undetected for 3-months, the risk of a severe outbreak (spreading to more than 10 river catchments) was 7%. However, if the disease was to go unnoticed for a year, this risk increased to nearly 90%.

River catchments were ranked in order of the likelihood of the sites within them receiving consignments of live fish from other farm sites. The Severn, Trent and Thames river catchments were identified by simulation to be the most likely to receive consignments of live fish. Details of the 10 catchments at highest risk of disease introduction are presented in Table 1.

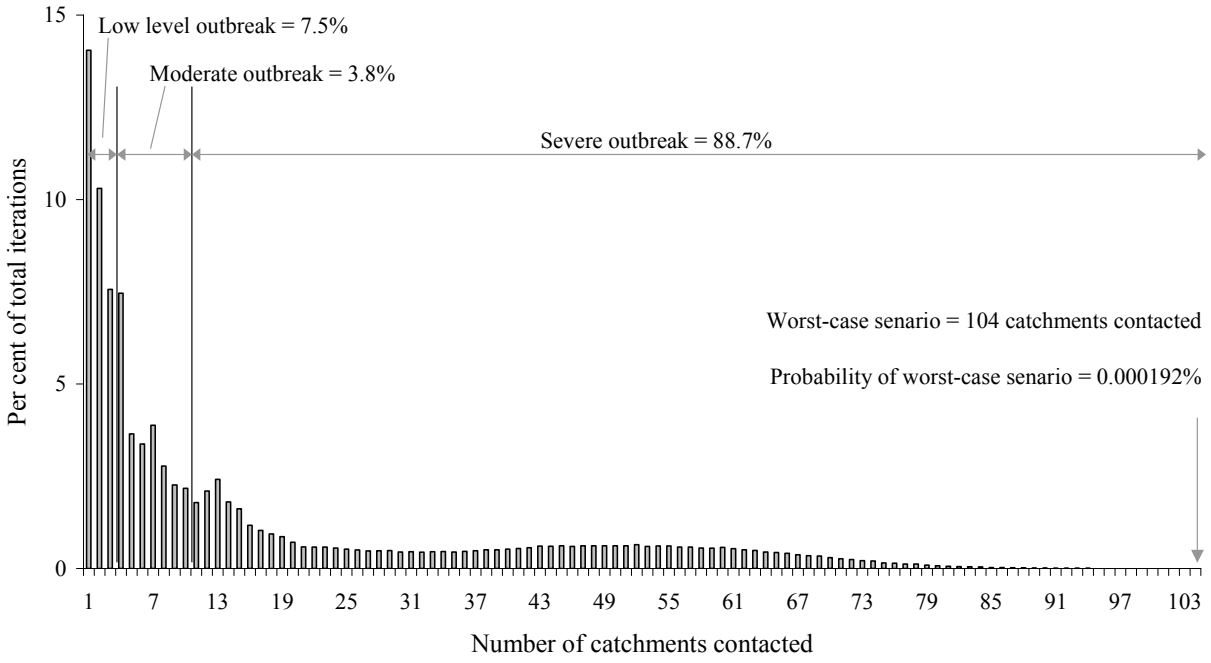


Fig. 3 Distribution of river catchments contacted after 12 months from single index farm inputs

Table 1. The top 10 (of a total 198) river catchments ranked by risk of acquiring potential infection by live fish movement

Rank	River Catchment	Total Received Contacts (Adjusted)[a]	Relative Position[b]
1	Severn	219747	100
2	Trent	219470	99.9
3	Thames	200894	91.4
4	Dee	173179	78.8
5	Avon (Bristol)	169749	77.2
6	Great Ouse	166187	75.6
7	Ribble	159217	72.5
8	Avon (Hants.)	153570	69.9
9	Mersey	152349	69.3
10	Parrett	149745	68.1

[a] Output adjusted to remove index-case seeding
[b] Risk relative to catchment at highest risk

DISCUSSION

This work is the first attempt to assess quantitatively the likely spread of an exotic fish pathogen within England and Wales via the movement of live salmonid fish. Previous trading

activities were used to establish a relationship matrix between farm and non-farm sites and model the potential spread of disease by live fish movement following the introduction of an exotic salmonid pathogen. The results clearly demonstrate that, unless a disease is detected and diagnosed quickly, and measures to prevent spread are implemented, a multi-focal outbreak involving many river catchments is highly likely. The results provide conservative estimates of the number of sites and catchments affected, since the model only considered one route of transmission – the anthropogenic movement of live fish. No attempt has been made to simulate either the spread of disease within catchments (e.g. by water currents or migration of wild fish) or other routes of transmission between catchment (e.g. movement of vehicles and people between sites) that may be important for some diseases. The frequency of movement between sites is not currently captured by the LFMD, therefore, it is not possible to predict seasonality in live fish movement, or to bias contacts towards more frequently supplied customers.

For many diseases, live fish movement has proved the most important route of spread. Trade in live fish and their gametes has resulted in the spread of fish pathogens within and between countries. For example, white spot virus (WSV) was rapidly spread by the international movement of shrimp larvae (mainly *Penaeus monodon*) and has resulted in the near collapse of the industry in a number of countries (Hill, 2002). *Gyrodactylus salaris* and furunculosis were introduced into Norway by the import of salmon smolts from Sweden (Mo, 1994) and Scotland (Egidius, 1987) respectively. The parasitic nematode *Anguillicola crassus* was introduced into Germany with the importation of Asiatic eels (*Anguilla japonicus*) from Taiwan (Koops & Haartmann, 1989) and subsequent spread through Europe is attributable largely to anthropogenic movement of European eels (*Anguilla anguilla*) for farming and restocking (Kirk, 2003). Long distance spread of infectious salmon anaemia (ISA) in farmed salmon in Scotland was mainly due to movement of live fish in well-boats (Murray et al., 2002). The large-scale movement of farmed fish in the England and Wales, between farms and from farms for restocking rivers and still-waters, provides a route for the rapid dissemination of an introduced exotic disease. A stochastic modelling approach has previously been taken to assess quantitatively the risk of transferring *G. salaris* between specific sites (Høgåsen & Brun, 2003; Paisley et al., 1999), but to our knowledge this is the first detailed predictive study of the potential distribution of disease on a national scale by live fish movement.

River catchments are areas joined by continuous freshwater flow, within which pathogens can disseminate on currents and are carried by wild or feral fish. In the case of *G. salaris*, disease spread within a catchment is practically impossible to control (Johnsen & Jensen, 1991) and, therefore, the catchment may be regarded as a discrete epidemiological unit. For this reason, management of a disease outbreak must largely operate at the catchment level, to minimise spread of infection between catchments. In Norway, *G. salaris* is also believed to have been transmitted by the migration of wild salmonids between catchments through regions of low salinity (Høgåsen & Brun, 2003). This route was ranked as the third most important for the spread of *G. salaris,* after anthropomorphic moments of salmonids and other species of live fish respectively by Peeler et al. (2004). Neighbouring catchments with shared estuaries may consequently need to be regarded as single units. The role of wild fish in the dissemination of viral diseases, including VHS and IHN, over long distances is likely to be less important, compared with *G. salaris*, and thus smaller, sub-catchment, epidemiological units may be appropriate for improved, disease specific models in the future. This could be achieved by combining elements of contact structure and geographical proximity.

The model's results show that the time to diagnosis of an exotic or emerging disease in the UK will be a critical factor in determining its geographical spread before control and eradication

efforts can be initiated. *Gyrodactylus salaris* causes significant mortalities in pre-smolt Atlantic salmon and has resulted in a 98% decline in infected river catchments in Norway over a time-scale of 2-5 years (Johnsen & Jensen, 1991; Mo, 1994). The parasite infects and completes its life cycle on rainbow trout at a much lower prevalence, causing no signs of clinical disease and is likely to go unnoticed in a farmed trout population. If introduced in to the UK, this parasite is unlikely to be detected until significant outbreaks in wild salmon stocks have occurred or population declines have been identified by survey, which could take up to a year or more.

For other exotic diseases time to first detection is likely to be shorter. For example, an outbreak of VHS or IHN in a farmed fish stock, reared at relatively high density and under close supervision is likely to be detected relatively quickly, and, ideally, farmed (rainbow) trout would act as sentinels for diseases affecting wild salmonid fish. However, although time to first mortality in a farmed population may be short, definitive diagnosis of an exotic pathogen may take much longer. Testing for exotic viral diseases would be unlikely until the involvement of endemic bacterial diseases with similar clinical signs (for example enteric red mouth (ERM) or rainbow trout fry syndrome (RTFS)) have been eliminated (possibly following failure to respond to antibiotic treatment, and subsequent resistance testing). Time to diagnosis of an outbreak of one of these viruses is therefore theoretically unlikely to be less than 3-weeks. Experience in Canada has shown that, in practice, time to diagnosis of IHN (determined by retrospective epidemiological investigation) following initial mortality may take several months (St-Hilaire et al., 2002). In addition, the impact of both VHS and IHN are temperature dependent. At relatively low temperatures (less than 12 ºC) both diseases cause outbreaks in farmed rainbow trout populations with high mortalities (Bootland & Leong, 1999; Smail, 1999). However, mortality is greatly attenuated at temperatures above 14 ºC, so the diagnosis of an outbreak occurring in the summer may be further delayed by a number of weeks.

There are two classes of model that may be developed for the prediction of disease spread: tactical and strategic. A tactical model is primarily used 'in peace time' in the absence of a disease to provide theoretical data that may, for example, be used for contingency planning. A strategic model is run in the face of a real outbreak and may be used to compare control strategies and thus inform decision-making (e.g. the UK FMD outbreak in 2001). This work will inform the contingency planning process for the introduction and control of exotic aquatic pathogens in England and Wales. Effective contingency plans must be based on realistic scenarios and, importantly, include a worst-case scenario. This work indicates that contingency plans for *G. salaris* control in England and Wales should be based on multi-focal outbreaks and wide geographical spread on first detection. The UK FMD epidemic in 2001 highlighted the need for planning to include a worst-case scenario. Prior to this episode, the FMD contingency plan was based a maximum of 10 sites becoming infected before the detection and identification of the disease (Anderson, 2002). In the event, 57 farms became infected prior to identification (Gibbens et al., 2001). The model developed in this paper predicted a worst-case scenario affecting more than 104 catchments for a disease that may go undetected for a year. Contingency planning for notifiable fish disease introduction must ensure that a response will accommodate the worst-case scenario disease outbreak. The worst case scenario would effect more than 50% of the salmonid farming industry in England and Wales and would clearly have a massive impact on the wild salmon population, combined trout and salmon rod and net fisheries (valued at £751 million in 2001, Anon., 2004) and associated secondary leisure and tourist revenue.

Future developments of the model will include the incorporation of disease specific transmission and infection characteristics and the modelling of local transmission routes (for

example, from infected farm populations to susceptible wild stocks in neighbouring rivers). These developments should create a model that can be used to compare alternative disease control strategies. In addition, network theory, which has recently been applied to examining the contact structure in terrestrial livestock industries (Bigras-Poulin et al., 2004; Webb & Sauter-Louis, 2004), has potential to improve further our understanding of the contact structure of the fish farming industry and needs to be assessed.

Results from the model have allowed us to rank catchments by their likelihood of receiving a potentially infected consignment of live fish during an outbreak. Despite its limitations, the live fish movement model described in this paper provides outputs that form a sound basis on which to base contingency planning and risk-based surveillance for exotic notifiable fish diseases. It is a an important first step in the modelling of aquatic disease spread on a national basis and provides a vital component of a future, more comprehensive, disease transmission model.

ACKNOWLEDGEMENTS

This work was funded by the Department for Environment, Food and Rural Affairs (Defra, contract FC1150).

REFERENCES

Anderson, I. (2002). Foot-and-Mouth 2001: Lessons to be learnt inquiry report. The Stationery Office, London. 187p

Anon (2004). Our nations' fisheries, the migratory and freshwater fisheries of England and Wales - a snapshot. The Environment Agency, Almondsbury, Bristol. 98p

Bigras-Poulin, M., Thompson, A., Chriel, M., Mortensen, S. and Griener, M. (2004). Network analysis of the Danish cattle and swine industry trade patterns as an evaluation of risk potential for disease spread: the heterogeneity issue. In: Society for Veterinary Epidemiology and Preventive Veterinary Medicine, Martigny, Switzerland. 189-199

Bootland, L.M. and Leong, J.C. (1999). Infectious haematopoietic necrosis virus. In: Fish Diseases and Disorders. Vol 3, Viral, Bacterial and Fungal Infections, Ed. Woo, P.T.K. and Bruno, D.W., CABI, Wallingford, Oxon. 57-121

Clers, S. (1993). Modelling the impact of disease-induced mortality on the population size of wild salmonids. Fish. Res. 17, 237-248

Davies, G. (2002). The foot and mouth disease (FMD) epidemic in the United Kingdom 2001. Comp. Immunol. Microbiol. Infect. Dis. 25, 331-343

Egidius, E. (1987). Import of furunculosis to Norway with Atlantic salmon smolts from Scotland. International Council for Exploration of the Sea report. ICES CM 1987/F:08

Gibbens, J.C., Sharpe, C.E., Wilesmith, J.W., Mansley, L.M., Michalopulou E. and Ryan, J.B.M. (2001). Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. Vet. Rec. 149, 729-743

Green, L.E. and Medley, G.F. (2002). Mathematical modelling of the foot and mouth disease epidemic of 2001; strengths and weaknesses. Res. Vet. Sci. 73, 201-205

Hamel, O.S. (2002). The dynamics and effects of bacterial kidney disease in Snake River spring chinook salmon (*Oncorhynchus tshawytscha*). Diss. Abst. Int. Pt. B - Sci. Eng. 62, 5446

Hill, B.J. (2002). National and International Impacts of white spot disease of shrimp. Bull. Eur. Assoc. Fish Pathol. 22, 58-65

Høgåsen, H.R. and Brun, E. (2003). Estimating the risk of inter-river transmission of *Gyrodactylus salaris* by migrating Atlantic salmon smolts using Monte Carlo simulation. Dis. Aquat. Org. 57, 247-254.

Jalvingh, A.W., Nielen, M., Maurice, H., Stegeman, A.J., Elbers, A.R.W. and Dijkhuizen, A.A. (1999). Spatial and stochastic simulation to evaluate the impact of events and control measures on the 1997-1998 classical swine fever epidemic in The Netherlands. I. Description of the model. Prev. Vet. Med. 42, 271-295

Johnsen, B.O. and Jensen, A.J. (1991). The *Gyrodactylus* story in Norway. Aquaculture 98, 289-302

Keeling, M.J., Woolhouse, M.E.J., Shaw, D.J., Mattthews, L., Chase-Topping, M., Haydon, D.T., Cornell, S.J., Kappey, J., Wilesmith, J.W. and Grenfell, B.T. (2001). Dynamics of the 2001 foot-and-mouth epidemic: stochastic dispersal and a hetergenous landscape. Science 294, 812-817

Kirk, R.S. (2003). The impact of *Anguillicola crassus* on European eels. Fisheries Manag. Ecol. 10, 385-394

Klinkenberg, D., Everts-van de Wind, A., Graat, E.A.M. and de Jong, M.C.M. (2003). Quantification of the effect of control strategies on classical swine fever epidemics. Math. Biosci. 186, 145-173

Koops, H. and Haartmann, F. (1989). Anguillicola infestations in Germany and in German eel imports. J. Appl. Ichthyol. 1, 41-45

Levy, D.A. and Wood, C.C. (1992). Review of proposed mechanism for sockeye salmon population cycles in Fraser River. Bull. Math. Biol. 54, 241-262

May, R.M. and Anderson, R.M. (1987). The transmission dynamics of HIV infection. Nature 326, 137-142

Mo, T.A. (1994). Status of *Gyrodactylus salaris* problems and research in Norway, In: Lewis, JW (Ed.) Parasitic diseases of fish. Samara Publishing, Tresaith, Dyfed, Wales, 43-48

Murray, A., Smith, R. and Stagg, R. (2002). Shipping and the spread of infectious salmon anaemia in Scottish aquaculture. Emerg. Infect. Dis. 8, 1-5

Nokes, D.J. and Anderson, R.M. (1988). The use of mathematical models in the epidemiological study of infectious diseases and in the design of mass immunization programmes. Epidemiol. Infect. 101, 1-20

Paisley, L.G., Karlsen, E., Jarp, J. and Mo, T.A. (1999). A Monte Carlo simulation model for assessing the risk of introduction of *Gyrodactylus salaris* to the Tana river, Norway. Dis. Aquat. Org. 37, 145-152

Peeler, E.J., Gardiner, R. and Thrush, M.A. (2004). Qualitative risk assessment of routes of transmission of the exotic fish parasite *Gyrodactylus salaris* between river catchments in England and Wales. Prev. Vet. Med. 64, 175-189

Smail, D.A. (1999). Viral haemorrhagic septicaemia. In: Fish Diseases and Disorders. Vol 3, Viral, Bacterial and Fungal Infections, Ed. Woo, P.T.K. and Bruno, D.W., CABI, Wallingford, Oxon. 123-147

St-Hilaire, S., Ribble, C.S., Stephen, C., Anderson, E., Kurath, G. and Kent, M.L. (2002). Epidemiological investigation of infectious hematopoietic necrosis virus in salt water net-pen reared Atlantic salmon in British Columbia, Canada. Aquaculture 212, 49-67

Webb, C.R. and Sauter-Louis, C.M. (2004). Investigations into the contact structure of the British sheep population. In: Society for Veterinary Epidemiology and Preventative Medicine, Martingy, Switzerland. 10-20

Woolhouse, M.E.J., Chase-Topping, M., Haydon, D.T., Friar, J., Matthews, L., Hughes, G., Shaw, D.J., Wilesmith, J.W., Donaldson, A., Cornell, S.J., Keeling, M.J. and Greenfell, B.T. (2001). Foot-and-mouth disease under control in the UK. Nature 411, 258-259

DIAGNOSTIC TESTS

TEST CHARACTERISTICS OF A PCR FOR *STREPTOCOCCUS SUIS* DEFINED BY BAYESIAN ANALYSIS

B. SWILDENS[1], B. ENGEL, A.J. STEGEMAN AND M. NIELEN

SUMMARY

The tonsils of 471 live sows from 4 herds were sampled with a swab. Sampled material from these swabs was subjected to a recently developed polymerase chain reaction (PCR), and enzyme-linked immunosorbent assays (ELISA) were also performed. After slaughter, the whole tonsils were collected. Material from these tonsils was subjected to the PCR and the ELISA and bacterial examination (BE) was also performed. No test could be considered the gold standard and a Bayesian analysis was performed to interpret test results.

In the final model, sensitivities (Se) of the PCR on swabs and tonsils were estimated at 0.63 and 0.88 respectively, Se of BE on tonsils at 0.65 and the specificity of all tests at around 0.96. The Se of a repeated swabbing procedure was higher than that of the first swab alone (0.85).

In this Bayesian analysis, BE on tonsils, which is commonly regarded as the gold standard, appeared to be a less sensitive method to sample sows than PCR on tonsils. Herd sampling protocols can now be designed and studies on the dynamics of infection within a herd can be implemented.

INTRODUCTION

Streptococcus suis is a pathogen associated with important economic losses in the swine industry. The diseases in which *S. suis* is involved are diverse, the most important being meningitis, arthritis, septicaemia and endocarditis. Although the pathogenesis of the infection is largely unknown, some characteristic features of the *S. suis* strains have been proposed as virulence factors. These include the capsular polysaccharides, the extracellular proteins muramidase-released-protein (MRP) and extracellular factor (EF), adhesins and a haemolysin. In most European countries, as well as in the United States and Australia, MRP and EF positive strains of the capsular serotype 2 are most frequently isolated from diseased pigs (Wisselink et al., 2000).

It was shown that pigs can carry *S. suis* on their tonsils without clinical signs for a very long time. These healthy carriers, especially sows, are considered the most important source of infection for susceptible young pigs (Clifton Hadley et al., 1984; Robertson & Blackmore,

[1] Bas Swildens, Department of Farm Animal Health, Faculty of Veterinary Medicine, Yalelaan 7, 3584 CL Utrecht, The Netherlands, E-mail: b.swildens@vet.uu.nl

1989). Detection of these carriers can contribute to the development of effective control measures.

Bacteriological techniques are routinely used to detect *S. suis* from clinical samples (Marois et al., 2004). Various other laboratory techniques have been developed. In addition to bacteriological examination (BE), two tests that were based on distinct biological characteristic features of the EF-positive *S. suis* serotype 2 strains were also used in this study. One test was an ELISA (Vecht et al., 1993) that isolates the EF in the supernatant of samples grown overnight and the other test was a PCR for the detection of the *epf*-gene of the EF-positive *S. suis* strains. This PCR demonstrated good sensitivity and specificity on tonsillar specimens when compared with BE of the same specimens (Wisselink et al., 1999). However, no test characteristics, such as sensitivity (Se) and specificity (Sp), of this PCR combined with sampling methods in live pigs had yet been determined.

To sample live sows for EF-positive *S. suis* serotype 2 strains, tonsil biopsies and tonsil swabs can be used, while the whole tonsil is available after slaughter. Biopsies are reported to be less sensitive compared to swabs, probably because *S. suis* tends to group together in some crypts of the tonsil, and these may be missed by a biopsy.

In the current study, the test characteristics of a PCR, an ELISA and a BE for the detection of EF-positive *S. suis* serotype 2 strains in tonsillar swabs and whole tonsils were assessed without a gold standard. A Bayesian analysis was used to determine sensitivity, specificity and repeatability of the PCR on tonsillar swabs.

MATERIALS AND METHODS

Sample collection

Four hundred and seventy one sows selected for slaughter were sampled from four farrow-to-finish farms. At these farms, weaned piglets suffered from clinical problems related to EF-positive *S. suis* serotype 2 strains. Sows were not medicated before or during the sampling period. In the waiting area of the slaughterhouse, the sows were caught and restrained using a nose snare. An iron wedge was pushed between the teeth. The swab (a sterilised 40 cm plastic stalk with a cotton wool tip of 5 cm) was inserted through the 5 cm round hole in the middle of the wedge, and light was provided with a torch. Both tonsils were swabbed during a sampling period of 10 seconds, while turning the swab between forefinger and thumb. In order to calculate the repeatability of the tonsil-swab procedure, randomly selected sows were swabbed a second time, 10 minutes after the first. Sows were slaughtered within one hour after tonsil swabbing, and both whole tonsils were collected.

Sample handling

Directly after sampling, tonsillar swabs and tonsils were transported to the laboratory. Tonsillar swabs were transported in Todd-Hewitt broth. Whole tonsils were transported in individual plastic bags on ice.

At the laboratory, swab samples were grown in the transport medium for 18 hours at 37°C. Whole tonsils were processed into homogenates as described by Wisselink et al.(1999) and 15% glycerol was added. All samples were stored in 2 ml Greiner cryo tubes at -70°C.

The PCR, BE and ELISA

The PCR assay and the preceding DNA preparations were performed as described by Wisselink et al. (1999). *Streptococcus suis* strains were isolated from the tonsillar specimens (bacteriological examination, BE) as described by Wisselink et al.(1999).

In order to calculate the repeatability of the PCR-assay, PCR analysis (including the preceding DNA preparations from the stored samples) was repeated on 299 randomly selected samples, equally distributed over positive- and negative Swab-PCR and Tonsil-PCR samples. Three hundred BEs were performed on the tonsil homogenates (Tonsil-BE); 152 of these 300 were randomly selected from Tonsil-PCR positive samples, whereas 148 out of these samples were randomly selected from Tonsil-PCR negative samples.

The detection of EF, produced by *Streptococcus suis* serotype 2 strains in the supernatant of samples that were grown in the Todd-Hewitt broth for 18 hours at 37°C, was performed with a Double Antibody Sandwich Enzyme-Linked Immuno Sorbent Assay (DAS-ELISA) as described by Vecht et al. (1993).

Statistical analysis

The combination of a sampling method and an isolation technique was regarded as one complete test. This led to five tests: Swab-PCR, Swab-ELISA, Tonsil-PCR, Tonsil-ELISA and Tonsil-BE. The values of Se and Sp of the five tests were determined by performing a Bayesian analysis using the Markov Chain Monte Carlo (MCMC) algorithm as implemented in the WinBUGS package (Spiegelhalter et al., 2003). Results are reported assuming conditional positive dependence between the PCR and BE, because a PCR assay was included in both tests.

The prior probability distributions for the Se of Tonsil-PCR and Tonsil-BE were based on the work of Wisselink et al. (1999). Further information about the Se of the Tonsil-BE based on studies of others (Davies & Ossowicz, 1991; Moreau et al., 1989) was inconclusive. Therefore, probability distributions that allowed for values on a wide interval (0.01 – 0.99) were assumed for all Se parameters. More constraining prior probability distributions were considered valid for the Sps of Swab-PCR and Tonsil-PCR, because of the good Sp of the PCR in a validation study by Wisselink et al. (1999). These distributions emphasized on values larger than 0.5. The Sp of Tonsil-BE was assumed to be greater than 0.73, because the combination of slide agglutination, hybridisation and PCR in this test were likely to result in a high Sp (Vecht et al., 1985; Wisselink et al., 1999). Herd-prevalence was calculated in a pilot study and, in the herds involved, was found to be at least 0.15 and at the most 0.80. Consequently, a distribution with equal probabilities between 0.01 and 0.99 for all herd-prevalences was a cautious assumption.

The study also involved two ELISA tests: Swab-ELISA and Tonsil-ELISA. These measurements were not binary but continuous. Various cut-off points for these tests were defined and used as binary ELISA results in the joint Bayesian analysis of all 5 tests. For both tests, normal prior probability distributions that allow for values on a wide interval (0.01 – 0.99)

were assumed. The ELISA tests performed poorly and were eventually discarded from the final model (Engel et al., 2005).

The final model included four farm prevalences, three analytical tests and parameters for dependencies between tests, all provided with the most plausible priors. The repeated swabbing procedure (second-Swab-PCR), and duplicated PCRs on the same samples were analysed by adding these data as a fourth analytical test to the final model.

RESULTS

Sensitivity and Specificity

Using Bayesian analysis, the calculated values for Se, Sp and 95% credibility intervals of the tests are summarised in Table 1. In the final model three tests were added: Swab-PCR and Tonsil-BE had a Se of 0.63 and 0.65, respectively; Tonsil-PCR had a Se of 0.88. Specificities of all tests were around 0.96.

Table 1. Sensitivities, specificities and 95% credibility intervals of Swab-PCR, Tonsil-PCR and Tonsil-BE in the Bayesian analysis

Test	Sensitivity (95% credibility interval)	Specificity (95% credibility interval)
Swab-PCR	0.63 (<0.52, 0.74>)	0.96 (<0.92, 0.99>)
Tonsil-PCR	0.88 (<0.75, 0.96>)	0.94 (<0.87, 0.99>)
Tonsil-BE	0.65 (<0.51, 0.76>)	0.97 (<0.91, 0.99>)

Repeatability of the swabbing procedure

The repeatability of the tonsil swab procedure was intermediate, with a kappa of 0.63 (Table 2A). Nineteen samples were negative in the first Swab-PCR and positive in the second Swab-PCR, whereas only 3 samples were positive in the first and negative in the second.

In the Bayesian analysis with the second Swab-PCR added as a fourth additional test, the Se of the second Swab-PCR was 0.85 (<0.67, 0.96>), greater than the first Swab-PCR, and the Sp was 0.93 (<0.84, 0.98>), similar to that of the first Swab-PCR (Table 1).

Repeatability of the PCR-assay

The results of the repeated PCR test on the same sample were very similar to the first PCR as indicated by a kappa of 0.81 for tonsillar swabs and of 0.82 for whole tonsil specimens (Table 2B and 2C). This agreement was also found when the repeated PCR test (on the same sample) was added as a fourth additional test in the Bayesian analysis (results not shown).

Table 2. Repeatability of Swabbing-procedure and PCR-assay: Cross tabulation of the results of Swab-PCR versus the second-Swab-PCR (A), first PCR-assay versus repeated PCR-assay on the same swab (B), first PCR-assay versus repeated PCR-assay on the same tonsil (C).

A		Swab-PCR	
		+	-
Second-Swab-PCR	+	29	19
	-	3	104
Kappa		0.63	

B		First PCR	
On swabs		+	-
Repeated-PCR	+	65	5
	-	9	70
Kappa		0.81	

C		First PCR	
On tonsils		+	-
Repeated-PCR	+	71	8
	-	4	67
Kappa		0.82	

The ELISA

In the plots of Swab- and Tonsil-ELISA-titres versus the other tests, no comparable distributions of positive and negative samples were apparent, except that some 6% of the animals showed markedly increased values for Tonsil-ELISA. These results tended to agree in all five tests (samples assumed to be positive). Although the test characteristics for Swab- and Tonsil-ELISA seemed reasonable in the Bayesian joint analysis with the other three tests, herd prevalences were unrealistically low and specificities for Swab- and Tonsil-PCR, and especially Tonsil-BE, showed a severe downward bias compared to the analysis without the ELISA tests (results not shown). Moreover, the receiver operating characteristic (ROC) curves (Greiner et al., 2000) (not shown) obtained from the Bayesian analyses corresponding to the chosen cut-off points showed that the two ELISA tests performed poorly.

Herd prevalence

The estimated prevalences (with 95% credibility intervals) in the four herds in the final model were 0.31 (0.23, 0.39), 0.34 (0.20, 0.48), 0.57 (0.42, 0.74) and 0.70 (0.53, 0.86).

As stated above, separate analyses with the two ELISA tests produced unrealistically low herd prevalences ranging from lowest 0.05 (0.01, 0.12) to highest 0.16 (0.07, 0.29).

DISCUSSION

The Se and Sp of a method to detect live sows carrying EF-positive *S. suis* serotype 2 strains by tonsillar swabbing and PCR were calculated. The Se of Swab-PCR was 0.63 (<0.52, 0.74>) and Sp was 0.96 (<0.92, 0.99>) (Table 1). The characteristics of four other test methods were also estimated by Bayesian analysis.

The Bayesian approach has the advantage over traditional statistical methods, which use gold standard comparisons, that more precise calculations can be made with the use of assumptions about the parameters based on scientific information combined with all test results. For example, in this study, the Se and Sp of Tonsil-BE were calculated to be 0.65 (<0.51; 0.76>) and 0.97 (<0.91, 0.99>) respectively. This indicates that Tonsil-BE cannot be used the as gold standard without taking its less-than-perfect test characteristics into account. The lower Se of

Tonsil-BE compared to Tonsil-PCR 0.88 (<0.75; 0.96>) might be due to less effective bacterial growth after storage of Tonsil-BE-samples compared to the growth before storage of PCR-samples. Moreover, even if bacteria die during storage, DNA will still be available for PCR.

The Tonsil-PCR showed a higher Se compared to the Swab-PCR: 0.88 (<0.75; 0.96>) and 0.63 (<0.52, 0.74>), respectively. The Se of the second-Swab-PCR (0.85 (<0.67, 0.96>)) also tended to be higher than that of the first Swab-PCR. In both cases, this may be due to the harvesting of more bacteria out of deep crypts and adjacent lymphoid tissue than from the surface of the tonsils of carrier sows (Arends et al., 1984; Williams et al., 1973), or of some other unknown time dependent process. Further research to unravel the underlying mechanisms of this phenomenon might demonstrate ways to improve Se of the Swab-PCR.

Specificities of all three tests for the EF-positive strains were calculated to lie around 0.96 (Table 1). The sampling method did not seem to have an effect on the Sp of the PCR. False positive results will not be problematic if the sows are sampled more frequently, as a result of the fact that, in the present study, dependence between tests in non-carriers was small (results not shown).

The agreement between results of the PCR-assay and its repeat on the same sample were excellent, as indicated by a kappa of 0.81 (Table 2B and 2C), which was also concluded from the Bayesian analysis. This suggests that differences in Swab-PCR results mainly reflect differences in the amount of bacteria on the tonsils, the sampling procedure and the sample processing before the PCR-assay.

Diagnostic tests are directed at certain aspects of a disease. The positive results for Swab- and Tonsil-ELISA in this study were associated with a small fraction of samples with very high EF concentrations in the supernatant and were quite different from the distribution of Swab- and Tonsil-PCR and Tonsil-BE results. This could be because the PCR is directed towards detection of the *epf*-gene encoding for the EF protein, whereas the ELISA detects the protein itself. The estimated low sensitivities of the ELISAs are then possibly due to the fact that the EF is not always produced even if the *epf*-gene is present.

IMPLICATIONS

The results from this study provide clear evidence for the possibility of testing live sows in a simple and repeatable manner for carriage of EF-positive *S. suis* serotype 2 strains. Transmission studies can now be performed at sow farms by tonsil swabbing and the *epf*-gene isolation by PCR. The Tonsil-PCR is easier and cheaper than Tonsil-BE and therefore certification of negative herds through sow sampling at the slaughterhouse becomes feasible.

ACKNOWLEDGEMENTS

The authors thank Henk Wisselink, Helmi Fijten, Dick van de Wiel and Willem Buist of the Animal Science Group at Lelystad for their work on bacterial examinations and assistance in performing the statistical calculations.

REFERENCES

Arends, J. P., Hartwig, N., Rudolphy, M. and Zanen, H. C., (1984). Carrier rate of *Streptococcus suis* capsular type 2 in palatine tonsils of slaughtered pigs. J Clin Microbiol. 20, 945-947

Clifton Hadley, F. A., Alexander, T. J., Enright, M. R. and Guise, J., (1984). Monitoring herds for *Streptococcus suis* type 2 by sampling tonsils of slaughter pigs. Vet. Rec. 115, 562-564

Davies, P. R. and Ossowicz, C. J., (1991). Evaluation of methods used for detecting *Streptococcus suis* type 2 in tonsils, and investigation of the carrier state in pigs. Res Vet Sci. 50, 190-194

Engel, B., Swildens, B., Stegeman, J. A., Buist, W. and De Jong, M. C., (2005). Estimation of sensitivity and specificity of three conditionally dependent diagnostic tests in the absence of a gold standard. In press

Greiner, M., Pfeiffer, D. and Smith, R. D., (2000). Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med. 45, 23-41

Marois, C., Bougeard, S., Gottschalk, M. and Kobisch, M., (2004). Multiplex PCR assay for detection of *Streptococcus suis* species and serotypes 2 and 1/2 in tonsils of live and dead pigs. J Clin Microbiol. 42, 3169-3175

Moreau, A., Higgins, R., Bigras Poulin, M. and Nadeau, M., (1989). Rapid detection of *Streptococcus suis* serotype 2 in weaned pigs. American-Journal-of-Veterinary-Research. 50, 1667-1671

Robertson, I. D. and Blackmore, D. K., (1989). Prevalence of *Streptococcus suis* types 1 and 2 in domestic pigs in Australia and New Zealand. Veterinary-Record. 124, 391-394

Spiegelhalter, D. J., Thomas, A., Best, N. G. and Lunn, D., (2003). User Manual, WinBUGS, Version 1.4. MRC Biostatics Unit, Cambridge

Vecht, U., van Leengoed, L. A. and Verheijen, E. R., (1985). *Streptococcus suis* infections in pigs in the Netherlands (Part I). Vet Q. 7, 315-321

Vecht, U., Wisselink, H. J., Anakotta, J. and Smith, H. E., (1993). Discrimination between virulent and nonvirulent Streptococcus suis type 2 strains by enzyme-linked immunosorbent assay. Vet Microbiol. 34, 71-82

Williams, D. M., Lawson, G. H. and Rowland, A. C., (1973). Streptococcal infection in piglets: the palatine tonsils as portals of entry for *Streptococcus suis*. Res Vet Sci. 15, 352-362

Wisselink, H. J., Reek, F. H., Vecht, U., Stockhofe-Zurwieden, N., Smits, M. A. and Smith, H. E., (1999). Detection of virulent strains of *Streptococcus suis* type 2 and highly virulent strains of *Streptococcus suis* type 1 in tonsillar specimens of pigs by PCR. Vet Microbiol. 67, 143-157

Wisselink, H. J., Smith, H. E., Stockhofe-Zurwieden, N., Peperkamp, K. and Vecht, U., (2000). Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. Vet Microbiol. 74, 237-248

ESTIMATING TEST ACCURACY AND PREDICTIVE VALUES FOR THE DANISH *SALMONELLA* DUBLIN SURVEILLANCE PROGRAMME IN DAIRY HERDS

L.D. WARNICK [1], L.R. NIELSEN, J. NIELSEN AND M. GREINER

SUMMARY

The Danish government and cattle industry instituted a *Salmonella* surveillance programme in October 2002 to help reduce the spread of *S.* Dublin. All dairy herds are tested by measuring antibodies in bulk tank milk and beef herds are tested using blood samples. The programme is based on well-established tests, but the overall test programme accuracy and misclassification had not been investigated. The objective of this study was to estimate misclassification in the *S.* Dublin surveillance programme in Danish dairy herds. Although the programme focus is *S.* Dublin, for this study, herds with *S.* Typhimurium were also considered to be infected, as cross-reactions may occur in the milk antibody test.

Simulation models were used to estimate the probability of testing positive for herds with each of 16 infection history patterns, e.g. infected previous four quarters, infected for three quarters and then non-infected in the most recent quarter etc. These results were then used in a second simulation step to estimate herd-level sensitivity, specificity and predictive values for a range of assumed herd-level prevalences.

For a true herd-level prevalence of infection of 15%, the simulation model estimated the test programme sensitivity (HSe) as 0.96 and specificity (HSp) as 0.93 for detecting herds with at least 5% of animals that would test positive on individual faecal culture or antibody ELISA. At 15% herd-level prevalence, approximately 99% of herds classified negative would be truly non-infected and 72% of herds classified positive would be truly infected. These predictive values were consistent with the stated goal of the programme, which is to be able to have confidence that herds classified as likely to be free of infection truly are not infected.

INTRODUCTION

Salmonella infections are of concern in livestock production systems because of potential transmission to humans and effects in animals. In addition to the public health impact of zoonotic infections, livestock morbidity, mortality and the resulting economic losses are substantial for certain strains. *Salmonella enterica* subsp. e*nterica* serotype Dublin is a host-adapted serotype in cattle which occasionally infects other species (McDonough, et al., 1999). Though relatively infrequently associated with human salmonellosis, *S.* Dublin may cause invasive infections in people resulting in serious illness and death. Among patients hospitalized

[1] Lorin D. Warnick, Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853 USA. Email: LDW3@cornell.edu

with salmonellosis, *S.* Dublin causes higher mortality than other serotypes (Helms, et al., 2003). *Salmonella* Dublin has received high priority in Danish cattle for several reasons. Human infections continue to occur with between 25 and 45 cases per year reported from 2001-2003 (Anon, 2004). In some countries, transmission to humans may occur after consumption of improperly pasteurized milk products, but in Denmark the main source is probably insufficiently cooked meat (Fierer, 1983; Helms, et al., 2003; Humphrey, et al., 2000). In cattle herds, *S.* Dublin causes economic losses in the form of disease and death among calves and young animals, as well as abortions and reproductive disorders among adult cattle, extra labour and increased veterinary expenses (Hinton, 1974; Peters, 1985; Visser, et al., 1997).

In an effort to control *S.* Dublin in cattle, the Danish government and cattle industry initiated a National Surveillance Programme for *Salmonella* Dublin in October 2002. In this programme, all cattle herds are classified into one of three *Salmonella* infection levels according to antibody measurements in bulk tank milk or blood samples, faecal culture results and movement of animals between herds. Test results and animal trade data are recorded in the Danish Cattle Database. The programme is regulated by the Danish Veterinary and Food Administration and administered by the Danish Cattle Federation.

Salmonella programme classification levels are calculated each quarter for dairy herds. Level 1 is considered most likely to be free of *Salmonella* Dublin. Dairy herds are classified as Level 1 if the average ODC% (background corrected optical density value from the ELISA) of the last 4 bulk tank milk measures is below 25 and no increase of more than 20 ODC% is found when comparing the last measurement to the average of the three previous measurements. Levels 2 and 3 are divided into two sub-levels. Level 2a includes herds with bulk tank antibody measurements which exceed either of the ELISA test cut-off values described above. Herds in Level 2b are not classifiable due to lack of data or have had registered contact with 2a or 2b herds. Such contact could include purchase of cattle or contact via common pasture, markets, dealers or shows. Herds in level 3a have had bacteriologically diagnosed clinical salmonellosis due to *S.* Dublin, and level 3b have had the bacteria detected by culture, but clinical salmonellosis is not necessarily present, or the herd has had contact with cattle from a level 3a or 3b herd.

The stated goal of the surveillance programme is to make sure the herds classified as most likely to be free of infection (Level 1) would provide a safe source of purchased animals with regard to transmission of *S.* Dublin infection. In other words, the negative predictive value for these herds should be very close to 100%. When the programme was developed, it was acknowledged that with available diagnostic tools for salmonella infections, it would not be possible to obtain a high positive predictive value for herds classified as likely to be infected (Level 2a or higher). However, estimates of the accuracy and predictive values in the current programme were not available. The objective of this study was to estimate the herd-level sensitivity, specificity and predictive values of the Danish *Salmonella* Dublin surveillance programme in dairy cattle.

MATERIALS AND METHODS

A risk analysis approach was used to estimate misclassification in the *Salmonella* Dublin surveillance programme for Danish dairy farms. This method was selected to make efficient use of existing data sets and because of the cost of alternative methods, such as field studies. The bulk tank ELISA test currently used in the surveillance programme cannot distinguish *S.* Dublin

infections from *S.* Typhimurium and possibly other serotypes (Konrad, et al., 1994). Therefore, the working definition of herd infection for the current project was expanded to include infection with *S.* Typhimurium and other cross-reacting serotypes. Strictly speaking, these infections may result in misclassification in the surveillance programme, but the practical effect is to expand the control programme to other *Salmonella* serotypes.

A simulation model was used to estimate herd-level test accuracy and predictive values. The model was developed in two modules. Module I was designed to estimate the probability of testing positive conditional on disease history. Data from a previous field study (Kongeå project) were used to estimate the distributions of bulk tank milk ELISA values for infected and non-infected herds. In these herds, all animals had been tested repeatedly with bacteriological culture of faecal samples and serology over a period of at least 15 months and, therefore, constitute a well-characterised sample of herds with regard to infection status (Nielsen, 2003; Nielsen & Ersbøll, 2004; Nielsen, et al., 2004). Distributions estimated from the herds were then used to simulate repeated quarterly bulk tank milk ELISA values and calculate level classification for herds at various times relative to infection. Module II estimated the overall test accuracy and predictive values based on assumed values for true prevalence. The herd-level predictive values were calculated using distributions of infection history patterns calculated from prevalence and average herd-level duration of infection.

Module I

The surveillance testing programme uses four bulk tank milk ELISA measurements from samples collected at quarterly intervals. Using repeated measurements over time and the nature of the antibody response makes it reasonable to expect that the test accuracy will depend on the time of sample collection relative to the onset of infection or to recovery from infection in the herd. Therefore, herd-level test accuracy was estimated by initially estimating the probability of testing positive separately for groups of herds representing 16 infection histories (infected yes/no for four consecutive quarters). A quarter of a year was selected as the time unit of interest because, 1) tests are usually performed quarterly and, 2) significant herd infection is assumed to have a relatively long duration, i.e. months to years. Patterns representing four quarters were chosen because the herd classification is normally based on measurements from four consecutive quarters. With two states and four time periods, there were 16 possible sequences of infection and non-infection.

A model was developed in @Risk version 4.5 to simulate bulk tank milk ELISA results for each of 16 four-quarter disease history patterns. The inputs for this model were the test cut-off values, parameters for distributions of bulk tank milk ELISA ODC% for infected and non-infected herds, and the rank correlation coefficients for repeated measurements within herd.

The distributions for the infected and non-infected herds were investigated using data from 30 herds enrolled in the Kongeå project. Herds were classified as infected at a particular sampling period if the within-herd apparent prevalence among individual cattle was greater than 5%. Within-herd apparent prevalence was calculated as the percentage of cattle in the herd with either isolation of *Salmonella enterica* by bacteriological culture of a faecal sample or an ELISA measurement of 50 ODC% or above for individual-animal serum or milk samples. Herds classified as non-infected had no faecal culture positive samples. The faecal culture test and individual-animal ELISAs used for this classification have been evaluated elsewhere (Nielsen & Ersbøll, 2004; Nielsen, et al., 2004). Bulk tank milk ELISA measurements taken within +/- 20

days of the within-herd prevalence estimate were used to estimate the ELISA result distributions for infected and non-infected herds. A bootstrap sampling method was used to estimate the distributions of the parameters needed for the simulation model. One hundred samples were drawn with one observation per herd selected for each sample (n=9 per sample for uninfected and n=23 per sample for infected herds). The sample sizes for each sample corresponded to the number of herds in the uninfected and infected categories. Two herds which changed from non-infected to infected during the observation period were classified as non-infected before the switch and as infected afterwards. The mean and standard deviation were calculated for each of the 100 samples from the infected herds and the mean for the 100 samples from non-infected herds. (Only one parameter was evaluated for the non-infected group because this was modelled using an exponential distribution in the simulation).

Spearman's rank correlation of repeated bulk tank milk measurements from the same herd was estimated for monthly and quarterly intervals using data from the Kongeå project. Herds were classified as infected if culture positive or if individual animals were observed to seroconvert (changes of greater than 25% ODC from one sample to the next) during the observation period. Compared with classifying herds only on the basis of culture results, the seroconversion criterion resulted in one herd being added to the infected group. Spearman's rank correlation was calculated for the current bulk tank ELISA measurements and lag values within the same herd 1 month previously and 1, 2 or 3 quarters previously. The average correlation was calculated using all coefficients separated by the same time period, i.e. all 1 month differences were averaged for the 1 month estimate, all 1 quarter lags were averaged for the 1 quarter estimate etc. This was done separately for measurements from infected and non-infected herds.

Simulation was used to select 4 quarterly ELISA measurements from the corresponding series of distributions for each of the 16 infection history groups. The series of four quarterly measurements were designated y_0 for the current quarter, and with y_{-1}, y_{-2}, and y_{-3} for the measurements from the previous quarter, two quarters previously and three quarters previously, respectively. Truncated normal and truncated exponential distributions were used for infected and non-infected quarters respectively. The @Risk correlation feature was used to enter a correlation matrix for each infection history group. Data from pairs of quarters consisting of an infected and a non-infected quarter were assumed to be uncorrelated. Likewise, the correlation was assumed to be zero between quarter pairs consisting of either both infected or both non-infected quarters, but separated by at least one quarter with the opposite status.

The test result was coded as 1 if the average of the 4 measurements was ≥25 or if the 4th measurement minus the average of the previous 3 was > 20 and was coded zero otherwise (Eq. 1). The model was run for 5000 iterations and the mean test result was used as an estimate of the proportion of positive tests for each disease history group. Latin hypercube sampling was used. This method ensures that sampling is from throughout the distribution (Vose, 2000).

Regular Test Criteria

$$1 \text{ if } (y_{-3}+ y_{-2} + y_{-1} + y_{0})/4 \geq 25 \text{ OR } y_0 - ((y_{-3}+ y_{-2} + y_{-1})/3)>20; \text{ 0 otherwise.} \quad (1)$$

The programme specifies an automatic retest based on a sample collected approximately one month after a herd changes from Level 1 to Level 2a classification. A similar procedure was used to estimate the proportion of retests testing positive. The retest measurement (y_{1m}) was selected from the corresponding distributions for infected and non-infected herds. The one-month lag correlation estimate was used for correlation with y_0 and the corresponding quarterly

lag coefficients were used for the correlation with y_{-1}, y_{-2}, and y_{-3}. The retest value was used to replace the previous quarterly measurement (y_0) and then the test result was calculated as before. The proportion of herds testing positive on the retest given a 'previous' positive result was calculated as the mean retest result among iterations where the previous test result was positive (Eq. 2).

Retest (calculated if regular test=1)

$$1 \text{ if } (y_{-3}+ y_{-2} + y_{-1} + y_{1m})/4 \geq 25 \text{ OR } y_{1m} - ((y_{-3}+ y_{-2} + y_{-1})/3)>20; 0 \text{ otherwise.} \quad (2)$$

Module II

The herd-level test sensitivity, specificity and predictive values were calculated for a selected range of herd-level prevalence values in the 2nd simulation module. The scenario pathway for this module is shown in Fig. 1.

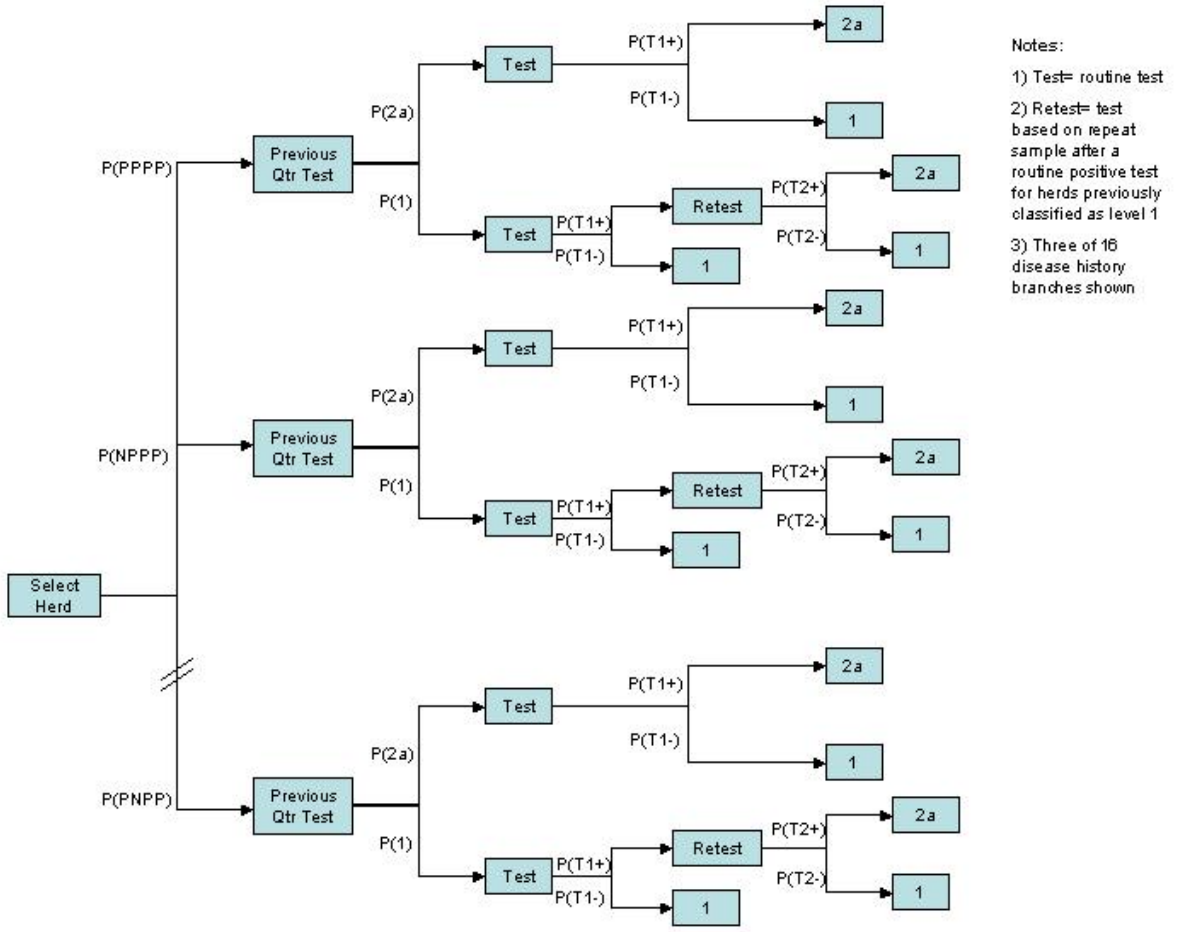


Fig. 2 Scenario pathway for estimation of herd-level test accuracy and predictive values for the *Salmonella* surveillance programme in Danish dairy cattle

The model included eight four-quarter disease history groups that were currently infected and eight disease history groups that were currently non-infected. The distribution of infection history patterns was estimated based on a two-compartment transition model (Fig. 2).

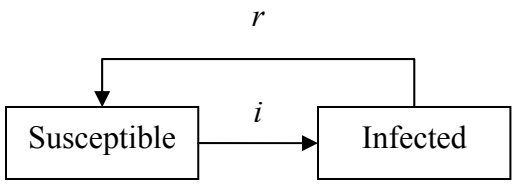


Fig. 3 Transition model for estimating frequency distribution of four-quarter disease history patterns

Movement from the susceptible to infected compartment occurred with probability *i* and movement from infected to susceptible with probability *r*. The recovery rate *r* was equal to 1/(average duration of infection). It is accepted that the total dairy herd population size was declining and that infection prevalence may have changed over time. However, for the simulation model we assumed a constant population size, where a steady state was reached at prevalence $p = (i/(i+r))$. The minimum, most likely and maximum average duration of infection were selected based on clinical experience with infected herds, personal communication with *S.* Dublin researchers, and published observations. Minimum, maximum and most likely recovery rates were calculated from average duration of infection estimates and then used as parameters for a PERT distribution in @Risk. The probability of each infection pattern was calculated as the probability of starting as infected or non-infected multiplied by the transition probabilities for subsequent states. Transition probabilities were assumed to be independent of past disease history. For example, at a herd-level prevalence of 0.15 and average duration of infection of three years (12 quarters), the recovery rate was calculated as 1/12 (0.083) and the incidence at steady state as shown in Eq. 3.

$$0.083 \times 0.15/(1-0.15) = 0.0146 \quad (3)$$

The probability of the disease history pattern NPPP would then be

$$(1-0.15)\times 0.0146\times(1-0.083)\times(1-0.083) = 0.01 \quad (4)$$

The frequency distribution for the 16 four-quarter disease histories were calculated at steady state for fixed populations with fixed values for prevalence and duration of infection modelled as a PERT distribution. The model was run at five selected herd-level prevalence values: 0.02, 0.08, 0.15, 0.3, and 0.5.

From the scenario path diagram (Fig. 1) it can be seen that there were 5 test result pathways for each disease history pattern resulting in 80 total pathways. @Risk version 4.5 was used to calculate the probabilities for each pathway using the test accuracy values generated by the Module I simulation described above. The probability of testing positive was then calculated as the sum of the probabilities for the '2a' branches. The positive predictive value (PPV) was calculated as the sum of the probabilities of 2a branches for currently infected herds divided by the probability of testing positive. The analogous calculations were done for the probability of testing negative and for negative predictive value. The proportions of herds in each disease history group previously classified 2a was estimated iteratively by starting with 0.5, calculating the apparent prevalence for each group, and then replacing the original previous 2a probabilities with the calculated values. The estimates stabilised within 5 iterations with differences between input and output values at that step < 0.000001.

Sensitivity Analyses

The potential effects of uncertainty about the distribution parameters and serial correlation coefficients were evaluated through a series of sensitivity analyses where models were run with various combinations of input values. For the mean and standard deviation of the infected herd distribution and beta for the non-infected distribution, models were run at all possible combinations of the mean, minimum and maximum values observed in the 100 bootstrap samples used to evaluate the variability of the distribution parameter. Models were also run with the mean values for the mean, SD and beta parameters, but with the maximums for the truncated distributions set to either the maximum observed value (in the Kongeå project for the non-infected distributions and from the surveillance data for the infected distributions). To evaluate the effect of the rank correlation coefficients, models were run with all coefficients set to either 0 or to 0.99. Herd-level prevalence was set to 0.15 for the sensitivity analyses.

RESULTS

Parameter estimates from field study

The distribution of bulk tank ELISA measurements for infected and non-infect herds as defined by apparent within-herd prevalence are shown in Fig. 3.

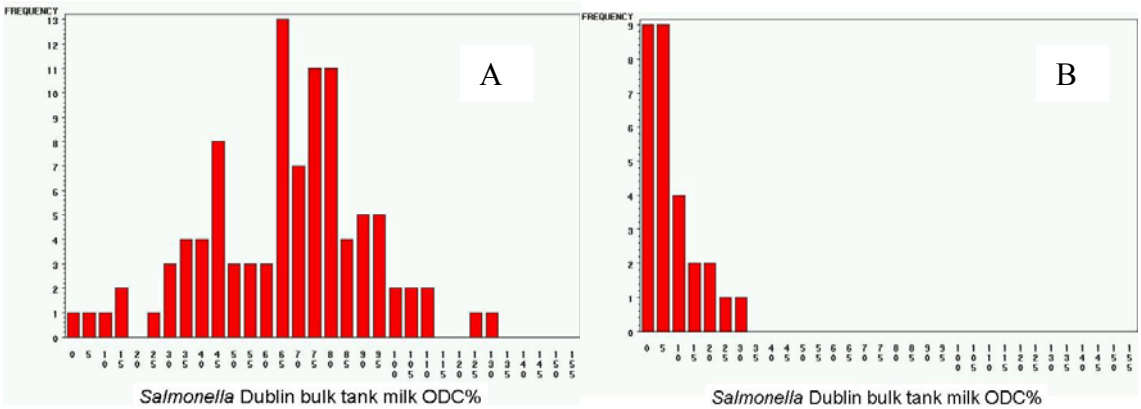


Fig. 4 Distribution of bulk tank milk ELISA responses in herds with A: 5% or higher within-herd apparent prevalence and B: less than 5% within-herd apparent prevalence (there were multiple observations per herd)

The bulk tank ELISA antibody measurements from infected herds were approximately normally distributed with mean 66.3 and SD of 25.2 while the measurements from non-infected herds had a skewed distribution with a mean 7.8 and SD of 8.1. The maximum measurements for infected and non-infected herds were 131 and 28, respectively. The distribution parameters as estimated using a bootstrap sampling technique consisting of 100 samples with one observation per herd are shown in Table 1. The within-herd rank correlation coefficients for repeated measurements within the same herd tended to be higher in non-infected herds than for infected herds (Table 2).

Table 1. Descriptive statistics for distributions of parameter estimates from 100 bootstrap samples of one randomly selected observation per herd.

	Mean	SD	Minimum	Maximum
Infected mean (n=23/sample)	65.6	3.0	58.4	72.5
Infected SD (n=23/sample)	28.4	2.1	23.5	34.6
Non-infected mean (n=9/sample)	8.9	1.9	5.0	13.4

Table 2. Spearman rank correlation coefficients for bulk tank milk ELISA measurements differing in time by 1, 2 or 3 quarters or by 1 month.

	Infected		Non-infected	
Lag	Month	Quarter	Month	Quarter
1	0.65	0.44	0.70	0.67
2		0.37		0.65
3		0.38		0.57

Module I results

The output from Module I of the simulation model consists of estimates for the probability of testing positive for the initial test and retest for each of the 16 four-quarter infection history patterns (Table 3). These results reflect the variability associated with the distributions of bulk tank milk ELISA measurements in infected and non-infected herds, but not the uncertainty about the distribution parameters.

The probability of testing positive on the initial test was 0.90 or higher for all patterns where the herd was infected in the current quarter. Patterns currently non-infected, but infected in previous quarters had a probability of testing positive from 0.39 to 0.95 which increased with the number and proximity of previous infected quarters.

Module II results

The estimates of HSe for the overall test programme varied little over the range of prevalence values evaluated and had a narrow distribution within each prevalence. Mean estimates were from 0.96 to 0.97 (Table 4). In contrast, the mean estimates for HSp ranged from 0.78 to 0.97 and were inversely related to herd level prevalence (Table 4). This occurred because higher prevalence was associated with a higher frequency of mixed infection history patterns which resulted in more false positive diagnoses. The herd-level negative predictive value mean estimates were from 0.96 to 0.999 and the positive predictive value mean estimates were 0.37 to 0.82 (Table 5). These results reflected the relatively wide range of HSp and narrow range of HSe as well as the effect of prevalence on predictive values. The distributions for these

estimates were associated with the input distribution for herd-level duration of infection. This distribution was selected to account for the uncertainty about this parameter.

Table 3. Probability of testing positive in the current quarter for the initial test (P(T1+) and the automatic retest P(T2+|T1+) for 16 infection history patterns.

Infection Pattern[a] Quarter 1, 2, 3, 4	P(T1+)	P(T2+|T1+)
PPPP	0.982	0.995
NPPP	0.964	0.984
NNPP	0.914	0.954
NNNP	0.918	0.948
NNNN	0.042	0.698
PNNN	0.395	0.926
PPNN	0.844	0.987
PPPN	0.954	0.997
NPNP	0.944	0.968
PNPN	0.895	0.989
PNNP	0.944	0.966
NPPN	0.850	0.986
NNPN	0.404	0.919
NPNN	0.391	0.929
PPNP	0.988	0.992
PNPP	0.982	0.990

[a]P=infected; N=non-infected, quarter 4=most recent quarter

Table 4. Estimates of herd-level sensitivity (HSe) and herd-level specificity (HSp) with credibility intervals at five different herd-level prevalence values.

Prevalence	HSe			HSp		
	5th pctl	Mean	95th pctl	5th pctl	Mean	95th pctl
0.02	0.95	0.96	0.97	0.96	0.97	0.97
0.08	0.95	0.96	0.97	0.94	0.95	0.96
0.15	0.95	0.96	0.97	0.91	0.93	0.95
0.3	0.95	0.96	0.97	0.84	0.88	0.92
0.5	0.96	0.97	0.97	0.68	0.78	0.85

Table 5. Estimates of herd-level positive predictive value (HPPV) and herd-level negative predictive value (HNPV) with credibility intervals at five different herd-level prevalence values.

Prevalence	HPPV			HNPV		
	5th pctl	Mean	95th pctl	5th pctl	Mean	95th pctl
0.02	0.348	0.366	0.380	0.999	0.999	0.999
0.08	0.589	0.638	0.677	0.996	0.997	0.997
0.15	0.662	0.722	0.770	0.991	0.993	0.995
0.3	0.717	0.783	0.837	0.977	0.983	0.987
0.5	0.752	0.815	0.869	0.942	0.957	0.968

Sensitivity analyses were run to evaluate the effect of uncertainty about model input parameters on test accuracy and predictive values at an assumed herd-level prevalence 0.15. HSe mean estimates ranged from 0.92 to 0.99, HSp from 0.87 to 0.97, HPPV from 0.57 to 0.84 and HNPV from 0.98 to 0.998.

DISCUSSION

This is the first study to estimate misclassification in the current *Salmonella* Dublin surveillance programme for Danish dairy herds. Previous field and laboratory studies addressed herd-level *Salmonella* Dublin testing, but may not be directly comparable to our results (Hoorfar et al., 1994; Hoorfar & Bitsch, 1995; Veling et al., 2002; Wedderkopp et al., 2001). In none of these studies were the bulk tank milk ELISAs used on repeated samples from the same herds. Herd classification was based either on bacterial culture results or on serology of individual animals and comparison was made to single bulk tank milk ELISA measurements.

A *S.* Dublin programme evaluation project was carried out by Danish researchers before instituting the national surveillance programme. One part of the study compared bulk tank milk measurements to *Salmonella* Dublin faecal culture results for selected herds (Anon, 2001). The investigation was based on herds that did not necessarily represent the full range of herds to which the test is applied, but nevertheless provides valuable results. The report recommended a single test cut-off of 55 ODC% to maximize overall test accuracy. For cut-offs of 20 and 30, the HSe was 92 and 83% and the HSp 53 and 60%, respectively. Only 4% of 'bulk tank milk test negative' herds were culture positive when test negative was defined as <30 ODC% and no significant increase between samples. In designing the surveillance programme, it was recognized from these results and other experiences with the bulk tank milk ELISA, that simultaneously high HSe and HSp would not be possible. Therefore, test criteria were selected to result in high HSe, and thereby high HNPV, so that at least the negative test results could be considered reliable.

Misclassification in the current system

Assuming a true herd-level infection prevalence of 15%, under the current system, about 20 to 35% of Level 2a herds would be non-infected (as defined by having less than 5% within-herd apparent prevalence) and less than 1% of Level 1 herds would actually be infected (2b herds were not considered in the model and would be distributed among Level 1 and 2a depending on their bulk tank measurements). In terms of numbers of herds, this would be expected to

represent no more than 52 false negative herds and 450 false positive herds per quarter of the year (these estimates are the mean 95th percentiles of false negatives and false positives estimated from a second order model assuming 15% herd-level prevalence and a total dairy herd population of 6750). This amount of misclassification is consistent with the goal of decreasing the risk of transmission from cattle purchased from Level 1 herds. However, from a cattle seller's point of view, it may represent a high probability of being free of infection when classified as Level 2a.

The results of these simulation models should be interpreted in light of assumptions about model input values and in consideration of the model design. The uncertainty about input values was either modelled by a distribution (e.g. average duration of herd-level infection) or by performing sensitivity analyses. The results of a series of sensitivity analyses models show the range of results from relatively extreme input values. For example, the HPPV ranged from 0.65 to 0.81 and the HNPV from 0.986 to 0.995 for assumptions of 0.99 and zero for serial correlation coefficients. Ignoring serial correlation completely would result in overestimation of predictive values. Nevertheless, the effect of small errors in the specification of the correlation coefficients would be unlikely to affect the model results seriously, particularly when considering that correlations were unlikely to be negative or to be near zero or one.

The selection of the maximum values to truncate the distributions of bulk tank milk measurements was somewhat arbitrary. It seemed unreasonable to use distributions with upper limits of infinity because the maximum BT ELISA measurement observed out of approximately 100,000 measurements was 204. On the other hand, it was unlikely that the distribution maxima would have been observed in the Kongeå project data used to estimate distribution parameters. Therefore, sensitivity models were run using either the observed maxima or a high estimate of 500 (selected to be relatively high compared with the maximum ever observed in the surveillance programme). These models showed almost no effect on HSe and HNPV. This would be expected because the AVG4 cut-off is to the left of the centre of the infected herd distribution so extension of the right tail would not have a large effect on the probability of exceeding the cut-off. The effect was larger on HSp and HPPV but the range was still relatively narrow with HSp estimates of 0.93 and 0.96 and for HPPV of 0.70 and 0.81 for the high maximum and low maximum models, respectively.

All possible combinations of the minimum, maximum and mean values for the infected distribution mean and standard deviation and the non-infected herd beta were also evaluated in sensitivity models. The minima and maxima were the most extreme parameter estimates from the bootstrap sampling procedure. The sensitivity analyses showed that HNPV estimates were not substantially different over the range of input parameters tests. The HPPV values had a wider range with the lowest estimate being 0.57.

Several other important assumptions are related to the model design rather than the values of the input variables. For example, Module I was based on the assumption that the same distribution of bulk tank milk measurements applies to all infected herd-quarters, regardless of the time since infection. The analogous assumption was made for the exponential distribution for the non-infected herd-quarters. In other words, we modeled an abrupt rather than gradual change in distributions when infection status changed. The model was designed in this way for the practical reason that there were not adequate field data to estimate the positive and negative bulk tank milk distributions separately for various times since infection or times since recovery from infection. If, in fact, the true distributions depend on time since infection we would expect that the model would have overestimated HSe (because the mean bulk tank measurement was

specified to be higher than the true distribution) and underestimated HSp (because the mean bulk tank measurement for quarters just preceding recovery was specified to be higher than the actual distribution). These biases would affect 'mixed' disease history patterns and would only have a substantial impact on the overall test accuracy and predictive value estimates under a high incidence or high recovery rate conditions where the proportion of mixed patterns is increased.

Another feature of Module I was that zero correlation was assumed for pairs consisting of one non-infected quarter and one infected quarter. The same applied to pairs of infected quarters separated by at least one non-infected quarter and for pairs of non-infected quarters separated by at least one infected quarter. The reasoning behind this assumption was that the conditions contributing to serial correlation (e.g. sampling a herd consisting mostly of the same group of non-infected cows) would have a small impact on measurements relative to the effect of changing infection status. Also, changes in herd-level states of infection, recovery and reinfection or recovery, infection and recovery would imply that a large-scale change in the infection status of individual animals either through recovery, infection, or replacement. Our assumption is that the effect of taking repeated measurements in the same herd would be small in comparison.

Module II used prevalence, incidence and recovery rates to calculate the expected frequencies of 16 disease history patterns. The model inputs were recovery rate (inverse of duration of infection and modelled as a PERT distribution) and fixed values for prevalence. The incidence was calculated from prevalence and recovery rate. This method assumed that the average duration of infection was independent of prevalence. Recognizing that the surveillance data results are subject to misclassification, we estimated transition probabilities (Level 1 to 2a and Level 2a to 1) as proxies for incidence and recovery rates separately for seven regions with a wide range of herd-level apparent prevalence values. The results showed that the probability of the 1 to 2a transition increased approximately five-fold over apparent prevalence values of 7.5 to 39%. This is consistent with the model assumptions. Over the same range, the probability of the 2a to 1 transition decreased by about a factor of two. This 2a recovery rate is the inverse of the average duration of 2a status. This would reasonably be expected to be shorter in low prevalence areas where a greater proportion of 2a herds are actually free of infection. The result may also be a result of a true increase in average duration of infection in high prevalence areas. However, the size of this change is likely to be well within the range of average duration of infection specified in the model. The estimation of infection incidence and recovery rates is the subject of future work planned by the project group. This may allow the model to be refined in the future by allowing recovery rate to vary as a function of herd-level infection prevalence.

The method for calculation of disease history frequencies also depends on assumptions of constant prevalence, independence of disease status and entry or exit from the population, and independence of transition probabilities from prior disease history. Changes in apparent prevalence over the course of the surveillance programme suggest that prevalence may in fact be gradually declining and suggest that there may be some seasonality in apparent prevalence. Assuming test performance has remained constant, this would imply a decline in true prevalence as well. However, we believe the change in prevalence over four quarters in the misclassification model would not cause important changes in the results or conclusions. The assumptions of independence of herd exit and entry from disease status and the independence of transition probabilities could not be evaluated in observational data as part of this project, but were considered to be reasonable simplifying assumptions for the modeling process.

ACKNOWLEDGEMENTS

Dr Warnick conducted this research as a Guest Scientist at the International EpiLab in Denmark which funded the project. The authors thank Mette Marie Andersen for preparing databases used for the study. The authors appreciate information from Jan Veling on the duration of *Salmonella* Dublin infections in dairy herds and input from Viggo Bitsch, Peter Lind, and Niels C. Feld on test performance.

REFERENCES

Anon (2001). Bakteriologisk undersogelse til beskrivelse af sammenhaengen mellem forekomsten af *Salmonella enterica* og salmonellaantistoffer i danske kvaegbesaetninger: Dokumentationsproject. Statens Veterinaere Serumlaboratorium, Fodevaredirecktoratet og Mejeriforenningen, Copenhagen.

Anon. (2004). Annual Report on Zoonoses in Denmark 2003. Ministry of Food, Agriculture and Fisheries, Soborg.

Fierer, J. (1983). Invasive Salmonella dublin infections associated with drinking raw milk. West J Med. 138, 665-669

Helms, M., Vastrup, P., Gerner-Smidt, P., and Molbak, K. (2003). Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. Brit. Med. J. 326, 357-361

Hinton, M. (1974). Salmonella dublin abortion in cattle: studies on the clinical aspects of the condition. Br Vet J. 130, 556-563

Hoorfar, J. and Bitsch, V. (1995). Evaluation of an O-antigen ELISA for screening cattle herds for *Salmonella typhimurium*. Vet. Rec. 137, 374-379

Hoorfar, J., Feld, N. C., Schirmer, A. L., Bitsch, V., and Lind, P. (1994). Serodiagnosis of Salmonella dublin infection in Danish dairy herds using O-antigen based enzyme-linked immunosorbent assay. Can J Vet Res. 58, 268-274

Humphrey, T., Wray, C., and Wray, A. (2000). Public-health aspects of *Salmonella* infection. Pages 245-263 *in Salmonella* in Domestic Animals. C. Wray and A. Wray, eds. CABI Publishing, New York.

Konrad, H., Smith, B. P., Dilling, G. W., and House, J. K. (1994). Production of Salmonella serogroup D (O9)-specific enzyme-linked immunosorbent assay antigen. Am. J. Vet. Res. 55, 1647-1651

McDonough, P. L., Fogelman, D., Shin, S. J., Brunner, M. A., and Lein, D. H. (1999). *Salmonella enterica* serotype Dublin infections: an emerging infectious disease for the Northeastern USA. J. Clin. Micro. 37, 2418-2427

Nielsen, L. R. (2003). *Salmonella* Dublin in dairy cattle: Use of diagnostic tests for investigation of risk factors and infection dynamics. PhD, The Royal Veterinary and Agricultural University, Copenhagen.

Nielsen, L. R. and Ersbøll, A. K. (2004). Age stratified validation of an indirect *Salmonella* Dublin serum ELISA for individual diagnosis in cattle. J. Vet. Diagn. Invest. 16, 205-211

Nielsen, L. R., Toft, N., and Ersbøll, A. K. (2004). Evaluation of an indirect serum ELISA and a bacteriological faecal culture test for diagnosis of *Salmonella* serotype Dublin in cattle using latent class models. Journal of Applied Microbiology. 96, 311-319

Peters, A. R. (1985). An estimation of the economic impact of an outbreak of Salmonella dublin in a calf rearing unit. Vet Rec. 117, 667-668

Veling, J., Barkema, H. W., van der Schans, J., van Zijderveld, F., and Verhoef, J. (2002). Herd-level diagnosis for *Salmonella enterica* subsp. *enterica* serovar Dublin infection in bovine dairy herds. Prev. Vet. Med. 53, 31-42

Visser, S. C., Veling, J., Dijkhuizen, A. A., and Huirne, R. B. M. (1997). Economic losses due to *Salmonella* Dublin in dairy cattle. Dutch/Danish Symposium on Animal Health and Managemeng Economics, Copenhagen, Denmark:143-151.

Vose, D. (2000). Risk Analysis: A Quantitative Guide. John Wiley & Sons Inc., New York. 418p

Wedderkopp, A., Strøger, U., Bitsch, V., and Lind, P. (2001). Testing of bulk tank milk for *Salmonella* Dublin infection in Danish dairy herds. Can. J. Vet. Res. 65, 15-21

NAIVE BAYESIAN CLASSIFIERS FOR THE CLINICAL DIAGNOSIS OF CLASSICAL SWINE FEVER

P.L. GEENEN[1], L.C. VAN DER GAAG, W.L.A. LOEFFEN AND A.R.W. ELBERS

SUMMARY

Naive Bayesian classifiers have been applied successfully for solving diagnostic problems in the medical domain, but are relatively new to the veterinary field. To demonstrate their potential, naive Bayesian classifiers were constructed for discriminating between Classical Swine Fever (CSF) infected and uninfected herds using data on 490 herds, collected during the 1997/1998 CSF epidemic in the Netherlands. A full naive Bayesian classifier and a selective one were constructed, and their classification accuracies were compared to that of a previously published diagnostic rule. The full classifier had a higher accuracy than the diagnostic rule, and the selective classifier proved to be comparable to the rule. In contrast with the diagnostic rule, the two classifiers had the advantage of taking both the presence and the absence of clinical signs into account, which resulted in more discriminative power.

INTRODUCTION

Naive Bayesian classifiers have proved to be powerful tools for solving classification problems in a variety of domains. They have been applied successfully in the medical domain for solving diagnostic problems, such as the diagnosis of heart disease in newborn babies (Spiegelhalter et al., 1993), of dementia severity (Shankle et al., 1998), of ischaemic heart disease (Kukar et al., 1999), and of breast cancer (Butler et al., 2003). Naive Bayesian classifiers have recently also found their way to the veterinary domain, and resulted in the cattle disease diagnosis system CaDDiS (McKendrick et al., 2000) and in a system for the diagnosis of scrapie (Kuncheva et al., 2004). A naive Bayesian classifier in essence is a model of a joint probability distribution over a set of stochastic variables. It is composed of a single class variable, modelling the possible outcomes for the problem under study, and a set of feature variables, modelling the domain features that provide for distinguishing between the various outcomes. In the model, it is assumed that the feature variables are mutually independent given the class variable (Friedman et al., 1997), and the term ‘naive’ in fact refers to the assumption of mutual independence. Although this assumption is not always valid, naive Bayesian classifiers tend to have a good classification performance and often outperform more sophisticated models (Domingos & Pazzani, 1997).

As an example, Fig. 1 depicts the structure of a simple naive Bayesian classifier for distinguishing between CSF-infected and uninfected pig herds. In the structure, CSF denotes the class variable, and the feature variables are cyanosis, ataxia and conjunctivitis. The arrows

[1] Institute for Information and Computing Sciences, Utrecht University, P.O.Box 80.089, 3508 TB Utrecht, The Netherlands. Email: petrag@cs.uu.nl

from CSF to the feature variables indicate that whether or not a herd is infected influences the probabilities with which cyanosis, ataxia and conjunctivitis respectively, will be observed. In addition to its graphical structure, the model includes various probabilities capturing the strengths of the diagnostic influences between the variables. These probabilities are typically estimated from data, but in this paper it will be demonstrated that they may also be obtained from the epidemiological literature. The observations of a specific herd are presented to the classifier as values for the modelled feature variables. To classify a herd in which cyanosis and conjunctivitis have been observed and no ataxia, the two positive values and the negative one are entered for the appropriate feature variables. The classifier then returns the posterior probability distribution over the class variable for the herd. For computing this posterior distribution, the classifier builds upon Bayes' rule, and in fact, the term 'Bayesian' refers to the prominent role of this rule of probability. From the established posterior distribution, the class with the highest probability determines the classification of the herd. Even if missing values occur, the classifier will return a probability distribution. It will then take the posterior distributions over the missing variables into account upon computing the posterior probabilities for the class variable.

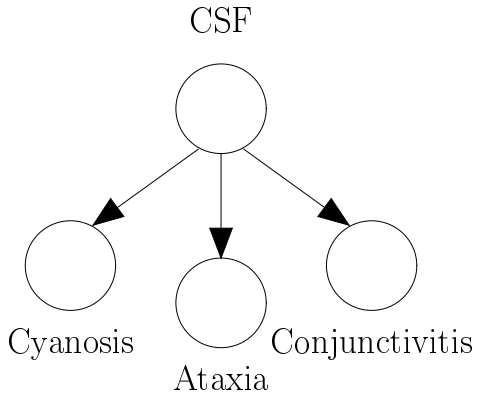


Fig. 5 The graphical structure of an example naive Bayesian classifier

When a naive Bayesian classifier is constructed to cover all features that have been gathered upon data collection, the resulting model is called a *full* naive Bayesian classifier. Real-life data, however, often record more features than are strictly necessary for classification. Moreover, the collected features may be correlated to some extent, thereby violating the independence assumption that underlies the classifier. However, from among the recorded features, the more discriminative features can be distinguished from the less informative ones by means of statistical concepts. A classifier built from such a subset of discriminative features, is called a *selective* naive Bayesian classifier. These less complex classifiers tend to have a better performance in general (Langley & Sage, 1994).

This paper will demonstrate how to construct a full and a selective naive Bayesian classifier from an epidemiological study, to discriminate between CSF-infected and uninfected pig herds. Clinical signs seen by the farmer or a veterinarian are usually the first indications of CSF-infection in a herd. Unfortunately, the clinical signs of CSF are mainly atypical and may vary from mild to severe (Van Oirschot, 1999), and, as a consequence, the disease can remain undetected for weeks. The classifiers described here aim to improve upon early detection and are based upon clinical signs only. For construction of the classifiers, data on 32 clinical signs of 490 herds collected during the 1997/1998 CSF epidemic in the Netherlands were used. An earlier analysis had resulted a number of diagnostic rules for the classification of CSF (Elbers et

al., 2002). The classification accuracies of classifiers will be compared with the reported optimally efficient diagnostic rule. Further, the selection of feature variables for the rule and for selective naive Bayesian classifier will be compared.

MATERIALS AND METHODS

The dataset

The data used for constructing the naive Bayesian classifiers, were collected and analysed earlier by Elbers et al. (2002). The data record the absence or presence of 32 clinical signs for 490 herds. These herds were visited by veterinary expert teams during the 1997/1998 epidemic in The Netherlands, due to a clinical suspicion of infection. For each herd, the body temperature of the diseased pigs was measured and an anamnesis was taken. On a standardised investigation form, the presence of disease signs within the herd was recorded. If one or more pigs within the herd were observed to suffer from cyanosis for example, then this feature was marked as being present in the herd. Pigs with apparent disease signs and/or fever were euthanased and submitted to the Animal Health Service for a post-mortem examination. The tonsils and samples of the spleen, ileum and kidney were collected and sent to the Institute for Animal Science and Health in Lelystad (currently CIDC-Lelystad) to be tested by means of a CSF-specific immune fluorescence assay (IFA). Later in the epidemic, blood samples also were collected for virus isolation and antibody detection. If one or more pigs from a submission from a single herd proved to be infected with the virus, that is if one or more pigs were positive in the IFA or in the virus isolation, then the herd was diagnosed as positive for CSF. If all pigs of the submission were negative upon examination and the herd remained to be so for at least six months after the submission, then the herd was diagnosed as negative for the disease. Of the 490 herds from which a completed investigation form was available, 245 herds were diagnosed as CSF positive and 245 herds were diagnosed as negative for the disease.

From the available investigation forms, a dataset was constructed for further analysis. Upon constructing the dataset, the recorded clinical signs were encoded as ‘1’s for the appropriate variables, and signs that were not recorded explicitly were assumed to be absent and were encoded as ‘0’s. The recorded clinical signs were relatively sparse, possibly as during the epidemic, most infected herds were detected rather early in the disease process. In the CSF-positive herds the mean number of recorded clinical signs was 3.5, whereas for the CSF-negative herds this number was 3.0, which is significantly lower (Mann Whitney test, $p < 0.01$). The number of ‘1’s in the dataset as a consequence constitutes just 10.2% of the total number of data.

The diagnostic rules

The dataset was analysed by Elbers et al. (2002). The goal of the analysis was to arrive at diagnostic classification rules composed of clinical signs that could be used as simple diagnostic tests for establishing the presence of CSF in a herd. As the recorded clinical signs were relatively sparse, disjunctive rules were constructed, so if at least one sign mentioned in such a rule is present in a herd, then the herd is classified as positive for CSF, but if all signs from the rule are absent, then it is classified as CSF negative. To select the clinical signs that serve to explain most variation in the classification, logistic regression with backward selection was applied to the data, with the classification of a herd for the response variable and the clinical signs for the explanatory variables. Subsequently, all possible disjunctive rules that could be

constructed from the selected signs were evaluated using receiver operating characteristic (ROC) analysis. Moreover, three arbitrary diagnostic rules were chosen, that are epidemiologically meaningful for disease detection: a rule that combines maximum sensitivity with the highest available specificity ('optimally sensitive'), a rule with maximum specificity and the highest available sensitivity ('optimally specific'), and a rule with maximised sensitivity and specificity ('optimally efficient').

Constructing naive Bayesian classifiers

Constructing a naive Bayesian classifier starts by defining the class variable with its possible values and the feature variables with their values. A binary class variable was created, modelling whether or not a herd is infected with CSF, and 32 feature variables, each modelling whether a specific clinical sign is present or absent in a herd. For the class variable, prior probabilities for the various classes discerned have to be specified, and for a binary diagnostic class variable these probabilities usually reflect the prevalence of the disease for the population under study. For the classifiers, the prior probabilities were computed from the numbers of positively and negatively diagnosed herds and were established to be p(CSF = yes) = p(CSF = no) = 0.5. As a consequence, the classifiers assume that an arbitrary herd is equally likely to be infected as it is to be uninfected, and we will return to this assumption in the discussion. To complete the construction of a naive Bayesian classifier, various conditional probabilities have to be obtained. For each feature variable included in the classifier, conditional probability distributions have to be defined over its values given the different classes. For the classifiers, these probabilities were taken to be the estimated sensitivity p(clinical sign = yes | CSF = yes) and specificity p(clinical sign = no | CSF = no) for each clinical sign. These sensitivities and specificities were reported by Elbers et al. (2002). For example, for conjunctivitis these probabilities are p(Conjunctivitis = yes | CSF = yes) = 0.229 (sensitivity) and p(Conjunctivitis = no | CSF = no) = 0.861 (specificity). It is noted that, as the reported sensitivities and specificities were established from a relatively small dataset, zero probabilities do not necessarily indicate a logical impossibility of the clinical sign occurring. Therefore, to prevent inconsistencies when entering the data into classifiers, these zero probabilities were replaced by 0.0001.

While a full classifier includes all possible feature variables, a selective naive Bayesian classifier includes just a carefully selected subset of the available feature variables. Building a selective naive Bayesian classifier therefore involves singling out the feature variables that best serve to separate the different classes under study. In third study, the so-called filter approach was used for this purpose. With this approach, the selection of appropriate feature variables is performed in a pre-processing step before the classifier is actually constructed. For each feature variable, its ability to separate the various outcome classes is investigated by means of an information-theoretic criterion. Only the variables that show a high discriminative ability will be included in the classifier under construction. In this study, the concept of mutual information was used to decide upon inclusion of a feature variable. The mutual information $I(X,Y)$ of two variables X and Y is defined as

$$I(X,Y) = \sum_{x,y} p(x,y) \cdot \ln \frac{p(x,y)}{p(x) \cdot p(y)} \qquad (1)$$

where the feature variables are taken for the variable X and the class variable is taken for the variable Y. To decide upon inclusion of a specific feature variable, the property that the quantity $2 \cdot N \cdot I(X,Y)$ asymptotically follows a $\chi^2_{(r-1)\cdot(r_0-1)}$ distribution was exploited, where r is the number of possible values of X (for the classifiers $r = 2$), r_0 is the number of values of Y (for classifiers $r_0 = 2$), and N is the number of observations (for the classifiers $N = 490$ equals the number of herds). To decide whether or not to include the feature variable under study in the classifier, a significance level of $\alpha = 0.01$ was used, and only feature variables X for which $2 \cdot N \cdot I(X,Y) > 6.64$ were thus included. It is noted that since $p(x, y) = p(x \mid y) \cdot p(y)$ and $p(x) = \sum_y p(x, y)$, all probabilities mentioned in Eq. (1) can be expressed in terms of sensitivity, specificity, and the numbers of positively and negatively diagnosed herds. The mutual information of the various feature variables with the class variable can therefore be calculated directly from the information reported in Elbers et al. (2002).

Data analysis

Based upon the information available from Elbers et al. (2002), a full and a selective naive Bayesian classifier were constructed as outlined above. Using the constructed classifiers and the previously published diagnostic rule respectively, each herd was classified as either CSF positive or CSF negative. From the results obtained, the sensitivity, the specificity and the overall accuracy of each of the three models were computed, where the accuracy was taken to be the fraction of herds diagnosed correctly. The established sensitivities, specificities and accuracies were compared, and the features selected for the rule and for the selective naive Bayesian classifier were evaluated. Both the classifiers and the diagnostic rule were programmed and run in the software package IDEAL, and the significance tests for comparing the results obtained were performed with S-plus.

RESULTS

The results of the study are summarised in Table 1., which reports the sensitivities, the specificities and the overall accuracies, with their respective 95%-confidence intervals, for the full and selective naive Bayesian classifiers and for the optimally efficient diagnostic rule. The full naive Bayesian classifier was found to have a significantly higher overall accuracy than the optimally efficient rule ($P < 0.05$, proportions test). The accuracy of the selective classifier proved to be comparable to that of the rule and to that of the full naive Bayesian classifier. It was further found that the optimally efficient rule had a significantly higher sensitivity and a significantly lower specificity ($P < 0.05$, proportions test) than the two classifiers.

Table 1. The sensitivities, specificities and accuracies, with their 95%-confidence intervals (95% CI) for the two constructed classifiers and the diagnostic rule

Classification model	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Full naive Bayes	**0.65** (0.59 - 0.71)	**0.73** (0.67 - 0.78)	**0.69** (0.65 - 0.73)
Selective naive Bayes	**0.63** (0.57 - 0.69)	**0.67** (0.61 - 0.72)	**0.65** (0.61 - 0.69)
Optimally efficient rule	**0.73** (0.67 - 0.78)	**0.53** (0.46 - 0.59)	**0.63** (0.59 - 0.67)

The feature variables that were selected for both the optimally efficient rule and the selective naive Bayesian classifier, are shown in Table 2. It was found that the variables modelling the clinical signs of ataxia, not responding to antibiotic treatment and low feed intake, were selected for both models. The variable coughing/respiratory problems was selected for the classifier only, while hard faecal pellets and conjunctivitis were selected only for the diagnostic rule. It should be mentioned that conjunctivitis would have been selected as the next clinical sign to be included in the selective classifier, based upon its mutual information with the class variable, and noted that it was not included on account of the mutual information being slightly lower than the threshold value of 6.64. The most striking difference between the two models is the absence from the diagnostic rule of the highly discriminative sign of coughing/respiratory problems.

Table 2. The selected feature variables and their mutual information, $I(X,Y)$, with the class variable

Optimally efficient diagnostic rule	Selective naive Bayesian classifier	$I(X,Y)$
Ataxia	Ataxia	21.6
Low feed intake	Low feed intake	18.9
Not responding to antibiotics	Not responding to antibiotics	9.0
-	Coughing/Respiratory problems	16.7
Conjunctivitis	-	6.5
Hard faecal pellets	-	4.3

DISCUSSION

In this study, the potential of naive Bayesian classifiers in veterinary medicine was illustrated by means of a full and a selective classifier for discriminating between CSF-infected and uninfected herds. These classifiers were constructed from the sensitivities and specificities of individual clinical signs and the numbers of positively and negatively diagnosed herds reported by Elbers et al. (2002). While the classifiers could be built from information provided in the literature, to establish their sensitivities, specificities and accuracies, a dataset was needed. Note that for such evaluation purposes, information on the absence or presence of clinical signs in both infected and uninfected herds is needed. For the evaluation of the classifiers, the original data that were analysed before by Elbers et al. (2002) were available These data indeed included observations from both infected and uninfected herds. With the available data, the constructed classifiers exhibited good performance. In fact, they had a comparable (for the selective classifier) or even better (for the full classifier) accuracy than the diagnostic rule that had been established from the same data by Elbers et al. (2002).

Upon singling out the most discriminative clinical signs for the selective classifiers, by means of the concept of mutual information, a large overlap was found with the clinical signs selected by Elbers et al. (2002) for their diagnostic rule. One of the highly discriminative features, that is the feature of coughing/respiratory problems, that was included in the selective naive Bayesian classifier, however, was not present in the rule. The data reveals that, in the 1997/1998 epidemic in The Netherlands, this sign was encountered more often in herds that

were uninfected than in infected herds. If coughing/respiratory problems were present in a herd, therefore, these problems were an indication against CSF rather than for the disease. In Bayesian classification in general, clinical signs that are indicative of the absence of a disease play an equally important role in discriminating between the various outcomes as signs that point to the presence of the disease. In the diagnostic rule, in contrast, the presence of a clinical sign can be used as an indicator only for the disease, because of its disjunctive character. As the occurrence of coughing/respiratory problems may vary strongly between CSF epidemics, and in the literature respiratory problems are said to be a possible indicator for CSF infection, the practical implications of selecting this clinical sign against CSF for early detection of CSF may be limited.

The data used in this study suffered from variance in the way clinical signs were observed and interpreted (Elbers et al., 2002). This variance was caused by the acuteness of the epidemic. In addition, there is some uncertainty as to whether clinical signs that were not recorded were really absent or were simply not noticed by the veterinarians. In a previous study, the effect of variation in the absence or presence of unrecorded clinical signs on the selection of discriminative feature variables was investigated using the filter approach taken in this paper (Geenen et al., 2004). It was shown that both the selection of feature variables and the accuracy of the resulting classifier were quite robust against a 10% variation.

When using the naive Bayesian classifiers in practice, the observed clinical signs would be entered into the computer programme that implements the classifier. This programme subsequently calculates the posterior probability of CSF in the herd. If the calculated probability exceeds a given threshold, the veterinary practitioner should be allowed to exclude CSF as possible cause for the disease problem by sending samples to the National Reference Laboratory. If there is a high probability of CSF given the observed clinical signs, a serious suspicion of CSF should be notified to the veterinary authorities and an expert team from the veterinary authorities should be urged to visit the farm and possibly take additional measures. The current classifiers are based on data that were collected during an epidemic. For the early detection of CSF, however, an inventory of the clinical signs seen in a situation without outbreaks is needed. In addition, the current classifiers are constructed to classify herds with maximised accuracy, resulting in maximised sensitivity and specificity. For early CSF detection however, one may wish to have a classifier with a somewhat higher sensitivity (and hence, a lower specificity). New methods for the selection of discriminative features are currently being developed that allow for weighting the sensitivity and specificity of the resulting classifiers. For the purpose of this study it was assumed that herds had equal probabilities of being infected and uninfected to allow for comparing the classification performance of the classifiers with that of the diagnostic rule. In practice, however, the usual prevalence of CSF would be much lower than 0.5. Moreover, the prevalence changes drastically at the onset of an epidemic. This variation over time has not been included within the classifiers as yet, and will pose an interesting subject for future research.

Judging from the accuracy attained from these relatively simple models, it is felt that naive Bayesian classifiers are promising tools for solving diagnostic problems in the veterinary field. Building upon experience with the classifiers discussed in the present paper, a more complex model for the early detection of CSF is being developed with the help of domain experts, which will hopefully further improve the accuracy of distinguishing between infected and uninfected herds based upon clinical signs only.

REFERENCES

Butler, S.M., Webb, G.I. and Lewis, R.A. (2003). A case study in feature invention for breast cancer diagnosis using x-ray scatter images. In: Gedeon, T.D. and Fung, L.C.C. (eds.) Proceedings of the 16th Australian Conference on AI, pp. 677-685

Domingos, P. and Pazzani, M. (1997). On the optimality of the simple Bayesian network classifier. Mach. Learn. 29, 103-130

Elbers, A.R.W., Bouma, A. and Stegeman, J.A. (2002). Quantitative assessment of clinical signs for the detection of classical swine fever outbreaks during an epidemic. Vet. Microbiol. 85, 323-332

Friedman, N., Geiger, D. and Goldszmidt, M, (1997). Bayesian network classifiers. Mach. Learn. 29, 131-163

Geenen, P.L., Van der Gaag, L.C., Loeffen, W.L.A. and Elbers A.R.W. (2004). On the robustness of feature selection with absent and non-observed features. In: Barreiro, J.M., Martin-Sanchez, F. and Maojo, V. (eds.) Proceedings of the Fifth International Symposium on Biological and Medical Data Analysis, Springer Verlag, Berlin, pp. 148 - 159

Kukar, M., Kononenko, I., Grošelj, C., Kralj, K. and Fettich J. (1999). Analysing and improving the diagnosis of ischaemic heart disease with machine learning. Artif. Intell. Med. 16, 25-50

Kuncheva, L.I., Whitaker, C.J., Cockroft, P.D. and Hoare, Z.S.J. (2004). Pre-selection of independent binary features: an application to diagnosing scrapie in sheep. In: Chickering, M. and Halpern, J (eds.), Proceedings of the 20th international conference on Uncertainty in Artificial Intelligence, AUAI Press, Arlington, pp. 325-332

Langley, P. and Sage, S. (1994). Induction of selective Bayesian classifiers. In: Mántaras, R.L. and Poole, D. (eds.) Proceedings of the 10th conference on Uncertainty in Artificial Intelligence, Morgan Kaufmann Publishers, San Francisco, pp. 399-406

McKendrick, I.J., Gettinby, G., Gu, Y., Reid, S.W.J. and Revie, C.W. (2000). Using a Bayesian belief network to aid differential diagnosis of tropical bovine diseases. Prev. Vet. Med. 47, 141-156

Shankle, W.R., Mani, S., Dick, M.B. and Pazzani, M.J. (1998). Simple models for estimating dementia severity using machine learning. In: Cesnik, B., McCray, A.T. and Scherrer, J.-R (eds.) Proceedings of the 9th World Congres on Medical Informatics, IOS Press, pp. 472-476

Spiegelhalter, D.J., Dawid, A.P., Lauritzen, S.L. and Cowell, R.G. (1993). Bayesian analysis in expert systems. Stat. Sci. 8, 219-283

Van Oirschot, J.T. (1999). Classical Swine Fever (Hog cholera). In: Straw, B.E., D'Allaire, S., Mengeling, W.L. and Taylor D.J. (eds.) Diseases of swine, 8th ed., Blackwell Science, Malden, MA, pp. 159-172

OPEN SESSION

USE OF A LOCOMOTION SCORING SYSTEM FOR DIAGNOSIS OF LAMENESS, AND EFFECT OF LAMENESS, ON REPRODUCTIVE PERFORMANCE IN POSTPARTUM HOLSTEIN COWS

J.A. HERNANDEZ[1], E.J. GARBARINO, J.K. SHEARER, C.A. RISCO AND W.W. THATCHER

SUMMARY

A longitudinal study was conducted to examine the relationship between lameness and delayed ovarian cyclicity during the first 60 days (d) postpartum and time to conception in Holstein cows. Two hundred and thirty-eight cows and 499 cows from a 600-cow dairy that calved during a 12-month (mo) period were used. Cows were classified into 1 of 3 categories of lameness during the first 5 and 8-weeks postpartum by using a 6-point locomotion scoring system. Cows were blood-sampled weekly for detection of plasma progesterone (P_4) concentrations during the first 300 d postpartum. Cows with a delayed resumption of ovarian cyclicity were defined as those with P_4 concentrations consistently < 1ng/ml during the first 60 d postpartum. The hypothesis that risk of delayed cyclicity is the same in cows classified as non-lame, moderately lame, or lame (after adjusting for potential confounding effects of loss of body condition and other variables related with delayed cyclicity) was tested using a logistic regression analysis. The hypothesis that time to conception (days) is higher in cows classified as lame or moderately lame, compared to non-lame cows during the first 8 weeks postpartum was tested using Cox proportional hazards regression analysis. The results of the study reported here support the hypothesis that lameness is associated with delayed ovarian activity in Holstein cows during the early postpartum period. Cows classified as lame had 3.5 times greater odds of delayed cyclicity, compared to cows classified as non-lame (OR = 3.5; 95% CI = 1.0 to 12.2; P = 0.04). Attributable proportion analysis indicated that delayed ovarian cyclicity in lame cows would have been reduced by 71% if lameness had been prevented. Non-lame cows became pregnant sooner than lame cows.

INTRODUCTION

Lameness is one of the top 3 health problems responsible for premature culling of dairy cows in the United States. The National Animal Health Monitoring System Dairy 2002 Study reported that lameness was the reason for culling 16% of dairy cows sent to slaughter (NAHMS 2002). Overall, 10% of cows were reported to be affected with lameness in the previous 12 mo (NAHMS, 2002). The economic importance of lameness is reportedly attributable to cost of treatment and control methods (Hernandez et al., 1999; Hernandez et al., 2000; Moore et al., 2001; Shearer & Elliot, 1998; Shearer et al., 1998), impaired reproductive performance

[1] College of Veterinary Medicine, Department of Large Animal Clinical Sciences, University of Florida. Gainesville, FL 32610. Email: hernandezj@mail.vetmed.ufl.edu

(Hernandez et al., 2001; Lee et al., 1989; Lucey et al., 1986; Melendez et al., 2003; Sprecher et al., 1997), decreased milk yield (Green et al., 2002; Hernandez et al., 2002 Warnick et al., 2001), increased risk of culling (Collick et al., 1989; Sprecher et al., 1997), and decreased carcass value of culled cows (Van Arendonk et al., 1984). In addition, because of the pain, discomfort, and high incidence of lameness in dairy cows, this disorder is an animal welfare issue of concern.

Delayed ovarian cyclicity in the pre-service postpartum period is a common ovarian dysfunction in dairy cows. In studies conducted in commercial dairy herds in Belgium, Canada, Japan, and the United States, for instance, 20 to 33% of study cows were reported to have delayed ovarian activity during the first 50 to 60 d postpartum (Etherington et al., 1991; Moreira et al., 2001; Nakao et al., 1992; Opsomer et al., 1998; Opsomer et al., 2000; Staples et al., 1990). Late resumption of ovarian activity postpartum has a detrimental effect on reproductive performance in dairy cows (Lucy et al., 1992; Stevenson & Call, 1983; Thatcher & Wilcox, 1973;). Cows ovulating earlier postpartum have fewer services per conception and a shorter calving-to-conception interval (Lucy et al., 1992). Minimizing the interval from calving to first ovulation provides ample time for completion of multiple ovarian cycles before insemination, which in turn improves conception rates (Butler & Smith 1989). Losses in body condition, puerperal disturbances, and ketosis have been identified as risk factors significantly associated with delayed ovarian cyclicity in dairy cows (Opsomer et al., 2000).

Previous studies have established an association between lameness and impaired reproductive performance (e.g., a prolonged calving-to-conception interval) (Lucey et al., 1986; Collick et al., 1989; Sprecher et al., 1997; Hernandez et al., 2001), but the relationship between lameness and ovarian activity in dairy cows has not been investigated using objective research methods. Results of previous studies in Florida suggest that as cows experience increasing positive energy status, there is increased ovarian follicular activity leading to early return to ovulation (Staples et al., 1990; Lucy et al., 1992). As energy status becomes more positive for cows in early postpartum, diameter of the largest follicle increases, the number of double ovulations increases, and time for detection of the first corpus luteum decreases (Lucy et al., 1991). These changes in follicle size and numbers and the number of ovulations are thought to be caused by increases in luteinising hormone (LH), insulin, growth hormone (GH), insulin-like growth factor-1 and possibly other yet-to-be determined compounds that are activated by an improved energy status (Beam & Butler, 1998).

Clinical observations by veterinarians and dairy farmers in Florida suggest that lameness has a detrimental effect on ovarian activity in lactating dairy cows. Veterinarians and dairy farmers prefer to avoid the use of synchronisation and timed-insemination protocols in lame cows, because it is known that lame cows experience a more severe loss of body condition, spend less time eating (Hassall, 1993) and are less likely to be cyclic, compared to non-lame cows, until lameness has been resolved. In the current study it was hypothesised that because lame cows experience a more pronounced loss in body condition (hence a prolonged state of negative energy balance) during the early postpartum period, lame cows are at higher risk of delayed ovarian cyclicity than non-lame cows. Under field conditions, evidence of corpus luteum function can be determined by monitoring plasma progesterone (P_4) concentrations weekly during lactation, before and after diagnosis of lameness in dairy cows. The objectives of the study reported here were, (1) to examine the relationship between lameness and delayed resumption of ovarian cyclicity during the first 60 d postpartum and, (2) to compare the calving-to-conception interval in postpartum Holstein cows.

MATERIALS AND METHODS

Cows and herd management

Cows in this study were from a high-yielding dairy herd (rolling herd average milk production, approx 12,000 kg/cow/year) of approximately 600 Holstein cows located in Florida. This herd was selected for the study on the basis of a history of lameness, quality of veterinary records, and willingness of the owner to participate in the study. Cows were milked and fed a TMR ration 3 times per day. Cows were housed in lots equipped with sprinklers, fans and shade cloths over the feed bunks to reduce the effects of heat stress. On this farm, most of the cows are enrolled in a programme to pre-synchronise the stage of the oestrous cycle followed by synchronisation of ovulation (Moreira et al., 2001). This programme involves the use of 2 injections of prostaglandin $F_2\alpha$ ($PGF_2\alpha$); the first injection is given at 30 to 35 d postpartum and the second at 44 to 49 d postpartum. Fourteen days later (58 to 63 d postpartum), cows in this group are injected with gonadotrophin releasing hormone (GnRH), 7 d later with $PGF_2\alpha$, and 2 d later with a second injection of GnRH followed by a timed artificial insemination 16 to 18 hours (hrs) later (68 to 73 d postpartum).

Study design

This study was designed as a longitudinal study. A total of 563 Holstein cows that calved from June 1st 2002 until May 31st 2003 were considered for inclusion in the study. To accomplish the first objective, 253 (45%) cows identified with an even ear-tag number were enrolled in the study as they calved (instead of cows randomly selected) to overcome logistical identification procedures and to reduce disruption of routine veterinary medical and management procedures on the study farm (it is easier to identify cows with even ear tag numbers than cows with either even or odd numbers, as it would be expected if a random selection process had been used). Cows were classified into 1 of 3 categories of lameness during the first 5 weeks postpartum by using a 6-point locomotion scoring system (Garbarino et al., 2004). Cows were blood-sampled weekly for detection of plasma P_4 concentrations during the first 60 d postpartum. Risk of delayed cyclicity during the first 60 d postpartum was compared between cows classified as non-lame, moderately lame, or lame. In the analyses, lame cows were those that had lameness prior to resumption of ovarian cyclicity.

To accomplish the second objective, 499 cows (89%) with complete records were used in the study. Cows were classified into 1 of 3 categories of lameness during the first 8 weeks postpartum by using the 6-point locomotion scoring system. Time to conception (days) was compared between cows classified as non-lame, moderately lame, and lame. The time-period of 8 weeks postpartum was chosen as exposure of interest because incidence of lameness was highest (60%) during this time period, and because the OvSynch protocol was initiated at 58 to 63 days postpartum, which was the end of the voluntary waiting period on the study farm.

Data collection

Using farm records, the following data were collected for each cow: lactation number, calving date, calving season (winter months: Jan-Apr and Oct-Dec; summer months: May-Sep), dystocia (yes, no), retained placenta (yes, no), metritis (yes, no), mastitis (yes, no), ketosis (yes, no), body condition score at calving using a scale of 1 to 5 with 0.25 increments (Edmonson et

al., 1989), change in body condition score in the first 47 to 53 d postpartum, use of $PGF_2\alpha$ (Lutalyse, Pharmacia, Kalamazoo, MI) before resumption of ovarian activity (yes, no), and 305-d mature equivalent (ME) milk yield. Cows with retained foetal membranes were cows that failed to expel foetal membranes within 24 h after parturition. Cows with metritis were cows with foetid discharge from the uterus. Cows with mastitis were cows with a deviation from milk conductivity by the Afimilk system and later confirmed by foremilk stripping by the attending farm worker. Cows with ketosis were cows diagnosed with ketonuria by using urine strips (Ketostix) based on Sodium Nitroprusside that detects acetoacetate. The range of body condition scores was from 1 (severe undercondition – emaciated) to 5 (severe overcondition), where a score of 3 was assigned to cows observed with a well-balanced covered frame. The change in body condition score was calculated by subtracting the score at calving from that at 47 to 53 d postpartum. For change in body condition, the score at 47 to 53 d postpartum was chosen because lameness exposure was measured during the first 35 d postpartum and because cows are at high risk of reduced ovarian activity during the first and second month after calving (Opsomer et al., 2000).

Diagnosis of lameness

During the first 5 and 8 weeks postpartum, study cows were examined weekly (Tuesday) for diagnosis of lameness by using a locomotion scoring system described by Garbarino et al. (2004) (Table 1). This system was tested weekly over a 2-mo period (April-May, 2002) in all lactating cows, before enrolment of the first cow in the study (1st June 2002). After testing the locomotion scoring system in the study herd, a new category was added to include cows that were observed with an arched-back posture that was evident both while standing and walking, but their gait seemed normal (score = 2, mildly lame). Cows were observed and scored by the same veterinarian as they walked-out of the washing pen to the holding area before milking. Cows with a locomotion score of 4 or 5 were further examined on a tilt table for diagnosis and treatment of lameness, noting lesions observed and date of occurrence. Lame cows with claw lesions had white line lesions or sole ulcers and were treated by use of corrective foot trimming techniques (Shearer & van Amstel, 2001). Lame cows with sub-acute laminitis were those with yellow and red discoloration of the sole and white line, and in most cases they had thin soles and were sensitive at examination with hoof testers (Toussaint-Raven et al., 1985). Lame cows with interdigital dermatitis were those with inflammation confined to the epidermis and, in some cases, hyperkeratosis, which creates a roughened appearance to the interdigital skin (Blowey, 1994); a foetid serous exudate could be present, and there was mild sensitivity to pressure. This condition was frequently accompanied by cracks in the heel, heel horn erosions, with potential under-running of the heel horn (Berry, 2001).

Collection of blood samples and detection of plasma P_4 concentrations

Cows were scored for body condition (Edmonson et al., 1989) and blood-sampled weekly (Thursday) for detection of plasma P_4 concentrations during the first 300 d postpartum. Cows were blood-sampled via coccygeal venipuncture using vacutainer collection tubes containing K_3 EDTA (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were refrigerated until and during transportation to a laboratory at the University of Florida where they were centrifuged for 20 min at 3000 RPM at room temperature for plasma harvest. Plasma samples were frozen at – 20 C until tested for P_4 concentrations (Coat-A-Count radioimmunoassay).

Table 1. Frequency distribution of cows classified as lame or non-lame by using a locomotion scoring system

Locomotion score	Clinical description	Assessment criteria	No. of cows (%) (n = 238)
0	Non-lame	The cow stands and walks with a level-back posture. Her gait is normal.	3 (1)
1	Arched-back while walking	The cow stands with a level-back posture but develops an arched back posture while walking. Her gait remains normal.	17 (7)
2	Arched-back while standing and walking	An arched-back posture is evident both while standing and walking. Her gait seems normal.	76 (32)
3	Moderately lame	An arched-back posture is evident both while standing and walking. Her gait is affected and is best described as short strides with 1 or more limbs.	101 (42)
4	Lame	An arched-back posture is always evident and gait is best described as 1 deliberate step at a time. The cow favours 1 or more limbs/feet.	41 (17)
5	Severely lame	In addition, the cow demonstrates an inability or extreme reluctance to bear weight on 1 or more of her limbs/feet.	0 (0)

Outcomes

The main outcomes of interest were: (1) resumption of ovarian cyclicity during the first 60 d postpartum and (2) time to conception. Cows with evidence of normal ovarian cyclicity during the first 60 d postpartum were those with: i) weekly plasma P_4 concentrations > 1ng/ml for 2 or 3 consecutive samples and followed by a decline in P_4; or ii) if a P_4 concentration > 1 ng/ml was followed by a marked decrease after a $PGF_2\alpha$ injection and this followed by an increase in P_4 concentration. Cows with a delayed resumption of ovarian cyclicity were those with concentrations of P_4 consistently < 1ng/ml during the first 60 days postpartum (Staples et al., 1990). A follow-up period of 60 d postpartum was chosen because an Ovsynch protocol was initiated at 58 to 63 d postpartum, which was the end of the voluntary waiting period on the study farm.

Statistical analyses

The hypothesis that risk of delayed cyclicity is the same in cows classified as non-lame, moderately lame or lame was tested by using logistic regression. In the analysis, non-lame cows were those with a score of 3 for 1 wk only or scores of ≤2. The rationale for classifying cows with a score ≤ 2 as non-lame in the analysis was that their gait seemed normal. Cows classified as moderately lame were those with a score of 3 in at least 2 consecutive weeks to reduce the risk of misclassification. Lame cows were those classified at least once with a locomotion score of 4 or 5. Additional independent variables (lactation number, calving season, milk yield, dystocia, retained placenta, metritis, mastitis, ketosis, body condition score at calving and loss of body condition, use of $PGF_2\alpha$) were examined in the analysis to address possible confounding effects that these factors might have on the risk of delayed cyclicity. The association between body condition loss of 0.50 and 0.75 points and delayed cyclicity was examined and, because the associated OR was similar for both, body condition score loss of 0.75 was used in the analysis. Association between a body condition score loss of ≥ 1.0 point was not examined, because the frequency of cows in this category was low (n = 9). Stepwise forward regression was used, and a variable had to be significant at the 0.20 level before it could enter the model. A variable remained in the model when its significance level was $P < 0.10$. Variables for lactation number and calving season were forced into the model because it was known that they can affect ovarian activity (Fonseca et al., 1983; Jonsson et al., 1997; Lucy et al., 1992; Moreira et al., 2001; Savio et al., 1990; Stevenson & Britt 1979). In the final model, adjusted OR and 95% confidence intervals (CI) were calculated. In this study, the OR was used as an epidemiological measure of association between a variable (i.e. lameness) and the outcome of interest (i.e. delayed cyclicity). In each variable, the reference category had an OR = 1. An adjusted OR > 1.0 indicates that the probability of delayed cyclicity increased, compared with cows in the reference category. The model's goodness of fit was explored using the Hosmer-Lemeshow goodness of fit χ^2 statistic and standardized residuals. The attributable proportion was estimated as (OR – 1)/OR and interpreted to represent the proportion of lame cows that experienced delayed ovarian cyclicity because of lameness (Martin et al., 1987). The hypothesis that time to conception (days) is higher in cows classified as lame or moderately lame, compared to non-lame cows during the first 8 weeks postpartum was tested by using Cox proportional hazards regression analysis.

RESULTS

Objective 1: Risk of delayed cyclicity

All 253 cows enrolled in the first study were followed-up successfully during the 60-d study period. Two hundred and thirty-eight (94%) cows met the criteria for ovarian cyclicity and were used in this study. A total of 101 of 238 (42%) cows were classified as moderately lame (locomotion score = 3) and 41 (17%) as lame (score = 4). The mean number of days postpartum when cows were classified as lame was 15 d. The most common lesions observed in lame cows (score = 4) were sub-acute laminitis (26/41 = 63%), and claw lesions such as sole ulcers and white line disease (9/41 = 22%). The overall incidence of delayed cyclicity was 11%. The incidence of delayed cyclicity was higher in cows classified as moderately lame (14/101; 14%) or lame (7/41; 17%), compared to non-lame cows (6/96; 6%). In the univariable analysis, cows classified as lame had 3.09 times greater odds of delayed cyclicity, compared to non-lame cows (OR = 3.09; 95% CI = 0.97 to 9.83) (P = 0.05) (Table 2). In addition, cows classified as

Table 2. Descriptive statistics and unadjusted odds ratios (OR) for risk of delayed cyclicity in postpartum Holstein cows

Variable	Delayed cyclicity Yes No. of cows (%) (n = 27)	Delayed cyclicity No No. of cows (%) (n = 211)	OR	95% CI	P
Lameness group					
Locomotion score ≤ 2	6 (22)	90 (43)	1.00	Reference	NA
3	14 (52)	87 (41)	2.41	0.90 – 6.55	0.08
4	7 (26)	34 (16)	3.09	0.97 – 9.83	0.05
Lactation number					
1	10 (37)	77 (36)	1.00	Reference	NA
≥ 2	17 (63)	134 (64)	0.98	0.43 – 2.23	0.95
Season					
Winter	18 (67)	123 (58)	1.00	Reference	NA
Summer	9 (33)	88 (42)	0.70	0.30 – 1.62	0.40
Milk yield					
Low	9 (33)	50 (24)	1.05	0.43 – 2.54	0.91
Medium	16 (59)	102 (48)	1.00	Reference	NA
High	2 (8)	56 (27)	0.22	0.05 – 0.96	0.04
Dystocia					
No	20 (74)	163 (77)	1.00	Reference	NA
Yes	3 (11)	17 (8)	1.44	0.39 – 5.34	0.58
Retained placenta					
No	23 (85)	181 (86)	1.00	Reference	NA
Yes	4 (15)	30 (14)	1.05	0.34 – 3.24	0.93
Metritis					
No	16 (59)	132 (63)	1.00	Reference	NA
Yes	11 (41)	79 (37)	1.15	0.51 – 2.59	0.73
Mastitis					
No	25 (93)	174 (82)	1.00	Reference	NA
Yes	2 (7)	37 (18)	0.38	0.09 – 1.64	0.19
Ketosis					
No	18 (67)	177 (84)	1.00	Reference	NA
Yes	9 (33)	34 (16)	2.60	1.08 – 6.28	0.03
BCS at calving					
< 2.75	3 (11)	34 (16)	0.68	0.19 – 2.40	0.55
2.75 – 3.5	20 (74)	151 (71)	1.00	Reference	NA
> 3.5	4 (15)	26 (12)	1.28	0.40 – 4.10	0.67
BCS change (0.75)					
No	20 (74)	173 (82)	1.00	Reference	NA
Yes	7 (26)	38 (18)	1.59	0.63 – 4.03	0.32
Use of $PGF_2\alpha$					
No	17 (63)	125 (59)	1.0	Reference	NA
Yes	10 (37)	86 (41)	0.86	0.38 – 1.95	0.70

moderately lame tended to have greater odds of delayed cyclicity, compared to non-lame cows (OR = 2.41; 95% CI = 0.89 to 6.55; P = 0.08); but this association was not significant.

In the multivariable analysis, lameness, lactation number, season, ketosis and milk yield were retained in the final model. Addition of 2-way interaction terms did not improve the fit of the final model for risk of delayed cyclicity, and these terms were removed from the model. Cows classified as lame had 3.50 times greater odds of delayed cyclicity, compared to non-lame cows (OR = 3.50; 95% CI = 1.00 to 12.21; P = 0.04). The attributable proportion of cows that experienced delayed ovarian cyclicity associated with lameness was 0.71. In addition, cows classified as moderately lame tended to have greater odds of delayed cyclicity, compared to non-lame cows (OR = 2.14; 95% CI = 0.74 to 6.14; P = 0.15). Finally, ketosis was, by itself, a risk factor for delayed resumption of ovarian cyclicity (OR = 2.76; 95% CI = 1.08 to 7.06; P = 0.03). The Hosmer-Lemeshow goodness-of-fit statistic was 2.45 (8 degrees of freedom; P = 0.96) and indicated that the overall fit of the model was very good.

Objective 2: Time to conception

A total of 154 (31%), 214 (43%), and 131 (26%) cows were classified as non-lame, moderately lame, and lame during the pre-service postpartum period, respectively. Most cows classified as lame had sub-acute laminitis (54%), claw lesions or disorders (33%). Median time to conception was 36 days longer in lame cows, compared with non-lame cows (P = 0.11).

DISCUSSION

Analysis of the results of the study reported here support the hypothesis that lameness has a detrimental effect on ovarian activity in Holstein cows during the early postpartum period. Cows classified as lame had 3.5 times greater odds of delayed cyclicity than non-lame cows. Attributable proportion analysis indicated that delayed ovarian cyclicity in lame cows would be reduced by 71% if lameness had been prevented. To the authors' knowledge, only 1 previous study has examined the relationship between lameness and ovarian activity. In a study conducted in 335 dairy cows in 6 high yielding dairy herds in Belgium, cows diagnosed with clinical mastitis, severe lameness or pneumonia by farmers were at higher risk of delayed cyclicity, compared to cows classified as clinically healthy (Opsomer et al., 2000); however, the actual number of cows affected with clinical mastitis, severe lameness or pneumonia was not reported.

In this study, that starting hypothesis was that because lame cows experience a more pronounced loss in body condition (hence a prolonged state of negative energy balance) during the early postpartum period, they are at higher risk of delayed cyclicity than non-lame cows. Although an association between lameness and delayed cyclicity was established, loss of body condition (or a modifying effect of lameness and loss of body condition) was not identified as a significant risk factor associated with delayed cyclicity. Ketosis was, by itself, a risk factor for delayed resumption of ovarian cyclicity. Thus, it is possible that lameness and ketosis may additionally interact with each other to affect risk of delayed cyclicity. Lameness can depress dry matter intake resulting in negative energy balance, and it is well accepted that negative energy balance contributes to increased ketone body formation and delays the onset of ovarian activity. A negative energy balance postpartum not only contributes to increased ketogenesis, but also delays the onset of ovarian cyclicity, especially if energy deficiency is prolonged. Lame

cows that experience delayed ovarian cyclicity during the pre-service postpartum period would be expected to have a longer calving-to-conception interval, if lameness is not resolved.

REFERENCES

Beam, S.W. and Butler, W.R. (1998). Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81,121-131

Berry, S.L. (2001). Interdigital Dermatitis. In: Anderson D, (Ed), Vet. Clin. N. Am-Food A. 17, 134-135

Blowey, R.W. (1994). Interdigital causes of lameness. Proceedings of the 8th International Symposium on Disorders of the Ruminant Digit. Banff, Canada. pp 142-154

Butler, W.R. and Smith, R.D. (1989). Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72, 767-783

Collick, D.W., Ward, W.R and Dobson, H. (1989). Associations between types of lameness and fertility. Vet. Rec. 125, 103-106

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G. (1989). A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72, 68-78

Etherington W.G., Christie, K.A., Walton, J.S., Leslie, K.E., Wickstrom, S. and Johnson, W.H. (1991). Progesterone profiles in postpartum Holstein dairy cows as an aid in the study of retained fetal membranes, pyometra and anestrus. Theriogenology 35, 731-746

Fonseca F.A., Britt, J.H., McDaniel, B.T., Wilk, J.C., and Rakes, A.H. (1983). Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66, 1128-1147

Garbarino E.J., Hernandez J.A. and Shearer J.K. (2004). Effect of lameness on ovarian activity in postpartum Holstein cows. J Dairy Sci 87, 4123-4131

Green, L.E., Hedges, V.J., Schukken, Y.H., Blowey, R.W. and Packington, A.J. (2002). The impact of clinical lameness on the milk yield of dairy cows. J. Dairy Sci. 85, 2250-2256

Hassall, S.A., Ward, W.R. and Murray, R.D. (1993). Effects of lameness on the behaviour of cows during the summer. Vet. Rec. 132, 578-580

Hernandez, J., Shearer, J.K. and Elliott, J.B. (1999). Comparison of topical application of oxytetracycline and four nonantibiotic solutions for treatment of papillomatous digital dermatitis in dairy cows. J. Am. Vet. Med. Assoc. 214, 688-690

Hernandez, J. and Shearer, J.K. (2000). Efficacy of oxytetracycline for treatment of papillomatous digital dermatitis lesions on various anatomic locations in dairy cows. J. Am. Vet. Med. Assoc. 216, 1288-1290

Hernandez, J., Shearer, J.K. and Webb, D.W. (2001). Effect of lameness on the calving-to-conception interval in dairy cows. J. Am. Vet. Med. Assoc. 218, 1611-1614

Hernandez, J., Shearer, J.K. and Webb D.W. (2002). Effect of lameness on milk yield in dairy cows. J. Am. Vet. Med. Assoc. 20, 640-644

Jonsson N.N., McGowan, M.R., McGuigan, K., Davison, T.M., Hussain, A.M., Kafi, M. and Matschoss, A. (1997). Relationships among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. Animal Reprod. Sci. 47, 315-326.

Lee, L.A., Ferguson, J.D. and Galligan, D.T. (1989). Effect of disease on days open assessed by survival analysis. J. Dairy Sci. 72, 1020-1026

Lucey, S., Rowlands, G.J. and Russell, A.M. (1986). The associations between lameness and fertility in dairy cows. Vet. Rec. 118, 628-631.

Lucy, M.C., Staples, C.R., Michel, F.M. and Thatcher, W.W. (1991). Energy balance and size and number of ovarian follicles detected by ultrasonography in early post partum dairy cows. J. Dairy Sci. 74, 473-482

Lucy, M.C., Staples, C.R., Thatcher, W.W., Erickson, P.S., Cleale, R.M., Firkins, J.L., Clark, J.H., Murphy, M.R. and Brodie, B.O. (1992). Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. Anim. Repro. Sci. 54, 323-331

Martin, S.W., Meek, A.H. and Willeberg, P. (1987). Disease causation. In: Veterinary Epidemiology. Ames, Iowa: Iowa State University Press. pp 121-148.

Melendez, P., Bartolome, J., Archbald, L.F. and Donovan, A. (2003). The association between lameness, ovarian cysts and fertility in lactating dairy cows. Theriogenology 59, 927-937

Moore, D.A., Berry, S.L., Truscott, M.L. and Koziy, V. (2001). Efficacy of a nonantimicrobial cream administered topically for treatment of digital dermatitis in dairy cattle. J. Am. Vet. Med. Assoc. 219, 1435-1438

Moreira F., Orlandi, C., Risco, C.A., Mattos, R., Lopes, F. and Thatcher, W.W. (2001). Effects of presynchronization and bovine somatotropin on pregnancy rates to timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84, 1646-1659

Nakao T., Moriyoshi, M. and Kawata, K. (1992). The effect of postpartum ovarian dysfunction and endometritis on subsequent reproductive performance in high and medium producing dairy cows. Theriogenology 37, 341-349

National Animal Health Monitoring System -Dairy 2002 Part I: Reference of Dairy Health and Management in the United States, 2002. United States Department of Agriculture: Animal Plant Health Inspection Service: Veterinary Services, Fort Collins. #N341.0701.

Opsomer, G., Coryn, M., Deluyker, H. and de Kruif, A. (1998). An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. Reprod. Dom. Anim. 33, 193-204

Opsomer, G., Gronhn, Y.T., Hertl, J., Coryn, M., Deluyker, H. and de Kruif, A. (2000). Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Thereogenology 53, 481-857

Savio J.D., Boland, M.P., Hynes, N. and Roche, J.F. (1990). Resumption of follicular activity in the early post-partum period of dairy cows. J. Reprod. Fert. 88, 569-579

Shearer, J.K. and Elliott, J.B. (1998). Papillomatous digital dermatitis: treatment and control strategies – Part I. Compend. Contin. Educ. Pract. Vet. 20, S158-S166

Shearer, J.K., Hernandez, J. and Elliott, J.B. (1998). Papillomatous digital dermatitis: treatment and control strategies – Part II. Compend. Contin. Educ. Pract. Vet. 20, S213-S223

Shearer, J.K. and van Amstel, S.R. (2001). Functional and corrective claw trimming. In: Anderson DA, eds. The Veterinary Clinics of North America. Philadelphia, Pennsylvania: WB Saunders Co. pp 53-72.

Sprecher, D.J., Hostetler, D.E. and Kaneene, J.B. (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47, 1179-1187

Staples, C.R., Thatcher, W.W. and Clark, J.H. (1990). Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73, 938-947

Stevenson J.S. and Britt, J.H. (1979). Relationships among luteinizing hormone, estradiol, progesterone, glococorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci. 48, 570-577

Stevenson, J.S. and Call, E.P. (1983). Influence of early estrus, ovulation, and insemination on fertility in postpartum Holstein cows. Theriogenology 19, 367-375

Thatcher, W.W. and Wilcox, C.J. (1973). Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 59, 608-610

Toussaint-Raven, E., Haalstra, R.T. and Peterse, D.J. (1985). Cattle Footcare and claw trimming. Ipswich, UK, Farming Press. pp 62.

Van Arendonk, J.A.M., Stokvisch, P.E. and Korver, S. (1984). Factors determining the carcass value of culled dairy cows. Livest. Prod. Sci. 11, 391-400

Warnick, L.D., Jansen, D., and Guard, C.L. (2001). The effect of lameness on milk production in dairy cows. J. Dairy Sci. 84, 1988-1997

A SURVEY OF ANTI-*OSTERTAGIA OSTERTAGI* ANTIBODIES IN BULK TANK MILK AND THEIR RELATIONSHIP WITH MILK PRODUCTION AND HERD MANAGEMENT FACTORS

J. CHARLIER[1], L. DUCHATEAU, E. CLAEREBOUT AND J. VERCRUYSSE

SUMMARY

Currently, the most promising diagnostic tool to detect dairy herds where the infection level with gastrointestinal nematodes interferes with productivity is a milk *Ostertagia ostertagi* enzyme-linked immunosorbent assay (ELISA). The objectives of the present studies were (1) to assess the repeatability of the ELISA, (2) to use the ELISA to determine the relationships of Ostertagia specific bulk tank milk antibody levels (ODR) with production parameters and (3) to investigate the relationship of the ELISA results with herd management factors.

The repeatability of the ELISA was found to be good. An increase in the ODR from the 25th to the 75th percentile was associated with a drop in the annual milk yield of 0.9 kg/cow/day. No important relationships were found between ODR and % fat or % protein. Differences in mean ODR were observed between herds with different pasture management.

The results indicate that the bulk tank milk *Ostertagia ostertagi* ELISA is a promising diagnostic technique to detect herds in which the infection level affects productivity.

INTRODUCTION

In contrast to first season grazing calves, infections with gastrointestinal (GI) nematodes in older cattle were for a long time considered to be of limited importance, merely due to the absence of clinical symptoms and the lower levels of infection usually found in these animals. However, several studies (e.g. Agneessens et al., 2000; Borgsteede et al., 2000) demonstrated however that GI nematodes are still widespread among adult cows in regions with a temperate climate, with a prevalence of infection between 80-100%. The most prevalent parasite species was *Ostertagia ostertagi.* Two reviews demonstrate that subclinical GI nematode infections in adult cows can have an effect on milk production. Gross et al. (1999) reported a significant increase in milk production after anthelmintic treatment in 70 of 87 experiments (80%), with a median increase of 0.63 kg/cow per day. A meta-analysis of the milk production response after treatment in 75 experiments estimated an overall treatment effect of 0.35 kg/cow per day (Sanchez et al., 2004a). Although there seems to be sufficient evidence to accept the impact of nematode infections on milk production, in many studies treatment responses show a large

[1] Department of Virology, Parasitology and Immunology; Faculty of Veterinary Medicine; Ghent University; Salisburylaan 133, 9820 Merelbeke, Belgium. Email: johannes.charlier@ugent.be

variation between different herds (e.g. Ploeger et al., 1989, 1990; Walsh et al., 1995). This might be due to differences in level of infection between herds (Ploeger et al., 1989).

However, a major problem in adult animals is to determine a threshold infection level above which productivity is affected. Only antibody levels against *O. ostertagi* are considered as a promising parameter (Eysker & Ploeger, 2000). Previously, significant correlations were found between the mean of individual serum antibody levels and the bulk tank milk antibody level (Kloosterman et al., 1993; Sanchez et al., 2002b). Also, relationships were found between bulk tank milk antibody levels and certain management practices known to be associated with infection levels, suggesting that bulk milk antibody levels were a reasonable measure of parasite infection levels in a dairy herd (Guitián et al., 2000; Sanchez & Dohoo, 2002). In addition, a negative relationship was established between bulk tank milk antibody levels and measures of productivity. However, previous work has been mainly conducted in Canada (Caldwell et al., 2002; Guitián et al., 2000; Nødvedt et al., 2002; Sanchez et al., 2002b), where herd infection levels and access to pasture appear to be lower than in West European studies (e.g. Agneessens et al., 2000; Borgsteede et al., 2000). Moreover, since milk production is influenced by many factors other than GI nematode burdens, there is a need to confirm these previous results in large-scale studies. In the present study, the repeatability of a milk *O. ostertagi* ELISA was investigated. Subsequently, the ELISA was used in a large-scale survey to determine the relationships between the specific bulk tank milk antibody levels and three production parameters (kg milk, % fat, and % protein). In addition, associations between certain management factors and the specific antibody levels were investigated.

MATERIALS AND METHODS

Selection of farms and sample collection

For the repeatability trial, 40 milk samples were collected by convenience sampling from 2 dairy herds. In the survey to determine associations with milk production parameters and management factors, the study population consisted of all the herds that participated in the milk production recording programme of the Flemish Cattle Breeders Organization (V.R.V.). From this population, 867 herds were randomly selected. In September 2003, a bulk tank milk sample was taken from each of the selected herds during the routine milk collection for the dairy cooperatives, in cooperation with the Milk Control Centre Flanders (MCC Flanders). All samples arrived at the laboratory between 24 and 72 hours after collection at the farms. During the handling procedures, the samples remained constantly chilled at 4°C. After arrival at the laboratory, the milk samples were centrifuged (16,000 *g*, 5 minutes), fat was skimmed off and the supernatant was collected and frozen at –20°C untill further analysis.

ELISA procedure

The milk samples were thawed and re-centrifuged (16,000 g, 5 minutes) before analysis. To determine specific antibody levels, a crude-antigen *O. ostertagi* ELISA was used. Briefly, a crude saline extract of an adult *O. ostertagi* preparation was used as the antigen (Keus et al., 1981). Microtiter plates (96 well) were coated with this antigen at a concentration of 1 µg/ml (pH 9.6) and incubated overnight at 4°C. Wells were washed 3 times with 0.4 ml PBST (phosphate buffered saline, 0.05% Tween-20). Non-specific binding sites were blocked using 3% foetal calf serum (FCS) in PBST. Plates were incubated for 1 hour at 20°C and washed as before. Control and milk samples were added to the wells, and plates were incubated and

washed as before. Rabbit anti-bovine IgG (1/1500 in PBST/1%FCS) coupled to horseradish peroxidase was used as the conjugate. Plates were incubated and washed as before. ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-sulfonic acid) was used as the substrate. After incubation for 30 min (20°C), absorbance was read at 405 nm and at 492 nm. The optical density (OD) was the result of the subtraction of the OD at 492 nm from the OD at 405 nm. The optical density ratio (ODR) was calculated following the formula ODR = OD – NC / PC – NC, where NC and PC are the OD values of the negative control and positive control respectively.

Repeatability trial

Forty milk samples were tested in duplicate per plate on 4 different ELISA plates per day. This was repeated on 2 days. From this test the between-duplicate, between-plate and between-day repeatability were determined and compared to the between-sample variability.

Collection of production and farm data

Production data were obtained from the milk production recording programme of the V.R.V. The data were obtained from October 2002 to September 2003. The following herd-level variables were computed based on the individual test-day cow production data: monthly averages of kg milk/cow/day, protein%, fat%, lactation number, days in milk (DIM), somatic cell count, number of milk producing cows and main breed. The main breed was defined as the breed that occurs most frequently, given that its proportion was higher than 80%. Otherwise, the main breed was mixed. The province of each herd was noted. The number of months for which production data could be obtained differed between the herds. This was due to herds that ceased to or began to participate in the milk production recording programme during the investigation period.

Information concerning herd management was obtained through a questionnaire. The questions were closed-ended and concerned pasture management. The questionnaires were administered by personal interview by the milk collectors of the V.R.V. during the period end of November to December 2003. In addition, information on herd type was obtained from an external dataset (SANITEL). A herd was considered as 'dairy and beef' if at least 5 beef cows were present along with dairy cows, otherwise the herd was classified as 'dairy'.

Data analysis

The test of repeatability over duplicates within plate, plates and days was modelled by a random effects model (Eq. 1):

$$Y_{ijkl} = \mu + s_i + d_j + p_{k(j)} + e_{ijkl} \quad (1)$$

with Y_{ijkl} the l^{th} measurement of sample i on plate k on day j (l=1,2; i= 1-40; k= 1-4; j= 1,2)

μ overall mean

s_i effect of sample i ~N(0, σ_s^2)

d_j effect of day j ~N(0, σ_d^2)

$p_{k(j)}$ effect of plate k within day j ~N(0, σ_p^2)

e_{ijkl} random error term ~N(0, σ^2)

Interaction terms were evaluated but were not included in the model because they were not significant. The variance components related to the different sources of variability were estimated by restricted maximum likelihood (Patterson and Thompson, 1971). Normality of the observed ODR values and the residuals was investigated by a histogram and a normal probability plot. Based on these diagnostics, there was no reason to reject the normal distribution assumption. Based on the variance component estimates, the amount of variance that could be attributed to each source of variability (replicate, plate, day) was expressed as a proportion of the total between sample variance. Overall measures with respect to duplicate-within-plate, plate and day variability were obtained as follows. The variance of the difference between two measurements from the same sample and same plate is given by Eq. (2), the variance of the difference between two measurements from the same sample and different plates on the same day is given by Eq. (3), the variance of the difference between two measurements from the same sample but on a different day is given by Eq. (4).

$$\mathrm{Var}\left(Y_{ijkl} - Y_{ijkl'}\right) = 2\sigma^2 \tag{2}$$

$$\mathrm{Var}\left(Y_{ijkl} - Y_{ijk'l'}\right) = 2\left(\sigma^2 + \sigma_p^2\right) \tag{3}$$

$$\mathrm{Var}\left(Y_{ijkl} - Y_{ij'k'l'}\right) = 2\left(\sigma^2 + \sigma_p^2 + \sigma_d^2\right) \tag{4}$$

The effect of bulk tank milk ODR on three different production parameters; milk production, protein% and fat%, was assessed by multivariable linear regression. The model contained the following covariates: average lactation number, average DIM, main breed, average somatic cell count, average number of producing animals and province, because these are considered possible confounders. The relationship between the ODR and the production parameters averaged over the whole preceding year was studied with covariates also averaged over the year. Most herds had production data for each month. In the case of missing information for a particular month, the values were imputed by the mean value of the production parameter in that month over all farms, multiplied by an adjustment factor. The adjustment factor is derived from the ratio of the mean of the production parameter in the particular farm over the months for which information was available and the mean over those months over all other farms.

The questionnaire was validated by determining the Spearman rank correlation coefficient between the herd type as determined by the questionnaire and by the SANITEL-dataset. The mean ODR was calculated per category for the herd management variables.

RESULTS

Repeatability

The random effects analysis resulted in a total variance estimate of 0.113. Ninety four percent of the total variance was attributable to the milk sample, 5% to the duplicates within a plate, 1% to the plate within the same day and 0% to the day. In other words, 94% of the variability was explained by the milk sample and 6% was explained by the assay variability. Based on these estimates, the overall variability of the difference between two measurements of the same sample as a function of their origin (replicate, plate, day) was deduced. The expected 95% range is - 0.14 to 0.14 for different ODR readings of the same sample on the same plate and - 0.16 to 0.16 for different ODR readings of the same sample on different plates or on different days.

ODR values and farm data

The average ODR of the 867 sampled dairy herds was 0.942 with a standard deviation of 0.245. The interquartile range was from 0.798 to 1.094 and the range from –0.133 to 1.899.

The herds for which production data could be obtained were located in the 5 Flemish provinces. The herds were distributed approximately proportionally in relation to the total number of herds in each province. The main breed was classified as mixed for most of the herds (61.5%), followed by Black Holstein (30.8%), Red Holstein (6.6%), East-Flemish (0.8%), Belgian Blue mixed (0.1%), Red-pied (0.1%) and Red (0.1%). The average and standard deviation of the annual production parameters are displayed in Table 1.

The response rate to the questionnaire was 93%. There were 779 herds for which both a completed questionnaire and an ODR value were available. The correlation coefficient between herd type as determined by the questionnaire and by the SANITEL-dataset was 73%.

Relationship between ODR and milk production parameters

The regression coefficients for ODR from the multivariable analysis to determine the relationships with milk yield, protein% and fat% are shown in Table 2. Significant effects were found for the covariates average number of producing animals, average days in milk, average somatic cell count, province and main breed. After controlling for these factors, a significant negative linear relationship was found between ODR and annual average milk yield. An increase in the ODR from 0.798 to 1.094 was associated with a decrease in milk production of 0.9 kg/cow/day.

A significant negative linear relationship was found between ODR and milk protein% with a regression coefficient of –0.0341 ($P < 0.01$) for ODR. No significant associations were found between ODR and average milk fat%.

Relationship between ODR and management factors

The mean ODR showed a gradual increase with increasing exposure to pasture. The mean ODR for herds in which cows were kept in total confinement (n=5) was 0.053 vs. 0.941 and

0.958 for herds in which cows had access to a small area with grass (n=164) or access to pasture (n=591), respectively. A gradual decrease of the mean ODR was observed for later turn out of the cows. The mean ODR of herds in which cows were turned out on pasture in March (n=87), April (n=508), May (n=146) and June (n=14) was 1.005, 0.974, 0.888 and 0.530, respectively.

Table 1. Year averages (and standard deviation) of the milk production parameters.

Variable	October 2002-September 2003
Average milk yield (kg/cow/day)	23.3 (4.80)
Average milk fat%	4.2 (0.34)
Average milk protein%	3.4 (0.15)
Average lactation number	2.6 (0.46)
Average days in milk	194 (38.2)
Average number of producing animals	38.2 (19.85)
Average somatic cell count/1000	265 (150.1)

Table 2. Regression coefficients (and 95% confidence interval) of the multivariable linear regression models to determine the relation of ODR with annual average milk yield (kg/cow/day), protein% and fat%.

	Regression Coefficient	P
Milk yield	-3.20 (-4.07; -2.32)	<0.001
Protein%	-0.034 (-0.060; -0.008)	<0.01
Fat%	0.037 (-0.027; 0.100)	0.26

DISCUSSION

In the present study, the repeatability of the *O. ostertagi* milk ELISA was studied. The species specificity was investigated by Keus et al. (1981), who reported cross-reactions with *Cooperia* spp. However, this is not considered a disadvantage, since the ELISA is used to measure total gastrointestinal parasite load, rather than just *O. ostertagi* infections. Sanchez et al. (2002a) demonstrated previously that adjusting the raw optical densities to optical density ratios (ODR) gave the most repeatable results for the ELISA studied here. Since the ODR values are the ones that are used in practice, in the repeatability trial, only the variability that is left after normalisation to ODR values was investigated. The random effect analysis allowed investigation of different sources of variability and assessment of the additional variability that is caused by each source of variation that is added in the model. According to standards laid down by Fleiss (1986), the amount of variability that was explained by the milk sample and the assay variability in the current study indicates an excellent repeatability of the ELISA test. No additional between day variability was observed. However, this result has to be considered with much caution since only two days were investigated. The between-duplicate variability was responsible for a considerable amount of additional variability. Therefore, each sample was tested in duplicate in the survey to determine relationships with milk production.

In the survey, the sample size of herds was large and the herds were randomly selected over the different provinces. However, only herds participating in a milk production recording programme were sampled. Since most of the modern dairy herds in Flanders participate in this programme, it is assume that the results are representative of modern Flemish dairy herds. Due to the similarity in the epidemiology of GI nematodes in the temperate climate regions of Western Europe (Shaw et al., 1998a), it is likely that the results of our study can also be applied in these regions.

September was chosen as the month of sample collection. It is known that antibodies against *O. ostertagi* follow a seasonal pattern, with highest levels in late summer and autumn, and lowest in spring and early summer. This pattern follows the expected epidemiological pattern of uptake of infective larvae (Agneessens et al., 2000; Borgsteede et al., 2000). For this reason, it is likely that the between-herd variation is greatest in late summer and autumn.

The relationship found between ODR and milk yield suggests that milk yield losses due to GI nematodes in a dairy herd can be estimated by determination of bulk tank milk antibody levels. The results are comparable with a previous report in Canada, where an increase in ODR from the 25th to the 75th percentile was associated with a drop in the milk production of 1.2 kg/cow/day (Sanchez & Dohoo, 2002). Although several possible confounding factors were controlled for in the analysis, the question remains whether there is a causal relationship between anti-*Ostertagia* antibody levels and milk production. Other effects, such as dilution of specific antibody titres with increasing milk production (Kloosterman et al., 1993) or mastitis, could also cause a negative relationship between ODR and milk yield. Since transport of IgG to the mammary secretion is a receptor dependent process (Butler, 1998), it is possible that this transport does not increase equally with milk production. A dilution effect of milk yield on the IgG concentration in milk has been suggested by Watson et al. (1972) and Caffin et al. (1983). However, in a recent study, it was suggested that individual ODR values are not greatly influenced by milk production (Sanchez et al., 2004b). Also, mastitis could bias the results. Serum total IgG concentrations are approximately 35 fold higher than total IgG concentrations in mature milk (Butler, 1986) and an infection of the udder can cause a flow of specific and non-specific antibodies from the serum to the milk (Caffin et al. 1983; Guidry et al., 1980). However, acutely mastitic cows generally do not contribute to the bulk tank milk and, in this study, the subclinical mastitis effect was controlled for by including the factor 'average somatic cell count' in the regression model. A causal relationship between anti-*Ostertagia* antibody levels and milk yield can only be determined by a treatment trial that investigates whether there is a greater treatment response in high ODR herds than in low ODR herds. Sanchez et al. (2002b) demonstrated an effect of the individual cow ODR on response to treatment. High ODR cows had an increase of 2.87 kg/day following treatment, while there was no apparent effect in low ODR cows. However, at the herd level, no significant association between bulk tank antibody level and treatment response has thus far been demonstrated. A significant positive correlation between the mean herd milk-production response to treatment with the mean herd serum *Ostertagia* antibody titre was demonstrated by Ploeger et al. (1989). Kloosterman et al. (1996) found a larger treatment response in high bulk tank milk antibody level herds than in low antibody level herds, although these differences lacked statistical significance.

The size of the association between ODR and milk protein % was small. An increase in ODR over the interquartile range was associated with a decrease in milk protein concentration of only 0.01%. No significant associations were found between ODR and milk fat%. Therefore, it is concluded that there exists no important relationship between ODR and milk solids

concentration. These results agree with 2 studies in which no increase in these concentrations was found after anthelmintic treatment (McPherson et al., 2001; Walsh et al., 1995).

In contrast to risk factors associated with infection level in first and second grazing calves, which are well described (e.g. Hansen et al., 1989; Ploeger et al., 2000; Shaw et al., 1998 a, b), very little information is available on factors that have an impact on the infection level of adult dairy cows. The results of this study suggest that some factors which are known to influence the infection level in calves can also be considered as risk factors in cows. The milk *O. ostertagi* ELISA seems to be a valuable measure to detect management factors associated with infection level of the cows. Based on these findings, alternative control measures can be proposed to lower the infection level and reduce production losses due to GI nematodes in dairy herds.

As a general conclusion, there exists in the Flemish dairy herds an important negative relationship between milk yield and GI nematode infection level, as estimated by the bulk tank milk *O. ostertagi* ELISA, while there is no strong relationship between infection level and milk solids percentage. The ELISA can be used to detect management factors associated with herd infection level. More research should be conducted to investigate whether the bulk tank milk *O. ostertagi* ELISA can be used as a diagnostic tool to predict the milk production response after anthelmintic treatment.

ACKNOWLEDGEMENTS

The authors thank Roland Bossuyt and Jean-Marie Van Crombrugghe of the Milk Control Centre Flanders (MCC Flanders) and Etienne De Mûelenaere of the Flemish Cattle Breeders Organisation (V.R.V.) for their cooperation. It would not have been possible to administer the questionnaires without the work of the milk collectors of the V.R.V. Jean-Marie Robijns is thanked for providing the SANITEL-data. We are also grateful to Iris Peelaers and Dirk De Meulenaere for their technical assistance. This study was funded by Merial.

REFERENCES

Agneesssens, J., Claerebout, E., Dorny, P., Borgsteede, F.H.M. and Vercruysse, J. (2000). Nematode parasitism in adult dairy cows in Belgium. Vet. Parasitol. 90, 83-92

Borgsteede, F.H.M., Tibben, J., Cornelissen, J.B.W.J., Agneessens, J. and Gaasenbeek, C.P.H. (2000). Nematode parasites of adult dairy cattle in the Netherlands. Vet. Parasitol. 89, 287-296

Butler, J.E. (1986). Biochemistry and biology of ruminant immunoglobulins. Prog. Vet. Microbiol. Immun. 2, 1-53

Butler, J.E. (1998). Immunoglobulin diversity, B-cell and antibody repertoire development in large farm animals. Rev. sci. tech. Off. int. Epiz. 17, 43-70

Caffin, J.P., Poutrel, B. and Rainard, P. (1983). Physiological and pathological factors influencing bovine immunoglobulin G_1 concentration in milk. J. Dairy Sci. 66, 2161-2166

Caldwell, V., DesCôteaux, L., Bouchard, E., DuTremblay, D., Dohoo, I. and Markham, F. (2002). Gastrointestinal nematodes in Québec dairy cattle: herd prevalence, level of infection estimated by bulk tank milk ELISA testing and related risk factors. Bovine Pract. 36, 117-125

Eysker, M. and Ploeger, H.W. (2000). Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitology 120, S109-S119

Fleiss, J.L. (1986). The design and analysis of clinical experiments. New York: Wiley, 432pp

Gross, S.J., Ryan, W.G. and Ploeger, H.W. (1999). Anthelmintic treatment of dairy cows and its effect on milk production. Vet. Rec. 144, 581-587

Guidry, A.J., Paape, M.J. and Pearson, R.E. (1980). Effect of udder inflammation on milk immunoglobulins and phagocytosis. Am. J. Vet. Res. 41, 751-753

Guitián, F.J., Dohoo, I.R., Markham, R.J.F., Conboy, G. and Keefe, G.P. (2000). Relationships between bulk-tank antibodies to *Ostertagia ostertagi* and herd-management practices and measures of milk production in Nova Scotia dairy herds. Prev. Vet. Med. 47, 79-89

Hansen, J.W., Zajac, A.M., Eversole, D.E. and Gerken, Jr. H.J. (1989). The effect of stocking rate and parasite control on the performance of replacement beef heifers on pasture. Vet. Parasitol. 34: 103-115

Keus, A., Kloosterman, A. and Van den Brink, R. (1981). Detection of antibodies to *Cooperia* spp. and *Ostertagia* spp. in calves with the enzyme-linked immunosorbent assay (ELISA). Vet. Parasitol. 8: 229-236

Kloosterman, A., Verhoeff, J., Ploeger, H.W. and Lam, T.J.G.M. (1993). Antibodies against nematodes in serum, milk and bulk milk samples as possible estimators of infection in dairy cows. Vet. Parasitol. 47, 267-278

Kloosterman, A., Ploeger, H.W., Pieke, E.J., Lam, T.J.G.M. and Verhoeff, J. (1996). The value of bulk milk ELISA *Ostertagia* antibody titres as indicators of milk production response to anthelmintic treatment in the dry period. Vet. Parasitol. 64, 197-205

McPherson, W.B., Gogolewski, R.P., Slacek, B., Familton, A.S., Gross, S.J., Maciel, A.E. and Ryan, W.G. (2001). Effect of a peri-parturient eprinomectin treatment of dairy cows on milk production. New. Zeal. Vet. J. 49, 106-110

Nødvedt, A., Dohoo, I., Sanchez, J., Conboy, G., DesCôteaux, L., Keefe, G., Leslie, K. and Campbell, J. (2002). The use of negative binomial modelling in a longitudinal study of gastrointestinal parasite burdens in Canadian dairy cows. Can. J. Vet. Res. 66, 249-257

Patterson, H.D. and Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. Biometrika 58, 545-554

Ploeger, H.W., Schoenmaker, G.J., Kloosterman, A. and Borgsteede, F.H.M. (1989). Effect of anthelmintic treatment of dairy cattle on milk production related to some parameters estimating nematode infection. Vet. Parasitol. 34, 239-253

Ploeger, H.W., Kloosterman, A., Bargeman, G., Wijckhuise, L.V. and Brink, R. van den (1990). Milk yield increase after anthelmintic treatment of dairy cattle related to some parameters estimating worm infections. Vet. Parasitol. 35, 103-106

Ploeger, H.W., Borgsteede, F.H.M., Sol, J., Mirck, M.H., Huyben, M.W.C., Kooyman, F.N.J. and Eysker, M. (2000). Cross-sectional serological survey on gastrointestinal and lung nematode infections in first and second-year replacement stock in The Netherlands: relation with management practices and use of anthelmintics. Vet. Parasitol. 90: 285-304

Sanchez, J. and Dohoo, I. (2002). A bulk tank milk survey of *Ostertagia ostertagi* antibodies in dairy herds in Prince Edward Island and their relationship with herd management factors and milk yield. Can. Vet. J. 43, 454-459

Sanchez, J., Dohoo, I.R., Markham, F., Leslie, K. and Conboy, G. (2002a). Evaluation of the repeatability of a crude adult indirect *Ostertagia ostertagi* ELISA and methods of expressing test results. Vet. Parasitol. 109, 75-90

Sanchez, J., Dohoo, I., Nødtvedt, A., Keefe, G., Markham, F., Leslie, K., DesCôteaux, L. and Campbell, J. (2002b). A longitudinal study of gastrointestinal parasites in Canadian dairy farms The value of an indirect *Ostertagia ostertagi* ELISA as a monitoring tool. Vet. Parasitol. 107, 209-226

Sanchez, J., Dohoo, I., Carrier, J. and DesCôteaux, L. (2004a). A meta-analysis of the milk-production response after anthelmintic treatment in naturally infected adult dairy cows. Prev. Vet. Med. 63, 237-257

Sanchez, J., Markham, F., Dohoo, I., Sheppard, J., Keefe, G. and Leslie, K. (2004b). Milk antibodies against *Ostertagia ostertagi:* relationships with milk IgG and production parameters in lactating dairy cattle. Vet. Parasitol. 120, 319-330

Shaw, D.J., Vercruysse, J., Claerebout, E. and Dorny, P. (1998a). Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprofylaxis. Vet. Parasitol. 75: 115-131

Shaw, D.J., Vercruysse, J., Claerebout, E. and Dorny, P. (1998b). Gastrointestinal nematode infections of first-grazing season calves in Western Europe: Associations between parasitological, physiological and physical factors. Vet. Parasitol. 75: 133-151

Walsh, T.A., Younis, P.J. and Morton, J.M. (1995). The effect of ivermectin treatment of late pregnant dairy cows in south-west Victoria on subsequent milk production and reproductive performance. Aus. Vet. J. 72, 201-207

Watson, D.L., Brandon, M.R. and Lascelles, A.K., (1972). Concentrations of immunoglobulin in mammary secretion of ruminants during involution with particular reference to selective transfer of IgG_1. Aust. J. exp. Biol. med. Sci. 50, 535-539

QUANTIFICATION OF THE BETWEEN-FLOCK TRANSMISSION OF AVIAN INFLUENZA A VIRUS (H7N7) DURING THE 2003 EPIDEMIC IN THE NETHERLANDS

J.A. STEGEMAN[1], A. BOUMA, A.R.W. ELBERS, M.C.M. DE JONG, G. KOCH AND M. VAN BOVEN

SUMMARY

In 2003 The Netherlands was struck by an epidemic of high-pathogenicity avian influenza (HPAI) A virus subtype H7N7. During the epidemic, 255 flocks became infected and over 30 million birds were culled before the virus was finally eliminated. The epidemic was combated by stamping out infected flocks and pre-emptive culling of flocks within a one km radius. Moreover, screening and tracing activities were implemented for the early detection of infected flocks and a transportation ban was enforced. At a further stage of the epidemic, poultry free buffer zones were created, contacts between different parts of the country were reduced by compartmentalisation and large areas were depopulated of all poultry.

To evaluate the effectiveness of the control measures, the between-flock transmission characteristics of the virus were quantified in two affected areas, using the reproduction ratio R_h (average number of secondary infections caused by one infected flock). The control measures markedly reduced the transmission of HPAI virus: R_h before detection of the first infected flock was 6.5 (95% confidence interval 3.1-9.9) in one area and 3.1 in another area, and decreased to 1.2 (95% CI 0.6-1.9) after detection of the first outbreak in both areas. The observation that R_h remained greater than 1 suggests that the containment of the epidemic was probably due to the reduction in the number of susceptible flocks by complete depopulation of the infected areas rather than to the reduction of transmission by the other control measures. The implications of these results for the control of HPAI outbreaks in densely populated poultry areas are discussed.

INTRODUCTION

In 2003 HPAI struck The Netherlands for the first time since 1926. On 28th February, suspicion for a notifiable disease in a layer flock was notified to the veterinary authorities. The hens in one house had refused their food and water since 22nd February. In addition, mortality increased and by 28th February approximately 90% of the 7,150 hens had died (Elbers et al., 2004). This outbreak appeared to be the onset of a huge epidemic, caused by an avian influenza (AI) A virus of type H7N7 (Stegeman et al., 2004). On the same day as the first report, four other flocks were reported as suspect. The epidemic was combated by movement restrictions, stamping out infected flocks and pre-emptive culling of flocks in the neighbourhood of infected flocks. Despite these measures, a total of 255 outbreaks were diagnosed and 1,255 commercial flocks and 17,421 flocks belonging to hobby farmers, accounting for 30 million birds, were

[1] Arjan Stegeman, Yalelaan 7, 3584 CL Utrecht, the Netherlands. Email: j.a.stegeman@vet.uu.nl

culled. In addition, the virus was also transmitted to Belgium and Germany and, even more seriously, to a large number of humans (Koopmans et al., 2004).

To reduce the probability that a future introduction of AI virus will develop into an epidemic of similar size, it is important to know whether the afore-mentioned measures are sufficient to stop secondary infections. To stop an epidemic, infected flocks should be detected and depopulated before they infect, on average, more than one other flock. Consequently, the average number of secondary infections caused by one infectious flock, the reproduction ratio (R_h), should be smaller than 1. In this study, the transmission of H7N7-HPAI virus between flocks was quantified, before and after detection of the first outbreak in the Netherlands in 2003.

MATERIALS AND METHODS

Outbreaks

In 241 of the 255 notified outbreaks clinical disease was noticed and H7N7-HPAI virus was detected by either virus isolation or PCR or both. On the basis of data collected from the outbreaks, the time of virus introduction was estimated for each of the infected flocks. The available data comprised the contacts of the flocks with previous outbreaks and the feed uptake and mortality data in the affected flocks. Van der Goot et al. (2003) observed, in transmission experiments, that contact-infected chicken died 4-8 days after the exposure to HPAI virus. By use of this period, the time of virus introduction could be estimated from the moment the mortality in a flock was first noticed to have increased. However, 14 outbreaks were detected by serological screening; no clinical disease was present, but one or more sera were positive in the H7 Hemaglutination Inhibition Assay. Estimation of the time of virus introduction is not possible in this situation and, consequently, these outbreaks could not be included in the analysis.

Control measures

Several measures were implemented in order to stop the virus circulation. To start with, infected flocks were depopulated. In addition, flocks within a radius of 1 km of an outbreak were culled pre-emptively. Furthermore, upon detection of an outbreak, the veterinary authorities performed forward and backward tracing. Moreover, a transport ban was implemented; a protection and a surveillance zone were established around an infected flock, and the flocks in these areas were examined for clinical disease. As the epidemic proceeded, more measures were implemented such as the establishment of buffer zones, compartmentalisation of the Netherlands and the culling of all poultry in large areas.

Analysis of data

The transmission parameter β (i.e. the average rate at which an infected flock infects susceptible flocks in a population consisting almost exclusively of susceptible flocks) of the stochastic SEIR model was estimated by means of a generalised linear model (GLM) (McCullagh and Nelder, 1997). In this instance, implementation was a straightforward generalisation of the GLMs described by Becker (1989) that have been used previously to analyse epidemics of classical swine fever (Stegeman et al., 1999) and foot-and-mouth disease (Bouma et al., 2003).

To obtain estimates of the transmission parameter, data on the date of detection and on the number of susceptible and infectious flocks were transformed into the format *[S(t), I(t), C(t)]*, where *S(t)* is the deduced number of susceptible flocks present at time *t* (i.e. taking into account culling and infection), *I(t)* is the deduced number of infectious flocks present at time *t*, and *C(t)* is the number of new cases (infected flocks) that have arisen between time *t* and time *t*+1. The total number of flocks is designated as N.

By standard reasoning, it is accepted that the number of cases C(t) arising in a fixed time period (1 day in the present study) is binomially distributed with the parameter

$$p_{\inf}(t) = 1 - e^{-\beta \frac{I(t)}{N}} \tag{1}$$

(the probability of infection) (Becker, 1989) and binomial totals *S*:

$$C(t) \sim \mathrm{Bin}(\mathrm{S(t)}, 1 - \mathrm{e}^{-\beta \frac{\mathrm{I(t)}}{\mathrm{N}}}) \tag{2}$$

Notice that the above model entails the following implicit assumptions: (*i*) all susceptible flocks are equally susceptible; (*ii*) all infected flocks are equally infectious; and (*iii*) each infected flock poses an independent and identical risk of infection to each susceptible flock. These assumptions can be relaxed, but this seems wise only if a large amount of data is available or if the fit of the model is unsatisfactory.

In the above model, $\ln(\beta)$ was estimated using a complementary log-log link function (i.e. $\ln(\ln(1- p_{\inf}))$, with $\ln\left(\frac{I(t)}{N}\right)$ as an offset variable. The fit of the model was checked by inspection of the residual deviance, which, under standard assumptions, is approximately χ^2-distributed with degrees of freedom given by the number of records minus the number of estimated parameters. All analyses were carried out in GenStat 6.

The infectious periods of the flocks were calculated as described above, based on the moment of detection and the moment of culling. The reproduction ratio R_h was calculated as the product of the estimates of the transmission parameter and the infectious period: $\hat{R}_h = \hat{\beta}\hat{T}$. The corresponding confidence interval was based on the identity (assuming that β and T were independent): $Var(\beta T) = (E\beta)^2 Var(T) + ET^2 Var(\beta)$ (Mood et al., 1985). Substitution of the estimated means and variances of β and T into this formula yields an estimate of the variance of R_h, which can be used to calculate the confidence interval. Because the model yields estimates of $\ln(\beta)$, the fact that (asymptotically) $\hat{\beta}$ is log normally distributed was taken into account. Hence, $E\beta$ and $Var(\beta)$ were calculated as $E\beta = e^{\mu + \frac{1}{2}\sigma^2}$ and $Var(\beta) = (E\beta)^2\left(e^{\sigma^2} - 1\right)$, where μ and σ^2 denote the (estimated) mean and variance of $\ln(\beta)$, respectively.

RESULTS

Description of the epidemic

The epidemic started in the centre of the Netherlands, in a region called the 'Gelderse Vallei'. This region has a very high density of poultry flocks (> 4/km^2). Figure 1 shows the course of the number of outbreaks throughout the epidemic and Fig. 2 shows the distribution of the infected flocks over the Netherlands.

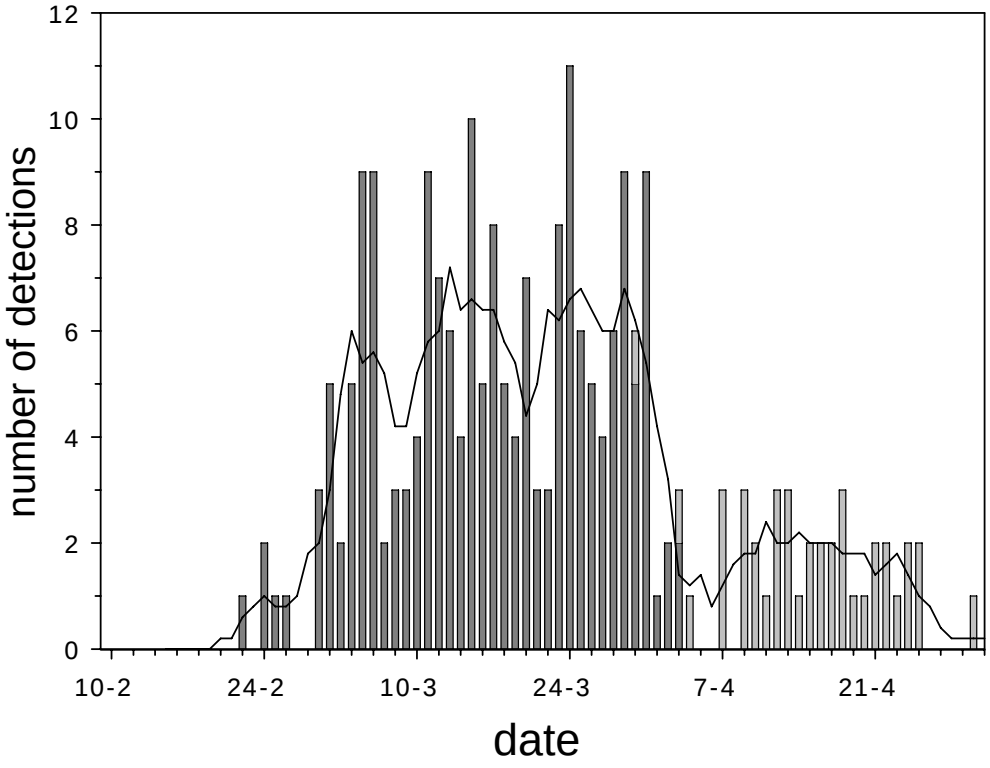


Fig.1 Course of the number of detected outbreaks during the 2003 epidemic of high-pathogenicity avian influenza in the Netherlands. Dark bars refer to the Gelderse Vallei and light bars to Limburg. The line represents a 5-day moving average.

The number of diagnosed outbreaks increased during the first week of March 2003. Subsequently, the number of outbreaks per day fluctuated between 2 and 11 until the end of March, without a clear trend up or down. During that period, outbreaks were only detected in the Gelderse Vallei. However, by the start of April, the number of outbreaks per day dropped markedly. The reason for this was that almost all flocks in the Gelderse Vallei had been culled at that time and consequently, the number of susceptible flocks became restricted. Nevertheless, after this drop, the number of outbreaks fluctuated throughout most of April between 0 and 5 outbreaks per day. The reason for this was that, by the end of March, the virus had escaped from the Gelderse Vallei to the southern part of The Netherlands. Here, the virus also continued to spread in the province of Limburg (lower part of Fig. 2). Again, until April 15th, no clear trend up or down is visible in the epidemic curve and the total number of outbreaks in Limburg mounted up to 43. However, subsequently, the epidemic faded out, with only a few outbreaks in

May. By then, in the Southern part of The Netherlands, large areas had been emptied of all commercial poultry.

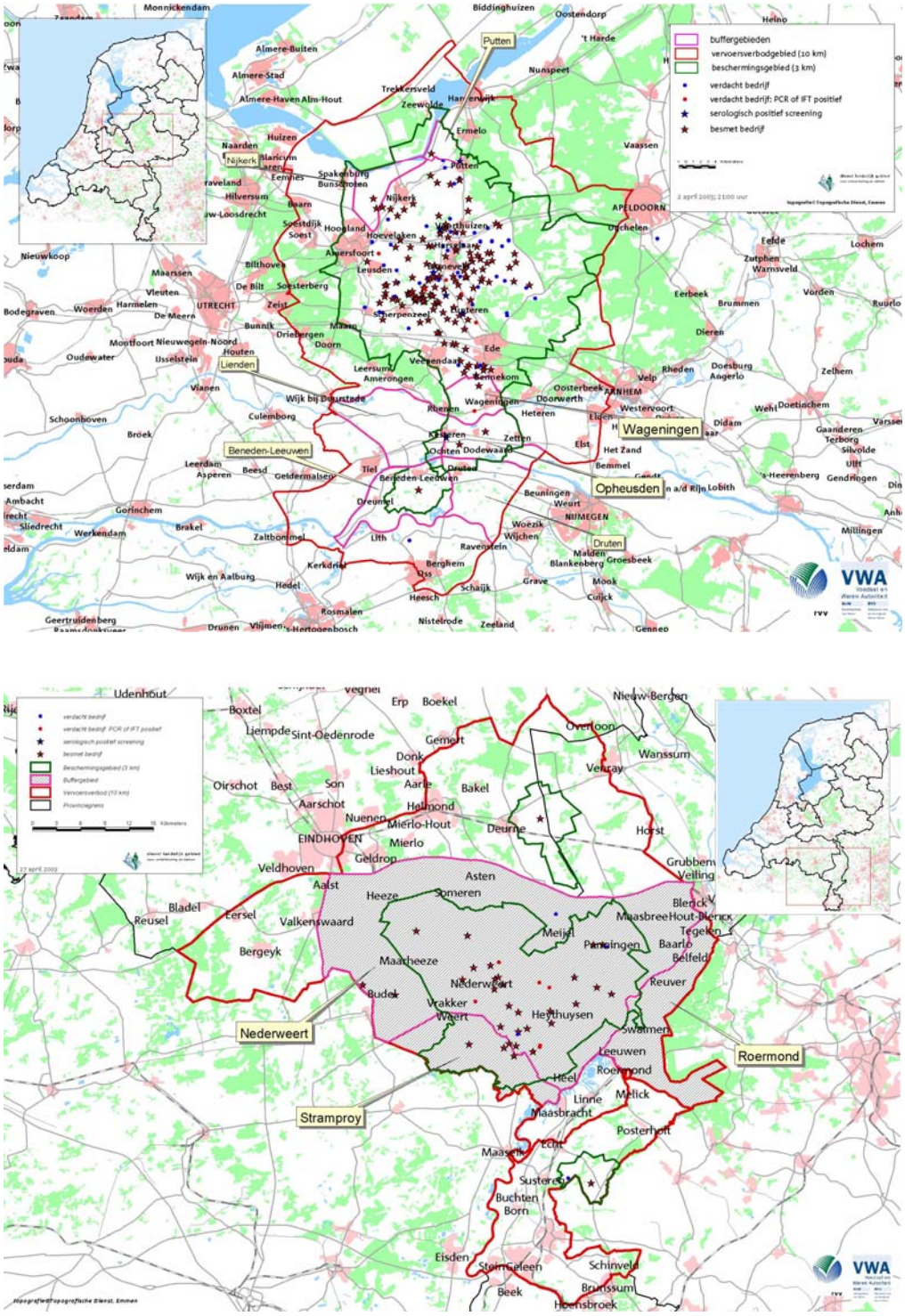


Fig. 2. Distribution of the flocks infected during the 2003 epidemic of high-pathogenicity avian influenza in The Netherlands. The upper map shows the Gelderse Vallei and adjacent areas, the lower map shows the affected region in the Southern province Limburg.

Transmission parameters

The transmission parameter β decreased significantly after detection of AI in both areas (Table 1). In fact, the between-flock transmission parameter decreased from β_{before}= *0.47* (day^{-1}) before detection to β_{after}= *0.17* (day^{-1}) after detection in the Gelderse Vallei, and from β_{before}= *0.39* (day^{-1}) to β_{after}= *0.18* (day^{-1}) in Limburg. In addition, we studied the consequences of analysing the data as two separate analyses. The data for the period after virus detection in the Gelderse Vallei (February 28th) were divided into a period from February 28th up to March 14th, and a period from March 14th onwards, and the reproduction ratio was $\beta_{upto14\text{-}3}$= *0.15 (day^{-1}) (95% CI, 0.12–0.18)* and $\beta_{after14\text{-}3}$= *0.19 (day^{-1}) (95% CI, 0.14–0.26)*, respectively. Thus, splitting the dataset did not significantly alter the estimates of the transmission parameter. For the other periods, the relatively small number of records ($n<30$) made this type of analysis inappropriate.

Table 1. The transmission parameter (β), infectious period (T), and corresponding reproduction ratio (R_h) in the Gelderse Vallei and Limburg, before and after notification of circulation of HPAI virus. The 95% confidence intervals are given in brackets.

	Before Notification			After Notification		
Area	β/day	T, day	R_h	β/day	T, day	R_h
Gelderse Vallei	0.47	13.8	6.5	0.17	7.3	1.2
	(0.3-0.7)	(9.9-17.6)	(3.1-9.9)	(0.1-0.2)	(3.4-11.1)	(0.6-1.9)
Limburg	0.39	8.0	3.1	0.18	6.9	1.2
	(0.2-0.9)			(0.1-0.2)	(3.9-9.9)	(0.6-1.9)

In the Gelderse Vallei, the infectious period decreased from *13.8* days (*95% CI = 9.9-17.6*) for the five flocks suspected to have AI on the first day virus was detected to *7.3* days (*95%CI = 3.4-11.1*) for the period after detection. In Limburg, the infectious period for the first two affected flocks was *7* and *9* days. In the subsequent period, the average infectious period was *6.9* days (*95%CI = (3.9-9.9)*).

Although in both areas between-flock transmission decreased significantly after virus was detected, the reproduction ratio was still higher than *1* (R_h = *1.2* for both areas) (Table 1). This suggests that the control measures were inadequate to interrupt the chain of infection. The containment of the epidemic was, therefore, probably due to the reduction in the number of susceptible flocks by depopulation of the infected areas rather than to the reduction of the transmission level by the other control measures (see Table 1).

DISCUSSION

In this study the transmission of H7N7-HPAI virus between poultry flocks before and after the detection of the first outbreak of AI in the Netherlands in 2003 was quantified. Virus transmission apparently decreased considerably after an outbreak was first detected and there is a strong indication that the infectious period decreased after culling of infected flocks. As a consequence, the reproduction ratio decreased considerably in the period after detection as compared to that in the period before diagnosis of the disease. This decrease is probably a consequence of the control measures implemented once the disease has been identified.

Unfortunately, it was not possible to establish the contribution of individual measures to the overall reduction in virus transmission.

Although the control measures were effective in reducing transmission, the estimates of the reproduction ratio after their implementation were still higher than 1. This suggests that the control measures were probably not sufficient to halt the epidemic by themselves. In fact, containment of the epidemic may have been due to the depletion of susceptible flocks as a result of culling rather than to a decrease in the transmission rate. Therefore, the main value of the control measures may be in preventing the spread of virus to unaffected areas rather than in preventing the spread of the virus within an area. This may be especially true for areas with a high flock density, such as the Gelderse Vallei, where an epidemic may well be impossible to stop once it has begun. This is in agreement with the findings in Italy in 1999, where an outbreak of H7N1-HPAI virus spread quickly and extensively and could only be controlled by depopulation of nearly all flocks in the affected area of 5,500 km^2 (Zanella et al., 2001).

A number of assumptions were made in the current study that could limit the scope and validity of the results. Apart from the assumptions of equal susceptibility and infectivity of herds, and of the independence of transmission events mentioned earlier, two other issues are important. First, our analyses were based on rather simple and rigid assumptions on the relation between the moment of detection and the latent and infectious period before detection. Ideally, it would be preferable to obtain more precise estimates of the (distribution of the) moment of virus introduction in a flock, as well as the infectious output of infected flocks as a function of time. This would require data on the within-flock spread of the virus or within-flock mortality data in conjunction with within-flock transmission models. Nevertheless, in view of the qualitative robustness of the results with respect to assumptions about the infectious and latent period, it is considered highly unlikely that more refined analyses would yield qualitatively different results and conclusions.

Second, spatial considerations were ignored in these analyses, whereas it is known that the proximity of infected flocks substantially increases the risk of infection in susceptible flocks. It is not known to what extent the results of our study would be affected by the introduction of a spatial component (e.g. along the lines of Keeling et al. (2001)), but these analyses are currently being extended by including an estimate of a spatial 'infection kernel'. In the long run, more detailed analyses of the local causes and risk factors of transmission of HPAI virus from flock to flock would improve our understanding of the effectiveness of control measures.

Results of analyses presented here indicate that outbreaks of HPAI viruses are difficult – if not impossible – to control in poultry-dense areas with the usual control measures, and control is only likely to be achieved by depopulation of the whole affected area. Moreover, new outbreaks can be expected, because the wild fowl population is endemically infected with AI virus strains (Alexander, 2000). It might be worthwhile to consider reducing the flock density in order to reduce the probability of an epidemic of this size, or to consider vaccination of poultry as an additional control measure. Vaccination was used during outbreaks of LPAI in Italy from 2000-2002 (Capua & Marangon, 2003), in Utah in 1995 (Frame, 2000) and HPAI Mexico in 1994 (Arriola, 2000). Vaccination significantly reduces the excretion of virus (e.g. Di Trani et al., 2003; Swayne et al., 2001), which may reduce virus spread in an infected area, thereby reducing the risk of human exposure. The risk of introduction of AI virus from wild fowl might be reduced by keeping poultry indoors. However, this may be unacceptable to the general public, who prefer the idea of free-range poultry for (presumed) welfare reasons.

ACKNOWLEDGEMENTS

This study was financed in part by a grant from the Dutch Ministry of Agriculture, Nature and Food Quality.

REFERENCES

Alexander, D.J. and Brown, I.H. (2000). Recent zoonoses caused by influenza A viruses. Rev. Sci. Tech.19, 197-225

Arriola, J.M. (2000). The Mexican experience. World Poultry, Elsevier special, 23-24

Becker, N.G. (1989). Analysis of infectious disease data. Chapman & Hall, London, 139-174

Bouma, A., Elbers, A.R., Dekker, A., de Koeijer, A., Bartels, C., Vellema, P., van der Wal, P., van Rooij, E.M., Pluimers, F.H. and de Jong, M.C. (2003). The foot-and-mouth disease epidemic in The Netherlands in 2001. Prev. Vet. Med. 57, 155-166

Capua, I. and Marangon, S. (2003). Vaccination policy applied for the control of avian influenza in Italy. Dev. Biol. (Basel) 114, 213-219

Di Trani, L., Cordioli, P., Falcone, E., Lombardi, G., Moreno, A., Sala, G. and Tollis, M. (2003). Standardization of an inactivated H17N1 avian influenza vaccine and efficacy against A/Chicken/Italy/13474/99 high-pathogenicity virus infection. Avian Dis. 47 (Suppl), 1042-1046

Elbers, A.R.W., Fabri, T.H.F, De Vries, T.S., De Wit, J.J., Pijpers, A. and Koch, G. (2004). The Highly Pathogenic Avian Influenza A (H7/N7) virus epidemic in The Netherlands in 2003 – lessons learned from the first five outbreaks. Avian Dis. 48, 691-705

Frame, D. (2000). H7N3 outbreak halted by vaccine. World Poultry, Elsevier special, 20-21

Keeling, M.J., Woolhouse, M.E., Shaw, D.J., Matthews, L., Chase-Topping, M., Haydon, D.T., Cornell, S.J., Kappey, J., Wilesmith, J. and Grenfell, B.T. (2001). Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogeneous landscape. Science 294, 813-817

Koopmans, M., Wilbrink, B., Conyn, M., Natrop, G., van der Nat, H., Vennema, H., Meijer, A., van Steenbergen, J., Fouchier, R., Osterhaus, A. and Bosman, A. (2004). Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in The Netherlands. Lancet 363, 587-593

Stegeman, J.A., Bouma, A., Elbers, A.R.W., De Jong, M.C.M., Nodelijk, G., De Klerk, F., Koch, G. and Van Boven, M. (2004). Avian Influenza virus (H7N7) epidemic in The Netherlands in 2003: Course of the epidemic and effectiveness of control measures. J.I.D. 190, 2088-2095

McCullagh, P. and Nelder, J.A. (1997). Generalized linear models. Chapman & Hall, London.

Mood, A.M., Graybill, F.A. and Boes, D.C. (1985). Introduction to the theory of statistics. McGraw-Hill Inc, New York, 180p

Stegeman, J.A., Elbers, A.R.W., Smak, J.A. and De Jong, M.C.M. (1999). Quantification of the transmission of Classical Swine Fever Virus between herds during the 1997-1998 epidemic in the Netherlands. Prev. Vet. Med. 42, 219-234

Swayne, D.E., Beck, J.R., Perdue, M.L. and Beard, C.W. (2001). Efficacy of vaccines in chickens against high pathogenicity Hong Kong H5N1 avian influenza. Avian Dis. 45 355-365

Van der Goot, J., de Jong, M.C.M., Koch, G. and van Boven, M. (2003). Comparison of the transmission characteristics of low and high pathogenicity avian influenza A virus (H5N2). Epidemiol. Inf. 131, 1003-1013.

Zanella, A., Dall'Ara, P. and Martino, P.A. (2001). Avian influenza epidemic in Italy due to serovar H7N1. Avian Dis. 45, 257-261

FOOT-AND-MOUTH DISEASE

FACTORS ASSOCIATED WITH THE EARLY DETECTION OF FOOT-AND-MOUTH DISEASE DURING THE 2001 EPIDEMIC IN THE UK

M. MCLAWS [1], C. RIBBLE, S.W. MARTIN AND J. WILESMITH

SUMMARY

An essential objective of an effective foot-and-mouth disease eradication campaign is to shorten the infectious period by rapidly detecting and destroying cases of disease. The purpose of this investigation was to identify factors associated with the rapid detection of clinical disease during the 2001 outbreak in the UK. A logistic regression analysis was performed using early versus late detection of disease as the outcome of interest.

Premises were more likely to be detected early if: (1) cattle (particularly dairy cattle) were infected compared to sheep, (2) a recently confirmed infected premise was within 3 km of the new case and (3) the case was initially reported by the farmer, rather than a disease control centre-initiated surveillance activity (i.e. patrol, tracing, pre-emptive cull). Our findings suggest that reporting by farmers, and initiatives that increase farmer education and awareness, should be encouraged.

INTRODUCTION

On February 20, 2001, foot-and-mouth disease (FMD) was confirmed in pigs in an abattoir in England. This was the beginning of a large outbreak that resulted in the identification of 2026 infected premises in Great Britain (and four in Northern Ireland) and the destruction of more than four million animals for disease control purposes. Britain's 'FMD-free status without vaccination' was restored by the Office International des Epizooties (OIE) on 22 January 2002 (Scudamore & Harris, 2002) following a control effort that involved more than 10,000 veterinarians, soldiers, field and support staff (National Audit Office, 2002).

Foot-and-mouth disease is an extremely contagious viral disease. It spreads between herds through direct animal contact, or indirectly through movements of people, vehicles and other fomites contaminated with the virus. Given appropriate conditions, windborne transmission may also occur (Radostits et al., 1994). Shortly after a herd is infected, typically 3-6 days in cattle, virus begins to be excreted that may infect other herds (Radostits et al., 1994). This may precede the appearance of clinical signs by up to four days (Kitching & Hughes, 2002; Kitching, 2002a).

[1] Melissa McLaws, Dept. of Population Medicine, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Email: mmclaws@uoguelph.ca

An essential target of an effective FMD eradication campaign is to shorten the infectious period of each herd by rapidly detecting and destroying cases of disease (Haydon et al., 1997). Because the disease is so contagious, upon the discovery of any infected animal(s) within a herd, it is usual that the entire herd is considered infected and subject to slaughter. The infectious period may be divided into four components: (1) time from initial viral shedding to the development of clinical signs, (2) time from first clinical signs to the report of suspected disease, (3) time from report of suspicion to the official confirmation of the presence of disease, and (4) time from confirmation of disease to the completion of slaughter and disposal. Different strategies are required to reduce each of these phases.

To minimise the intervals between the report of suspected disease and the completion of slaughter and disposal is a logistical challenge. Methods to shorten the time from initial viral shedding to the report of suspicion of disease are less straightforward. Traditional approaches to reduce this period consist of raising public awareness and conducting intensive active surveillance (Geering et al., 2002). Additionally, in the 2001 epidemic, pre-emptive culling was used extensively on premises contiguous to infected properties. While serving as a means to remove infected animals before they exhibited clinical signs (i.e. the earliest possible detection), this led to the destruction of many, at least apparently, uninfected animals, and the policy was therefore controversial (Anderson, 2002).

There has been considerable interest in the development of a pen-side diagnostic test that would be accurate in diagnosing infection even before the appearance of clinical signs (Kitching, 2002b; Woolhouse & Donaldson, 2001). Such a test may be applied to both suspect herds and those at high-risk of contracting the disease, allowing the early destruction of only infected herds and also eliminating the dilemma of whether or not to wait for laboratory results in ambiguous cases.

In future epidemics, both pre-emptive culling and laboratory testing of high-risk stock may be utilised to facilitate the early detection of infected herds. However, both strategies depend on risk-analysis and tracing practices to identify target premises. As it is likely that disease will still occur on premises where it is not expected, the early detection of suspect cases on the basis of clinical signs will remain important.

During the 2001 epidemic in the UK, there was considerable variation in the time from infection to reporting of suspect cases (Haydon et al., 2003). The purpose of this investigation was to explore the nature of this variation and to identify factors associated with the rapid detection of clinical disease. Factors that we considered included the species infected, the time of the outbreak in relation to the national epidemic curve, the method of detection (found by local disease control centre-initiated surveillance activities or not), the proximity of the premise to other previously confirmed infected premises, and finally, which local disease control centre was responsible for implementing the disease control procedures in that area. Multivariate logistic regression models were developed to explore the association of these variables with early detection, whilst controlling for other inter-variable relationships.

MATERIALS AND METHODS

For each infected premise, the date of report, the date of confirmation, origin of report (farmer, patrol etc.), local disease control centre involved, species infected, the age of oldest lesion, laboratory results and map reference were obtained from the database compiled by the

epidemiology team at the national disease control centre (Gibbens et al., 2001). The database was accessed in November 2002.

All analyses were conducted using Stata (StataCorp. 2003. Stata Statistical Software: Release 8.0. College Station, TX, USA: StataCorp LP.) unless otherwise noted. Any incompatibilities within the data (for example if cattle were listed as the species infected but it was not recorded that there were any cattle on the farm) were investigated using the 'Final Epidemiology Report' database. This second database was compiled by the field epidemiology teams at each local disease emergency control centre using information from the Disease Control System*, forms completed at the time of investigation, and local knowledge. If more detailed information could be gleaned from the Final Epidemiology Report database that clarified the inconsistency, the data were adjusted.

A logistic regression analysis was performed using early versus late detection of disease as the outcome of interest. If the age of oldest lesion on a premise was less than 3 days, it was classified as 'early detection'. If the oldest lesion was greater than or equal to 3 days, it was classified as 'late'. As antibodies may typically be first detected 3-5 days following the appearance of clinical signs (Alexandersen et al., 2003b), premises detected by serology were considered to have been detected 'late'.

The predictor variables that we considered are presented in Table 1. The method of disease detection was dichotomized into active and passive surveillance. Many cases were identified as a result of a report of suspect disease by a farmer or other member of the public. As the disease control authorities did not initiate the visit to the premises, this method of detection was referred to as passive surveillance. Other cases of FMD came to the attention of the authorities during patrol visits, tracing visits or pre-emptive culls; all visits initiated by the local disease control centre. It was considered that these cases were detected by active surveillance (Tsutsui et al., 2003).

The type of animal infected was represented by a categorical variable. Premises with more than one species or type of animal infected were classified according to the species/ type in which it should be the easiest to detect disease, based on clinical signs and typical husbandry practices. Disease was considered most obvious in dairy cattle, followed by beef cattle and pigs. Sheep were considered to be the species in which it was most difficult to detect disease. For example, if both sheep and dairy cattle were infected on a single premises, dairy cattle would be recorded as the 'species infected', as the clinical signs of FMD are usually easier to detect in dairy cattle than sheep. The ease of diagnosis was considered similar between beef cattle and pigs. The single premises with both beef cattle and pigs infected was classified as 'beef', as disease had been initially suspected in beef in that case.

A variable was created that represented whether or not the infected premises was within 3 km of another infected premise that had been declared infected within the previous two weeks. This distance was chosen because 'Protection Zones' were created by the disease control authorities in a 3 km radius around infected premises. All premises with susceptible stock within these zones were subject to more stringent restrictions on an ongoing basis, and may have also received patrol visits in the days and weeks immediately following detection of the infected premises that initiated the Protection Zone. The Euclidean distance between infected premises

* The computer system designed and used to manage the outbreak, described by Gibbens et al. (2001)

was calculated using the XY coordinates. These calculations and other data manipulations to create this variable were done using SAS Version 8.2 (SAS Institute 2002, Cary, NC, USA).

Table 1. Description of variables evaluated for an association with early detection, and results of the univariable analyses. The outcome is a laboratory-confirmed infected premise being detected early (oldest lesions < 3d) during the 2001 FMD epidemic in the UK.

Predictor Variable	Categories	Premises detected early	Premises detected late	Crude Odds Ratio (95% CI)	P-value[a]
Method of detection	Passive surveillance	674	414	1	P<0.001
	Active surveillance	91	142	0.39[b] (0.29, 0.53)	
Premises within 3 km of another IP[c] detected in the previous 2 wks	No	207	208	1	P<0.001
	Yes	558	348	1.61 (1.26, 2.05)	
Stock infected	Dairy	313	98	5.70 (4.21, 7.72)	P<0.001
	Beef	304	189	2.87 (2.19, 3.76)	
	Pigs	0	5	N/A	
	Sheep	148	264	1	
Time	Prior to Mar 18	139	116	0.79 (0.59, 1.06)	P=0.25
	Mar 19 – 29 Apr	376	249	1	
	After April 29	250	191	0.87 (0.67, 1.10)	
Local disease control centre	17 dummy variables				P=0.02

[a] Overall significance of variable.
[b] An OR of 0.39 means that the odds of cases being detected early by active surveillance were less than half the odds of cases detected by passive surveillance.
[c] Infected premises

To explore the effect of different methods of representing time, three categorical variables were created. First, two variables were created based on changes observed in the number of infected premises that were reported each day and a smoothed scatter plot of the log odds of early detection based on the report date. The scatter plot was lowess smoothed, with a bandwidth of 0.8, using Cleveland's tricube weighting function (Dohoo et al., 2003, p. 356). One of these variables had 3 categories, and the other had 4 (Figure 1). The third variable consisted of 8 equal time periods of approximately 4 weeks each.

Only infected premises that had the presence of foot-and-mouth disease virus confirmed by the laboratory were used for the analysis, because if FMD was not actually present on a premises, it could not be found 'early'. Five additional premises on which the origin of report was unknown were also excluded from the analysis.

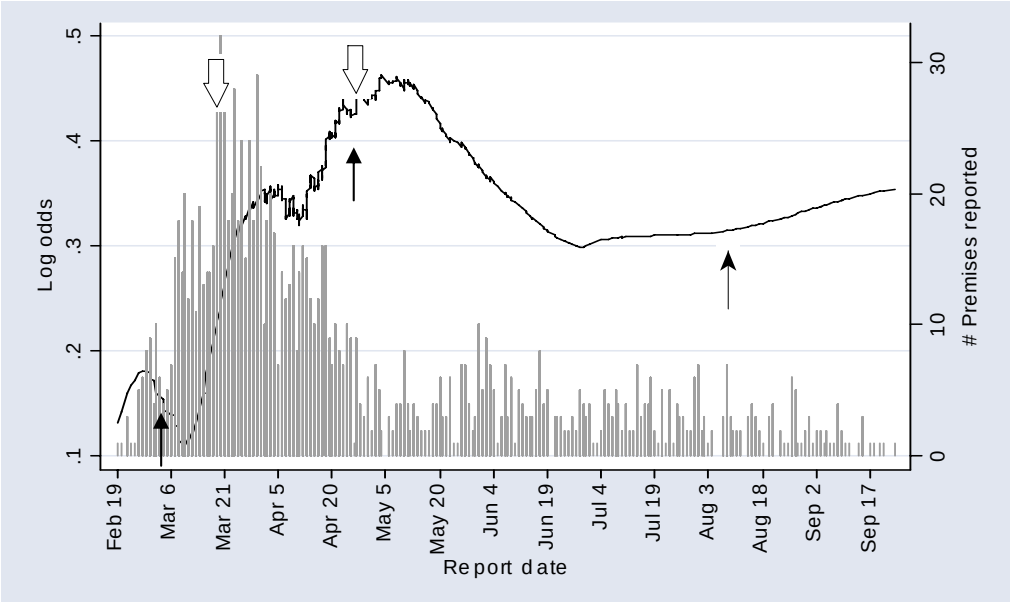


Fig. 1 Smoothed† scatter plot of the log odds of early detection based on the report date, overlaid on the curve representing the number of infected premises reported each day. Closed and open arrows show the cutpoints used to create two different variables representing time.

The logistic regression models were constructed using recognised methods (Dohoo et al., 2003). Univariable associations with the outcome were calculated, using a chi-square test for dichotomous predictors and simple logistic regression for predictors with more than two categories. As the number of predictor variables was small, all were subject to inclusion in the multivariable model. Of the variables created to represent time, the one with the most significant relationship with the outcome in the univariable analysis was used in the multivariable analysis. Indicator (dummy) variables were created to represent categorical variables with more than two levels. Variables not statistically significant were removed from the model one at a time, beginning with the least significant, until all remaining variables were significant at p less than or equal to 0.05. The statistical significance of each variable was assessed by either the Wald test (dichotomous variables) or the likelihood ratio test (non-dichotomous predictors).

To assess confounding, odds ratios were monitored as each variable was removed from the model. If the odds ratio of a remaining predictor variable changed by more than 20%, it was considered to have a confounding relationship with the excluded variable. After the model had been determined, all possible two-way interaction terms of the main-effects were created and their significance assessed using the likelihood ratio test.

Time did not remain in the model as either a significant main-effect or a confounder. To further explore the effect of time, two-way interaction terms between the main effects and the variable representing time were created. Their contribution to the model was assessed using the likelihood ratio test.

† Lowess smoothed, bandwidth 0.8, using Cleveland's tricube weighting function (Dohoo et al., 2003)

The overall fit of the model was assessed by the Hosmer-Lemeshow goodness-of-fit test. The impact of various covariate patterns on the model was assessed by looking for potential outliers and by calculating the delta-beta value of each covariate pattern. The delta-beta is a measure of the difference between the observed set of regression coefficients and the set that would be obtained if the observations in the covariate pattern of interest were deleted (Dohoo et al., 2003).

RESULTS

Data from 1321 infected premises were used in the analysis. Thirty-eight of these premises were identified by the detection of antibodies to the FMD virus in blood samples taken from sheep. The samples were taken either during a pre-emptive cull, because of routine testing prior to lifting restrictions, or for special tests ordered for surveillance purposes. The mean age of the oldest lesion on the remaining 1283 premises was 2.82 days (SD = 1.83), with a minimum age of 1 day and a maximum of 14 days. Five hundred and fifty-six (42.1%) premises were detected early (oldest lesion less than three days). The mean age of oldest lesion on these premises was 1.80 days (SD = 0.40). The remaining 765 (57.9%) premises were detected late (oldest lesions greater than or equal to 3 days), with the mean age of oldest lesion of these premises equal to 4.33 days (SD = 2.06).

The results of the descriptive and univariable analysis are presented in Table 1. Of the variables created to represent time, the one with the most significant relationship with the outcome is presented. With the exception of the variable that represented time, all of the predictor variables were significantly associated with the outcome.

The final logistic regression model (Table 2) was based on 1316 observations with 35 different covariate patterns. The overall model deviance was 805.65. The overall model likelihood ratio chi-square was highly significant (G = 178.11, 8 df, P<0.0001). The Hosmer-Lemeshow goodness of fit test indicated that the model fit the data adequately (Π^2 = 4.69, 6 df, P = 0.58).

No extreme outliers were observed. The model was re-run with observations from the three covariate patterns with the largest delta-beta values excluded (not shown). The goodness-of-fit test indicated that this reduced model also fit the data (Hosmer-Lemeshow test: Π^2 = 5.43, 7 df, P = 0.6074). In this model, the coefficients of all of the parameters, except that representing active surveillance, moved slightly closer to the null compared to the full model. The coefficient representing active surveillance moved slightly away from the null. However, the overall interpretation of the results did not change.

With the exception of local disease control centre, all predictors significant in the univariable analysis remained in the final model. There was evidence of confounding between the method of detection and the species infected. None of the two-way interaction terms between the main-effect variables were significant; however, there was significant interaction between the type of surveillance and time in the epidemic.

Infected premises were more likely to be detected early (within 3 days of showing clinical signs) at any time during the outbreak if there was another recently confirmed infected premise within 3 km, or cattle were infected (dairy or beef) versus sheep. The latter association was stronger if dairy rather than beef cattle were infected. During the tail of the outbreak only,

premises were more likely to be detected early if the disease was found by passive versus active surveillance (Table 3).

Table 2. Results of the logistic regression analysis comparing laboratory confirmed infected premises detected early (oldest lesion <3d) to those detected late (oldest lesion ∃ 3d) during the 2001 epidemic in the UK (n = 1316 premises).

Variable	B	SE	P (Wald's X^2)	95% CI (of B)	Odds Ratio[a]	95% CI (of OR)
Species infected						
Dairy	1.60[b]	0.16	<0.001	1.28, 1.91	4.93[c]	3.60, 6.76
Beef	0.98	0.14	<0.001	0.70, 1.26	2.67	2.01, 3.54
Sheep	0.00	-	-	-	1.00	-
Premise within 3km of IP detected in previous 2 wks						
Yes	0.52	0.13	<0.001	0.26, 0.78	1.69	1.30, 2.18
No	0.00	-	-	-	1.00	-
Time in the outbreak						
Prior to Mar 18	0.15	0.17	0.38	-0.19, 0.49	-	-
Mar 19 –Apr 29	0.00	-	-	-	-	-
Post Apr 30	0.23	0.16	0.15	-0.10, 0.55	-	-
Method of detection						
Active surveillance	0.08	0.35	0.82	-0.61, 0.77	-	-
Passive surveillance	0.00	-	-	-	-	-
Interaction (Method of detection X Time)						
Active X prior to Mar 18	-0.42	0.51	0.41	-1.43, 0.59	-	-
Active X post Apr 29	-1.06	0.42	0.01	-1.87, -0.25	-	-
Constant	-0.85	0.16	<0.01	-1.17, -0.53	-	-

[a] Odds ratios are not given for factors involved in interaction terms.

[b] ∃ = 1.60 indicates that the log odds of an infected premises being detected early were 1.6 units higher when dairy cattle were infected compared to if sheep were infected.

[c] An OR of 4.93 means that the odds of an infected premises being detected early were almost five times greater if dairy cattle were infected than if sheep were infected.

Table 3. Interpretation of the significant interaction between the method of detection and time in the epidemic. The outcome is early detection (infected premises with oldest lesions < 3d) during the 2001 epidemic in the UK

Variable		Odds Ratio	95% CI
Method of detection	Prior to Mar 18	0.71	0.34, 1.49
Active vs. passive surveillance	Mar 19 – Apr 29	1.08	0.54, 2.17
	Post Apr 29	0.38[a]	0.24, 0.59

[a] An OR of 0.38 means that, during the tail of the epidemic, the odds of an infected premises being detected early were 0.38 times as great for premises detected by active surveillance, compared to premises detected by passive surveillance.

DISCUSSION

Early detection of FMD during the 2001 epidemic in the UK was associated with cattle (particularly dairy cattle) being infected compared to sheep, the presence of another recently confirmed infected premises nearby, and detection by passive surveillance. The difference between species has been previously reported, and was attributed to disease in sheep being typically subtler, and to cattle being more frequently or intensely observed (Gibbens et al., 2001; Gibbens & Wilesmith, 2002). The difference between dairy and beef cattle has not been reported previously and may be attributed to differences in husbandry practices, with dairy cattle being milked twice-daily, and thus typically observed more closely than beef cattle.

The association of early detection with another recently confirmed infected premise within 3 km probably represents the effect of increased farmer vigilance as the disease entered their immediate vicinity. Such premises were under more stringent restrictions and were also more likely to also be inspected by patrols. Presumably, the restrictions and visits heightened awareness about the disease, and educated producers about typical clinical signs.

Passive surveillance may have been more likely to detect disease early because it detected cases with more obvious clinical signs, no matter what type of animal was infected, leaving the more subtle cases to be detected by the active surveillance system. Thus, we hypothesized that active surveillance 'mopped-up' cases missed by passive surveillance, rather than acting as a firewall that prevented the spread of infection. This may be contrary to the common perception of the role of active surveillance during an outbreak (Morris et al., 2002).

Due to limited resources, relatively few cases were diagnosed by active surveillance until the tail of the outbreak. Therefore, the lack of association between the type of surveillance and early detection during the first month and peak of the outbreak may have been due to lack of statistical power. Additionally, it may have been related to a shift in the activities of active surveillance over time, from primarily tracing visits to more pre-emptive culls and patrol visits.

During the epidemic, lesions were aged by six veterinarians in the headquarters epidemiology team, based on the reports received from the field. Some premises were visited by specialists from the FMD World Reference Laboratory at Pirbright, in which case their estimate of the age of oldest lesion was used (Gibbens et al., 2001). When performed by experienced clinicians, the ageing of lesions up to 5 days old should be accurate to plus or minus

one day. The accuracy of the estimation declines for lesions older than 5 days (Alexandersen et al., 2003a).

The accuracy of ageing disease on a premise is also dependent on the animal with the oldest lesion being examined. Whilst veterinarians in the field examined several animals prior to and during culls on infected premises, it was not possible to examine every animal slaughtered during this epidemic (Gibbens et al., 2001). Animals were most likely to remain uninspected when large sheep flocks were culled. Therefore, the animal(s) with the oldest lesion may have been more likely to remain unexamined on premises with infected sheep than other premises. If so, this would lead to an underestimation of the time to detection on these premises and differential misclassification of the outcome. As sheep are the reference group, this would result in a conservative estimate of the odds ratios between categories of 'stock infected' and the outcome.

It was considered that premises were detected 'early' when the oldest lesion was less than 3 days, as we believe that it this is a valid and realistic target for disease detection during an outbreak of FMD. It was decided to dichotomize the outcome rather than modelling the age of lesion directly (by constructing a linear regression model, for example), because we believed that it would yield more useful information in view of the inaccuracy inherent in determining the actual age of oldest lesions. It is believed that premises were much more likely to be classified accurately as either early or late, than by the actual age of lesion. Additionally, the linear regression modelling technique treats the difference between each unit increase of the outcome equally. For example, as much weight would be placed on the difference between premises with lesions aged 13 days and 14 days, as between premises with lesions aged 2 days and 3 days. As the expected accuracy of the age of lesions more than 6 days old is lower than for younger lesions, it was not felt that this was appropriate. Further, the decision to dichotomise the outcome allowed inclusion data from the premises detected by serology, on which clinical signs or lesions were never observed.

To minimise bias, we omitted farms from the analysis that did not have infection confirmed by laboratory testing. Some of the omitted farms did not have samples submitted to the laboratory, and others had tested laboratory-negative. We assumed that the results of our study should be applicable to all farms truly infected with FMD during this outbreak, regardless of whether they were laboratory tested or not. The decision as to whether or not samples were submitted was based primarily on available laboratory capacity at the time.

These results should be useful for the development of recommendations and contingency plans for the management of future outbreaks. It appears that active surveillance plays a critical role in 'mopping-up' cases missed by passive surveillance, but it should not be relied upon for early detection of disease. Rather, programs that enhance producer education and awareness should be developed and promoted. Farmers are in the best position to be the first to detect any deviations from normal, as they observe stock daily and are the most knowledgeable about the usual condition of the animals under their care. Producer training should be prioritised even when resources are scarce, although it may be targeted at beef and sheep operations where disease is more likely to be found late.

ACKNOWLEDGEMENTS

The research was supported by a fellowship provided by the Ontario Veterinary College, and the authors are also grateful to DEFRA for financial assistance. We thank Bruce McNab and Craig Stephen for valuable comments on the manuscript, and William Sears for statistical support.

REFERENCES

Alexandersen, S., Kitching, R.P., Mansley, L.M. and Donaldson, A.I. (2003a). Clinical and laboratory investigations of five outbreaks of foot-and-mouth disease during the 2001 epidemic in the United Kingdom. Vet. Rec. 152, 489-496

Alexandersen, S., Zhang, Z., Donaldson, A.I. and Garland, A.J.M. (2003b). The pathogenesis and diagnosis of foot-and-mouth disease. J. Comp. Pathol. 129, 1-36

Anderson, I., (2002). Foot-and-mouth disease 2001: lessons to be learned inquiry report. [online], <http://archive.cabinetoffice.gov.uk/fmd/nav/report.htm>

Dohoo, I., Martin, W. and Stryhn, H. (2003). Veterinary epidemiologic research. University of Prince Edward Island, Charlottetown, P.E.I. 706p

Geering, W.A., Lubroth, J., Food and Agriculture Organization of the United Nations and Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (2002). Preparation of foot-and-mouth disease contingency plans. Food and Agricultural Organization of the United Nations, Rome.

Gibbens, J.C., Sharpe, C.E., Wilesmith, J.W., Mansley, L.M., Michalopoulou, E., Ryan, J.B.M. and Hudson, M. (2001). Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. Vet. Rec. 149, 729-742

Gibbens, J.C. and Wilesmith, J.W. (2002). Temporal and geographical distribution of cases of foot-and-mouth disease during the early weeks of the 2001 epidemic in Great Britain. Vet. Rec. 151, 407-412

Haydon, D.T., Woolhouse, M.E.J. and Kitching, R.P. (1997). An analysis of foot-and-mouth disease epidemics in the UK. IMA J. Math. Appl. Med. Biol. 14, 1-9

Haydon, D.T., Chase-Topping, M., Shaw, D.J., Matthews, L., Friar, J.K., Wilesmith, J. and Woolhouse, M.E.J. (2003). The construction and analysis of epidemic trees with reference to the 2001 UK foot-and-mouth outbreak. Proc. R. Soc. Lond. B. Biol. Sci. 270, 121-127

Kitching, R.P. (2002a). Clinical variation in foot-and-mouth disease: cattle. Rev. Sci. Tech. 21, 499-504

Kitching, R.P. (2002b). Future research on foot-and-mouth disease. Rev. Sci. Tech. 21, 885-889

Kitching, R.P. and Hughes, G.J. (2002). Clinical variation in foot-and-mouth disease: sheep and goats. Rev. Sci. Tech. 21, 505-512

Morris, R.S., Sanson, R.L., Stern, M.W., Stevenson, M. and Wilesmith, J.W. (2002). Decision-support tools for foot-and-mouth disease control. Rev. Sci. Tech. 21, 557-567

National Audit Office, (2002). The 2001 Outbreak of Foot-and-mouth Disease. NAO Report (HC 939 2001-2002), [online] <http://www.nao.org.uk/publications/nao_reports/01-02/0102939.pdf>

Radostits, O.M., Blood, D.C. and Gay, C.C. (1994). Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses. Baillière Tindall, London, England. 1763p

Scudamore, J.M. and Harris, D.M. (2002). Control of foot-and-mouth disease: lessons from the experience of the outbreak in Great Britain in 2001. Rev. Sci. Tech. 21, 699-710

Tsutsui, T., Minami, N., Koiwai, M., Hamaoka, T., Yamane, I. and Shimura, K. (2003). A stochastic-modeling evaluation of the foot-and-mouth disease survey conducted after the outbreak in Miyazaki, Japan in 2000. Prev. Vet. Med. 61, 45-58

Woolhouse, M. and Donaldson, A. (2001). Managing foot-and-mouth. Nature 410, 515-516

ASSESSING EFFECTIVENESS OF CONTROL STRATEGIES AGAINST FOOT-AND-MOUTH DISEASE IN SWITZERLAND USING A DYNAMIC SIMULATION MODEL

I. BACHMANN[1], J. RÜFENACHT, C. GRIOT, R. S.MORRIS AND K.D.C. STÄRK

SUMMARY

Recent foot-and-mouth disease (FMD) epidemics in Europe have shown the importance of being prepared for virus introduction into a disease-free country. The objective of this project was to examine the effectiveness of the FMD control measures as foreseen in the current Swiss legislation. On the basis of the output of different scenarios, recommendations in respect of control measures are proposed which eventually can be implemented in case of an outbreak. To achieve this goal, InterSpread Plus (ISP), a computer simulation model, was used to simulate the spread of FMD in different outbreak scenarios. Before applying the model, input parameters on FMD virus as well as spread mechanisms as required by ISP had to be compiled.

This work presents for the first time quantitative estimates for the epidemiological effects of control strategies, the duration of the time span between virus introduction and detection of the first case, and the capacity of the depopulation resources on the course of the simulated FMD epidemics in Switzerland. It also documents the importance of professionals such as artificial insemination technicians and veterinarians as transmitters of FMD virus.

The importance of early detection of disease was shown for densely populated areas as the silent spread period of 14 instead of 21 days was able to nearly halve the mean duration of the epidemic. Consequently, the mean size of the epidemic was reduced by a factor of 8.2 and the mean number of slaughtered animals decreased as well. The evaluation of the effect of unlimited depopulation resources showed that the resource capacity is crucial for the course of an epidemic. In densely populated livestock areas, the eradication of FMD with limited resources demonstrated that the measures outlined in current Swiss legislation are not sufficient. Instead, it was shown that protective ring vaccination - as vaccination to live - was the most effective control strategy in terms of size and duration of the epidemic as well as the number of animals culled. In contrast, the prevailing control measures applied to epidemics in a sparsely populated livestock area were shown to be the best strategy at least effort.

Although this computer simulation model is a useful tool for comparing the impact of different control strategies on the course of the simulated FMD epidemic, the epidemiology of the disease can vary depending on the FMD virus serotype involved, and therefore parameters used in the model might not be valid for other serotypes, and would need appropriate adjustment if used in a real emergency.

[1] Iris Bachmann, Federal Veterinary Office, PO Box, Bern, CH-3003, Switzerland. Email: IrisBachmann@gmx.ch

INTRODUCTION

Foot-and-mouth disease (FMD) poses a permanent threat to countries free from the disease, despite preventive measures such as embargos on imports of animals and animal products from countries where FMD is endemic. The FMD status of Switzerland is similarly challenged because of the country's intensive tourism and trade contacts with FMD-infected countries. FMD would easily and quickly spread in Switzerland because of the full susceptibility of cloven-hoofed animals. Additionally, animal movements are frequent and often cover considerable distances within the country thus facilitating rapid spread (Lüdi, 2004).

If FMD were introduced into Switzerland, the directive on animal epizootics (Tierseuchenverordnung, 1995) demands the slaughter of all susceptible animals on infected premises (stamping-out policy), reflecting Switzerland's intention to regain the "FMD-free without vaccination" status. Additional to stamping-out, there is a considerable range of measures that could be applied to aid the eradication of FMD. Besides the depopulation of infected premises (IPs), pre-emptive depopulation of dangerous contact farms (DCs) and of contiguous premises (CPs) may be implemented within a suitable radius (Jalvingh et al., 1999; Nielen et al., 1999). Suppressive vaccination ("vaccination to kill") or protective vaccination ("vaccination to live") (Mangen, 2002) may also be considered within protection zones, and a decision has to be made whether mass or ring vaccination should be implemented (Keeling et al., 2003; Morris et al., 2001). Because each outbreak is different, different control measures or combinations of strategies may be needed depending on the area of the outbreak or the species affected (Mourits et al., 2002).

The objective of this project was to assess the impact of the depopulation of IPs, DCs and CPs in Switzerland, and to compare the duration and the size of the outbreak with a baseline intervention scenario. Furthermore, the effect of ring vaccination in combination with depopulation of IPs only and also with pre-emptive depopulation of DCs and CPs was considered. On the basis of the output of the different scenarios, recommendations for emergency control measures were to be derived. To achieve this goal, the computer model InterSpread Plus (ISP) was used to simulate the spread of FMD. ISP was developed by Massey University, New Zealand. A pre-cursor of ISP was used in the assessment of vaccination in the UK FMD outbreak 2001 (MAFF, 2001; Morris et al., 2001; Wilesmith, 2003).

MATERIALS AND METHODS

Characteristics and input parameters of InterSpread Plus

InterSpread Plus can model the spread of any infectious disease transmitted between farms, whose data, including spatial location and size of susceptible animal populations are loaded into the model. Data from the agricultural information system of Switzerland (AGIS) were used, which provided over 54,000 records of numbers of dairy and beef cattle, pig, sheep and goats per farm.

InterSpread Plus allows farms to be classified by transmission risk. In these simulations, farms were classified by the main production type as (1) Dairy, (2) Beef, (3) Pig, (4) Sheep, (5) Goat, (6) Mixed Farm, or (7) Small Farm. These were used either alone or in combination to define 17 farm classes.

Spread mechanisms and control measures were defined in ISP for each control scenario (see

below). After setting the parameters for the FMD serotype (incubation time, probability of transmission, distances over which local and airborne spread are most likely to occur, etc.), the model was run. The outcome of the model is the spatial and temporal spread of the epidemic on a daily basis. ISP uses routines based on Monte Carlo simulation to account for the variation and uncertainty in spread and control mechanisms that can be described by empirical probability functions. It was found that the size of outbreaks - averaged over all iterations - stabilised after approximately 50 iterations. Therefore, 50 replications of each scenario were performed. Each scenario was modelled for 200 days.

In all scenarios, infection was initialised by a random assignment of the index infection on one farm within a defined area. Once a farm was infected, it continued to be infectious until the disease was detected and control measures were completed. Spread of disease resulted from one of four mechanisms: (1) movement of animals to another animal holding or to an abattoir or movement of persons and vehicles coming from infected farms; (2) spread by dairy tanker movements; (3) airborne spread (depending on weather conditions); and (4) local spread. In order to describe the spread of FMD within the study area, ISP used a set of transmission parameters, and a set of control measures. Input parameter values are provided in the following paragraphs.

Sixteen movement types of animals, persons or vehicles were specified and according to best judgement and according to other authors (Morris et al., 2001) grouped into (1) high risk, (2) medium risk, and (3) low risk movements (Table 1). Depending on the risk category, the probability of FMD transmission was set at 1.0, 0.409, and 0.005 for high, medium and low risk, respectively. These values were derived from the data set that was used to model the UK outbreak by Wilesmith et al. (Mark Stern, personal communication). Further parameters were compiled from other sources as specified below, and shown in Table 1 grouped as medium-low and very low risk.

Input parameters on animal movements were compiled from different sources. Records on cattle movements were available from the Swiss animal movement database (TVD). As the movements of the other susceptible species are not yet electronically recorded, information on the frequencies and distances of pig, sheep and goat transports were obtained from various organisations (Swiss Pig Health Service (SGD), Swiss Extension and Health Service For Small Ruminants (BGK)). Records of these organisations were analysed to derive the parameters needed. BGK provided information on the movements of sheep and goats involved in exhibitions, markets, summer grazing on alpine pastures and trade. In addition to the movements of sheep and goats to other animal holdings or the abattoir, ram and buck movements during the breeding season were also considered.

As FMD can be transmitted by people, the impacts of artificial insemination (AI) technicians as well as veterinarians were considered. The Swiss organisation for artificial insemination (SVKB) provided information on the average number of visits to one farm per year, the number of farms visited by one technician per day and the daily distance covered by their employees. In order to obtain information on veterinary routines, seven veterinary practices both in the Swiss midlands and the mountain areas were asked to provide information on their average daily number of farm visits and the average length of the daily routes. The chance of a veterinarian or AI technician contaminating him-/herself with FMD virus and transmitting the disease was estimated to be medium (0.409) before the detection of the first FMD case. Thereafter, veterinarians, AI technicians and farmers were assumed to have increased disease awareness and would take additional hygienic measures, including

disinfection of hands and boots and changing clothes between farms, and this was assumed to decrease of the probability of FMD transmission to 0.1.

Table 1. Movement types and their level of risk for FMD transmission.
Risk levels: High (0.1), Medium (0.409), Medium-Low (0.1), Low (0.005), Very Low (0.001)

Movement name	Description	Risk level
Cattle Movement	Dairy or beef cattle being moved from one farm to another	High
Ram	Rams being moved from one farm to another during breeding season of sheep	High
Sheep Movement	Sheep being moved from one farm to another	High
Pig Movement	Pigs being moved from one farm to another	High
Sheep Butcher	Sheep being moved from a farm to an abattoir	Medium
Cattle Butcher	Dairy or beef cattle being moved from a farm to an abattoir	Medium
Pig Butcher	Pigs being moved from a farm to an abattoir	Medium
AI Before Detection	Person that carries out the artificial insemination on dairy or beef cattle before detection of disease	Medium
Vet Before Detection	Veterinarian before disease awareness	Medium
Vet In Zone	Veterinarian after disease awareness and operating within either a protection or a surveillance zone	Medium-Low
AI After Detection	Person that carries out the artificial insemination on dairy or beef cattle after disease awareness	Medium-Low
Vet After Detection	Veterinarian after disease awareness	Medium-Low
Tanker Before Detection	Milk tanker before disease awareness	Medium-Low
Farm Visit	Farmer visiting elsewhere, extension worker, family, friends, neighbours, etc	Low
No Contact	Non animal transport, feed transporters, contractor, animal product transport except milk & mail delivery, etc, animal contact missing	Low
Tanker After Detection	Milk tanker after disease awareness	Very Low

Information needed for the specification of farm visits without animal contact was taken both from a study conducted in the Netherlands (Nielen et al., 1996) and from a study conducted in Switzerland (Stärk, 1998). The transmission risk by such movements was rated as low (0.005).

Dairy tankers visiting various farms on a regular basis pose a risk of spreading FMD, potentially over larger distances, and were therefore modelled in ISP. In Switzerland, approximately half of the dairy farmers have their milk collected by dairy tankers. As for the movements of professionals, the probability of virus transmission was considered to be higher before the first detection of FMD infection (probability=0.1). After detection, the "concept of

the collection of milk in case of the presence of FMD" (BVET, 2001) would be implemented, i.e. tankers collecting milk from farms within a protection or a surveillance zone would need to have virus filters fitted to their vents, reducing the probability of transmission to 0.001.

Local spread is defined as a spread mechanism occurring between infected farms within 3 km (Gibbens et al., 2001). The exact mechanisms causing this kind of spread are not fully understood, but are assumed to be mostly local aerosol spread between contiguous animals, or contamination in the vicinity of IPs. The probability of transmission by Local Spread was determined based on both expert opinion (Griot, personal communication) and data used to model the Korean outbreak in spring 2002.

Virus can also be spread in aerosols by the wind depending on weather conditions. In order to model airborne spread in Switzerland, monthly average meteorological data, including the relative frequency of wind directions, the wind strength, and the relative humidity, were obtained from the Swiss Meteorological Institute (SMA). Parameters for a hypothetical "average" weather were derived for 7 different wind zones, as the wind situations recorded at the various stations differ significantly from each other. During a real outbreak, actual weather data would be obtained and used for more realistic simulations.

Control scenarios

Starting from a baseline scenario outlined in the current Swiss legislation, alternative control measures were defined. The emphasis was put on the following factors: the depopulation of farms, the duration of a nation-wide ban on livestock movements, vaccination policy, and the location of the first outbreak (densely vs. sparsely populated livestock area).

Culling strategy: In the baseline scenario, the disease was controlled by depopulating IPs only. In the alternative scenarios, depopulation of DCs and CPs as well as IPs was implemented.

Enforcement of a nationwide ban on livestock movements: Control measures such as a nationwide ban of livestock movements lasting for 3 days and the establishment of both protection and surveillance zones with appropriate movement restrictions and surveillance of the farms lying within these zones were implemented in each scenario (Perler, personal communication).

The duration of the standstill is not defined by the Swiss legislation but would be based on the recommendation of the authorities. Scenarios modelling a longer duration of either 7 or 14 days of standstill were defined to assess the effect of the length of the movement ban on the course of the epidemic.

Vaccination: There were two scenarios: one where vaccination was not implemented, and the other where a strategy of ring vaccination of cattle was applied. The ring vaccination strategy was applied locally in a ring around identified sources of infection. Vaccination was commenced 7, 14, or 21 days after the detection of the first case. Considering the logistical constraints - mobilisation of vaccine production and veterinary effort - a minimum delay of 7 days would be expected between detection of FMD and the start of vaccination. These estimates were considered the best case scenario, and two further scenarios with longer pre-vaccination delays of 14 and 21 days were modelled. Mass vaccination was not considered because it was thought not to be feasible due to the limited number of vaccine doses in the Swiss emergency

vaccine bank.

Livestock density: As livestock densities in Switzerland vary greatly depending on the region, the index case was set on a dairy farm located in either a densely populated livestock area (DPLA) such as the Mittelland (midlands, approximately 7 farms/km^2) or a sparsely populated livestock area (SPLA) such as the canton of Grison (1.3 farms/km^2) (Raumentwicklung, 2004a/b).

A period of silent spread (i.e. time between infection of index case and detection of index case) of 20 days was assumed for all scenarios unless stated otherwise. The depopulation of farms was assumed to be conducted by 13 teams as the depopulation resources in Switzerland are at present limited to 13 sets of depopulation equipment.

RESULTS

The impact of strategies applied in a densely populated livestock area

Comparison of the outputs of scenarios with limited resources showed little variation, ranging from 200-300 farms infected, although some scenarios resulted in potentially much larger outbreaks of >1000 farms (Table 2). In general, the epidemics with limited resources were relatively short, with a mean duration of 40 – 60 days and a maximal duration of 149 days.

Four scenarios were selected for a more detailed assessment - a) the baseline scenario, b) the additional depopulation of DCs and CPs, c) the depopulation of IPs with ring vaccination starting 7 days after first detection, and d) the depopulation of IPs, DCs and CPs with ring vaccination starting 7 days after first detection. In addition, these scenarios were re-run with unlimited depopulation resources. Based on these results, it was decided to model further scenarios (see below).

In scenarios with limited resources, the number of outbreaks was reduced by additional culling or vaccination by less than 20% in comparison to the baseline scenario (Table 2). Ring vaccination produced the greatest decrease in the mean number of outbreaks (18%) and also appeared able to reduce the maximum number of outbreaks. It was striking that scenarios with rigorous depopulation of DCs and CPs did not result in smaller outbreaks when resources were limited. Control strategies with additional vaccination terminated the epidemic up to 31% faster than in the baseline scenario when resources were limited.

In general, local and airborne spread and spread by veterinarians and AI technicians were most prevalent, while spread by animal movements (<5%) and farm visitors (< 1%) was not important. In the baseline scenario, local and airborne spread were responsible for 66% of all outbreaks, and 31% of disease transmission was due to veterinarians, AI technicians or dairy tanker movements. Depopulation of DCs was expected to decrease the proportion of airborne and local spread. However, when resources were limited, pre-emptive depopulation of DCs and CPs did not show the expected effect. This indicated that the capacity of the depopulation resources was insufficient for this strategy to become effective. Both ring vaccination strategies were able to reduce the proportion of airborne and local spread to 60%, indicating that effective buffer zones could be established.

Table 2. Impact of the resource capacity on the size of the epidemic (DPLA)

Scenario[a]	Number of farms affected					
	Average	Min	5%	Mean	95%	Max
IP_A3 (=Baseline Scenario)	272.5	7	14.3	130	1094.7	1571
IP_A3_Unlimited	159.3	7	15.3	109	498.1	791
IP_A3_C7	224	7	14.2	116.5	679.7	949
IP_A3_C7_Unlimited	139.1	7	11.5	110	342.3	575
IP_DC_CP_A3	267.1	1	7	128	1144.7	1584
IP_DC_CP_A3_Unlimited	131.9	7	13.5	105.5	316.8	536
IP_DC_CP_A3_C7	236.8	7	17.5	149.5	719.8	1169
IP_DC_CP_A3_C7_Unlimited	123.6	7	12.9	88	310.8	471
IP_A3_TTD14	32.7	2	3	19	99.6	280
IP_CP_A3_Unlimited	128.1	7	14.7	103.5	307.5	448
IP_DC_A3_Unlimited	134.4	7	12.45	104	332.7	508
IP_No_Standstill_Unlimited	172.3	12	20.2	143	422.1	799

[a] IP=depopulation of infected premises, DC=depopulation of dangerous contact farms, CP=depopulation of contiguous premises, A3= nationwide standstill lasting for 3 days, C7=ring vaccination starting 7 days after detection of index case, TTD14=silent spread of 14 days, Unlimited=unlimited resources

Table 3. Impact of the resource capacity on the size of the epidemic (SPLA)

Scenario[a]	Number of farms affected					
	Average	Min	5%	Mean	95%	Max
IP_A3 (=Baseline Scenario)	127.2	2	15.8	87.5	365.3	588
IP_A3_Unlimited	94.6	2	11	76.5	226.1	446
IP_A3_C7	122.1	2	3	72.5	405.2	680
IP_A3_C7_Unlimited	119.7	2	16.2	84.5	325.4	526
IP_DC_CP_A3	124.3	8	16.9	100	333.1	426
IP_DC_CP_A3_Unlimited	27.6	1	4	22	82.9	100
IP_DC_CP_A3_C7	98.5	6	23.5		226.5	346
IP_DC_CP_A3_C7_Unlimited	96.4	2	8.3	78.5	275.7	320

[a] IP=depopulation of infected premises, DC=depopulation of dangerous contact farms, CP=depopulation of contiguous premises, A3= nationwide standstill lasting for 3 days, C7=ring vaccination starting 7 days after detection of index case, TTD14=silent spread of 14 days, Unlimited=unlimited resources

While about 20,000 animals had to be culled when applying the baseline control measures with limited resources, the additional implementation of ring "vaccination to live" resulted in the slaughter of about 17,000 animals, a decrease of 13%. With a "vaccination to kill strategy", >350,000 animals would eventually have to be slaughtered and disposed of. Both of the scenarios involving the depopulation of DCs and CPs with limited resources resulted in the

culling of >70,000 animals.

Effect of unlimiting the depopulation resources

Unlimiting the resources resulted in a general decrease in the average size of the epidemics (Table 2). The ability immediately to depopulate each farm that was either detected as an IP or listed for pre-emptive culling brought about a reduction in the number of infected farms by 35 to 50%. The mean duration of the epidemics eradicated with unlimited depopulation resources ranged from 25-40 days. The greatest reduction in the duration of the epidemic (31%) was in the scenario in which IPs, DCs and CPs were depopulated. Unlimiting resources reduced the duration and size of the epidemic without pre-emptive culling, and resulted in a decrease of >40% in the number of animals culled.

Unlimiting the culling resources decreased the proportion of both airborne and local spread, ranging from 35-60%. The greatest decrease was observed when IPs, DCs and CPs were depopulated. Ring vaccination 7 days after first detection did not influence the proportion of airborne and local spread compared to the baseline scenario. In contrast to the equivalent scenario with limited depopulation resources, ring vaccination did not reduce the number of susceptible farms in the vicinity of IPs during the epidemic.

Effect of reduction of silent spread

An additional scenario was simulated where the first detection of FMD occurred 14 instead of 20 days after arrival of the virus and eradication of the epidemic was achieved by the use of baseline measures. This change decreased the size of the epidemic by 88% and consequently 88% fewer animals had to be culled. Earlier detection of the disease also reduced in the mean duration of the epidemic by 50%.

Effect of absence of a nationwide ban on livestock movement

The lack of a nationwide ban on livestock movements resulted in an 8% increase in the total number of outbreaks, and a 4% increase in the duration of the epidemic compared to the baseline scenario with unlimited resources. Surprisingly, the proportion of virus transmission due to animal movement was not increased compared to the baseline scenario including the standstill.

The impact of strategies applied in a sparsely populated livestock area

Comparison of the outputs showed that differences between scenarios in the mean number of IPs were limited (90-130 farms), but again some scenarios resulted in potentially larger outbreaks of >400 farms. A general comparison showed that the size of epidemics in a SPLA was approximately half the size of the equivalent epidemics in a DPLA. Additionally, the confidence intervals of the epidemics in the SPLA were smaller and more consistent than those of the epidemics in the DPLA (Table 3).

The comparison of the cumulative course of the epidemics in a SPLA showed that the choice of contingency plan strategy was not as critical as in a DPLA. The same four scenarios were assessed and additional scenarios with unlimited depopulation resources were defined for

the SPLA as previously for the DPLA (Table 3). Ring vaccination combined with pre-emptive culling led to a decrease in the size of the epidemic by 23% compared to the baseline control measure which was more than in the equivalent scenarios in the DPLA (13%). In contrast to the DPLA-epidemics, only 40% of the infections were due to either local or airborne spread and >55% of disease transmission was caused by veterinarians, AI technicians or dairy tanker movements.

The results also showed that unlimiting the depopulation resources did not have as big an effect on the size of the epidemic in the SPLA as it had in the DPLA. Comparing the scenarios with unlimited versus limited resources showed that the pre-emptive culling of all herds at risk had the greatest impact on the size of the epidemic, which was reduced by more than 70% compared to the baseline scenario. Unlimiting the capacity of the depopulation resources resulted in a general reduction in the duration of the epidemic ranging from 15 - 30% when compared with the equivalent scenario with limited resources.

Only a slight decrease of <15% in the proportion of both the airborne and local spread was observed when comparing the scenarios with unlimited depopulation resources to the equivalent limited-resource scenarios. It can therefore be assumed that neither airborne nor local spread influenced the course of the epidemic in a SPLA very much. The spread of disease was more likely linked to movement contacts (>60%) such as veterinarians, AI technicians and dairy tanker movements.

DISCUSSION

This paper presents for the first time quantitative estimates for the epidemiological effects of control strategies, the duration of the silent spread period and the capacity of the depopulation resources on the course of simulated FMD epidemics in Switzerland. The importance of an early detection of disease was shown for DPLAs as the silent spread period of 14 instead of 21 days was able to nearly halve the mean duration of the epidemic from 58 to 28 days. Consequently, the mean size of the epidemic was reduced from 272 to 33 outbreaks and the mean number of slaughtered animals was reduced from about 20,000 to about 2,400. The delay in detection was partly responsible for the large scale epidemic in the UK in 2001 (Gibbens et al., 2001). Late detection of FMD may be due to farmers and professionals in FMD-free countries being slow to recognise FMD due to a lack of disease awareness. It is essential to bring disease awareness to a high level and to keep it there, and it is recommended that repeated campaigns calling attention to the characteristics and significance of the disease.

Evaluation of the effect of unlimited depopulation resources showed not only that resource capacity was a very sensitive parameter, but also had a greater impact on epidemics in DPLAs than in SPLAs. This corresponds to findings of a Dutch study (Mourits et al. 2002). The results of epidemics in DPLAs with limited depopulation resources showed that baseline control measures alone - as outlined in the current Swiss legislation - were not sufficient. Unlimiting the resources brought about a reduction of the epidemic sizes in DPLAs by half. Currently, Swiss authorities are able to use 13 depopulation teams and sets of equipment. One option to increase resources would be to increase the number of sets of depopulation equipment. Alternatively, veterinarians could be recruited to euthanase animals. The carcasses would then have to be stored adequately, but the IP would lose a great part of its infectivity. The carcasses would then be transported to rendering plants as disposal crews became available.

Given unlimited resources, strategies including pre-emptive slaughter or vaccination achieved comparable results in DPLA. All strategies including pre-emptive culling resulted in the slaughter of >40,000 animals. From an economical and ethical point of view, there are many arguments against the application of those strategies. The number of animals culled when depopulating either only DCs or only CPs was similar and as large as with the combined pre-emptive culling strategy. However, in consideration of the large number of animals killed with any of the pre-emptive culling strategies, their application in a potential epidemic in Switzerland would have to be considered carefully.

Ring vaccination in terms of protective vaccination proved to be an effective control measure regarding the size, the duration and the total number of slaughtered animals in epidemics in DPLAs. As the cost-effectiveness of this strategy has not been estimated for Switzerland, further analyses with an economic model need to be conducted to predict the financial consequences of the ring vaccination strategy there. For SPLAs, there appears to be hardly any justification for ring vaccination, probably due to the shorter duration of epidemics and the time needed for the immunity of animals to develop. Therefore, application of the control measures as currently described in the Swiss legislation for epidemics in SPLAs is recommended.

Ring vaccination was only efficient regarding the total number of slaughtered animals when 'vaccination to live' was practised. Before protective ring vaccination is implemented, authorities have to inform the consumers about the relevant issues. As the market is tailored to the expectations of consumers, authorities would need to reach an agreement with consumers' organisations that the meat and other products from vaccinated animals are acceptable for human consumption if this strategy were to be applied.

This study documented for the first time the importance of professionals such as AI technicians and veterinarians as transmitters of FMD virus. Particularly in SPLAs, they were responsible for >40% of all infections in most of the scenarios. For both components, the probability of disease transmission was not based on research but on expert opinion. Hence, detailed studies on this topic need to be carried out in order to be sure about the accuracy of this parameter. Biosecurity measures on cattle farms are very limited in Switzerland. A general discussion on the risk of disease transmission and on the influence of certain working practices would be indicated.

Throughout all scenarios, the importance of animal movements in the spread of disease seemed to be minor. The proportion of outbreaks caused by movements was <10%, most likely due to the low frequency of animal movements off farms. This may reflect reality, or it may be due to the over-estimation of local and airborne spread. Both local and airborne spread were of little importance in SPLAs due to the sparseness of neighbouring farms. In the course of the 2001 FMD epidemic in the UK, the transmission pathway could not be fully explained for many cases detected in the vicinity of an infected herd. In order to define the method of transmission, those infections were attributed to local spread (Gibbens et al., 2001). As the same modelling parameters were used as in the 2001 epidemic, it has to be considered that the significance of local spread in Swiss circumstances, and for certain FMD virus strains, may be over-estimated in these results.

The implementation of a 72-hour standstill of all animal movements did not result in a significant reduction in the mean number of outbreaks probably due to the prolonged phase of silent spread. This in accordance with Mourits et al. (2002) who found that the implementation

of a nationwide ban on livestock movements for 72 hours did not significantly reduce the size of epidemics in DPLAs.

Limitations of InterSpread Plus

InterSpread Plus has been expanded by adding some more flexible mechanisms such as specification of farm types and disease spread mechanisms. The model has become more complex in design and perhaps more realistic (IDL, 2002; Taylor, 2003). However, the added complexity demands detailed epidemiological and biological data in order to parameterise the model. Despite the recent epidemics in Europe, there is still very little published which would help in estimating these epidemiological parameters. In the scenarios explored in the current study, the parameter values used were derived either from intensive investigation or expert opinion, or were data that had already been used to model FMD epidemics by other authors. The results of the control scenarios have to be treated with caution as epidemiological data on the virus and disease related parameters are specific for the FMD virus serotype. The scenarios of this study were run with exactly the same spread parameter values. Consequently, despite remaining uncertainty regarding the exact values of parameters, relative comparison of effect of intervention strategies should be fairly robust. In case of a new epidemic, the parameters need to be adjusted before decision support can be provided in an emergency situation.

The great level of detail that is achievable with ISP is a temptation to integrate a large quantity of data. However, it was pointed out by Professor Gary Smith (Plenary Speech, Annual SVEPM Conference, 2004) that it is not necessary to know everything about a system, only what is relevant. Instead, it is important not to attempt to incorporate biological data that is irrelevant. The calibration of parameters describing both movement numbers and routes did not significantly influence the course of the epidemic in these simulations. Instead, the transmission probabilities of local spread and the capacity of the depopulation teams turned out to be the most sensitive parameters. More effort is needed to obtain improved parameters for these spread mechanisms rather than for movements.

ACKNOWLEDGEMENTS

The authors extend their special thanks to Mark Stern, Massey University, NZ, for his help and great commitment. In addition, the authors thank Bryan O'Leary of Massey University, NZ, for his technical assistance. This project was funded by the Swiss Federal Veterinary Office.

REFERENCES

BVET, B.f.V., F.-D.S. Käsespezialisten, S.M. (SMP), and V.d.S.M. (VMI). (2001). Konzept für die Milchsammlung bei einem Ausbruch der Maul- und Klauenseuche (MKS) in der Schweiz.

Gibbens, J.C., Sharpe, C.E., Wilesmith, J.W., Mansley, L.M., Michalopoulou, E., Ryan, J.B. and Hudson, M. (2001). Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. Vet Rec 149:729-43

IDL (2002). Note of points made at meeting with Professor John Wilesmith on January 2002 http://www.royalsoc.ac.uk/inquiry/554.pdf.

Jalvingh, A.W., Nielen, M., Maurice, H., Stegeman, A.J., Elbers, A.R. and Dijkhuizen, A.A. (1999). Spatial and stochastic simulation to evaluate the impact of events and control measures on the 1997-1998 classical swine fever epidemic in The Netherlands. I. Description of simulation model. Prev Vet Med 42:271-95

Keeling, M.J., Woolhouse, M.E., May, R.M., Davies, G. and Grenfell, B.T. (2003). Modelling vaccination strategies against foot-and-mouth disease. Nature 421:136-42.

Lüdi, F. (2004). Demographie, räumliche Verteilung und Dynamik der Schweizerischen Rindviehpopulation in den Jahren 2002 und 2003. Inaugural-Dissertation.

MAFF. (2001). Foot-and-mouth disease 2001 - epidemiological forecasts.

Mangen, M.-J.J. (2002). Economic welfare analysis of simulated control strategies for Classical Swine Fever epidemics. PhD Thesis Chapter 2.

Morris, R.S., Wilesmith, J.W., Stern, M.W., Sanson, R.L. and Stevenson, M.A. (2001). Predictive spatial modelling of alternative control strategies for the foot-and-mouth disease epidemic in Great Britain, 2001. Vet Rec 149:137-144.

Mourits, M.C.M., Nielen, M. and Léon, C.D. (2002). Effect of control measures on the course of simulated Foot and mouth disease epidemics that started on different farm types in various Dutch areas. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, 190-200

Nielen, M., Jalvingh, A.W., Horst, S.H., Dijkhuizen, A.A., Maurice, H., Schut, B.H., van Wuijckhuise, L.A. and de Jong, M.C.M. (1996). Quantification of contacts between Dutch farms to assess the potential risk of Foot-and-mouth disease spread. Prev Vet Med 28:143-158

Nielen, M., Jalvingh, A.W., Meuwissen, M.P., Horst, S.H. and Dijkhuizen, A.A. (1999). Spatial and stochastic simulation to evaluate the impact of events and control measures on the 1997-1998 classical swine fever epidemic in The Netherlands. II. Comparison of control strategies. Prev Vet Med 42:297-317

Raumentwicklung, B.f. 2004a/b. Eckdaten, Kanton Graubünden/ Kanton Luzern. http://www.are.admin.ch/are/de/raum/kantonsprofile/unterseite20/.

Stärk, K.D.C. (1998). systems for the prevention and control of infectious diseases in pigs. Thesis for the degree of Doctor of Philosophy:203-215.

Taylor, N. (2003). Review of the use of models in informing disease control policydevelopment and adjustment. DEFRA http://www.defra.gov.uk/science/Publications/2003/UseofModelsinDiseaseControlPolicy.pdf.

Tierseuchenverordnung. (1995).

Wilesmith, J.W. (2003). The use of the InterSpread simulation model during the 2001 FMD outbreak. Science Directorate, DEFRA FMD modelling workshop.

NETWORK ANALYSIS OF CATTLE MOVEMENT IN GREAT BRITAIN

R.M. CHRISTLEY[1], S.E. ROBINSON, R. LYSONS AND N.P. FRENCH

SUMMARY

Movement of animals represents considerable risk for the movement of infectious agents between farms and over considerable distances. Social network analysis provides tools to model and interpret contacts between farms associated with animal movements and other forms of contact. Using these methods we investigated the network of cattle movements throughout Great Britain (England, Wales and Scotland) in 2002. The structure of the movement networks was very heterogeneous, with most animal holdings having few on and off movements, but a few having very many. Investigation of a single four-week period identified the formation of giant weak and strong components, which were several orders of magnitude larger than the next largest components. The giant strong component for this period was a "small-world" network, and the degree distribution of this network followed a power-law distribution. The majority of movements occurred over the range of 10 to 100 km. Although some errors and omissions are known to exist in the movement records used in these analyses, the results of this study suggest clear features of the network of cattle movements in Great Britain that will have important implications for the transmission of infectious agents.

INTRODUCTION

Animal movements form complex networks linking farms and represent considerable risk for the movement of infectious agents (and genes, such as those encoding for antimicrobial resistance) between farms and over considerable distances. The role of direct and indirect contact between farms was graphically illustrated in the 2001 FMD outbreak, when spread during the critical first days of the outbreak was influenced by contact patterns, largely in the form of animals movements (Kao, 2002; Mansley et al., 2003). Similarly, animal movement is an important risk factor for transmission of a range of other diseases, such as brucellosis (Sheahan et al., 2002) and equine influenza (Guthrie et al., 1999). Furthermore, such complex non-random patterns of contact have fundamental effects on transmission dynamics (May et al., 2001; May & Lloyd, 2001).

Many spatially explicit epidemiological models assume that contact, and hence transmission, is simply a function of the Euclidean (straight-line) distance between points (Keeling et al., 2001). Whilst this contact definition is appropriate in some circumstances, transmission between farms occurring via movement of livestock (as well as equipment, people and vehicles) is more complex and geographically remote locations may be closely linked by animal movements. Social network analysis (SNA) provides tools to model and interpret contacts between farms associated with such factors.

[1] Robert Christley, Epidemiology Group, Faculty of Veterinary Science, University of Liverpool, Leahurst, Neston, CH64 7TE, UK. Email: robc@liv.ac.uk

The analysis of the network structure may help identify targets for interventions, such as control procedures and surveillance, and may inform mathematical models and simulations to investigate disease transmission through populations (Bell et al., 1999; Bell et al., 2002; Liljeros et al., 2001; Lloyd & May, 2001; Pastor-Satorras & Vespignani, 2001; Zanette & Kuperman, 2002). By identifying individuals (either animals or farms) with high vulnerability and infectivity, SNA can enhance targeted surveillance and prevention programmes. Within a network, vulnerability is a measure of the likelihood an individual will become infected and infectivity is the probability that an individual will infect others (Bell et al., 1999). In certain circumstances, vulnerability and infectivity are highly associated with several network parameters and network information may be fundamental to the development of rational control practices (Albert et al., 2000). Using SNA, we investigated the network of cattle movements in 2002 in Great Britain using the Cattle Tracing System (CTS) data, sourced from the Department for Environment, Food and Rural Affairs' (DEFRA) RADAR (Rapid Analysis and Detection of Animal Risk) database. The CTS is a register of all cattle within the national herd in Great Britain, with recordings of all births, deaths and movements between locations.

MATERIALS AND METHODS

Data

In Great Britain, all cattle must be individually identified and animal keepers must report each animal's birth, movements and death to a centralised data recording service. At present, movement notifications only record an animal's identity, the date, the identity of the holding, and the type of movement (on or off the premises). Hence, each movement record incorporates just one end of any movement event (leaving a holding, or arriving at another) but not both.

Cattle movement data were sourced from DEFRA's RADAR database. Data were available for most of 2002, although the data for December 2002 were incomplete. Within this database, locations are identified as slaughterhouses or animal holdings. Animal holdings (AH) include farms, markets and dealers. For these analyses, only movements between AHs were of interest; movements reported by slaughterhouses were not considered. The network of animal movements was re-constructed using animal and location identifiers, date and movement direction data. Anomalies occurred in the reconstructed data when on and off movements could not be linked. Additional anomalies occurred where particular animals reappeared at a location. In such circumstances, these latter movements were removed from the dataset. Due to these reconstruction errors, a proportion of animal movements were not included in the final network models. For example, 3% of the movements of animals onto farms recorded in the RADAR database in February 2002 were not present in the movement network for that month.

Data analysis

Annual Data: Microsoft Access 2002 (Microsoft Corporation, Redmond, WA) was used for database management. Network analyses were performed using the Pajek Programme for Large Network Analysis (v1.00, http://vlado.fmf.uni-lj.si/pub/networks/pajek/) and UCInet (v6.57, Analytic Technologies, Harvard, MA). Data for the entire year (January to December 2002) were plotted to assess the temporal trends in animal movements. Basic network features were also assessed for this period.

Network analyses: Descriptive statistics (degree, betweenness and farness) were calculated for each node using data available for 2002. In network analysis, the unit of interest (in this case animal holdings) are called *nodes*. Node *degree* is the number of other nodes directly contacted by each node. In this network, which is directed, both the in-degree (the number of holdings from which animals move onto a particular holding) and the out-degree (the number of holdings to which animals move from a particular holding) can be defined. *Betweeness* for node *i* is defined as the proportion of shortest distances between all pairs of nodes (excluding node *i*) that pass through node *i*. *Farness* is the sum of the shortest network distances between node *i* and all other nodes in the network. Hence, in a directed network, both in-farness (the sum of the shortest distance from all nodes to node *i*) and the out-farness (the sum of the shortest distances from node *i* to all other nodes) can be calculated. More detailed descriptions of these network parameters are available elsewhere (Wasserman & Faust, 1994).

The location of each animal holding was identified, based on the address of the holding, using QuickAddress (v3.3, QAS Ltd, Manchester, UK). This provided the easting and northing of each holding, to the nearest 100m. The distance between each pair of holdings was calculated from the easting and northing, using Pythagoras' theorem.

The network formed during the study period assumed that a contact between two animal holdings was present for the entire year. In order to examine the network over a time-scale more relevant to the epidemiology of infectious diseases, we analysed networks of movements arising from sequential 4-week periods. These periods provided "snapshots" of the network, whilst being sufficiently long to enable considerable connectedness to be present between holdings in the network. Initially, the number of strong and weak components was measured. Strong components are sections of the network where every holding can be reached from every other holding via directed paths, whereas weak components are sections of the network which are linked, but not every farm can be reached from every other farm (Fig. 1). In many networks a single large weak component exists, which contains a single large strong component. These are called the giant weak component (GWC) and the giant strong component (GSC) respectively. Other weak and strong components may exist, but these often include substantially fewer nodes than the giant components. Initially, detailed network analyses were performed on the GSC formed during each period. Results of analysis of a 4-week period in February 2002 are presented here. In order to assess the class of network represented by the largest strong component during the 4-week study period, the clustering coefficient and the average path length between nodes in this network was compared to an equivalent random network (i.e. with same number of nodes and movements).

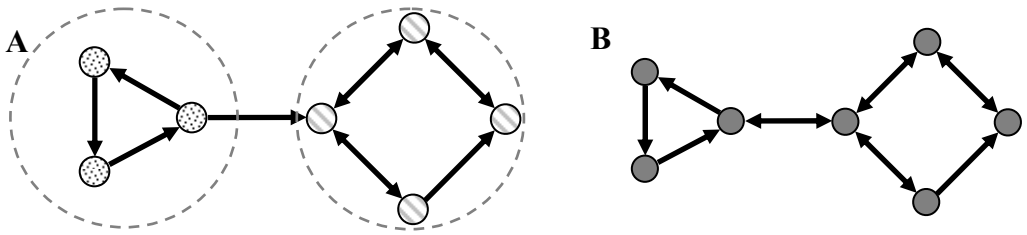


Fig. 1 Strong and weak components. In figure A there are two strong components (dashed circles) linked by a directed movement to form a single weak component. In figure B, there is a single strong component as all nodes are reachable from all other nodes.

The E-I index (Krackhardt & Stern, 1988) was calculated using post code data for each individual AH (measuring the share of movements made by that holding that are with AHs

outside its postcode area) and for the each post code area (the share of all movements involving AHs in an area that are with AHs outside that postcode area). The postcode area was obtained from the first letters of the postcode and represents a broad geographic area. The E-I index compares the number of movements (ties) between holdings within the same area to the number of movements between holdings in different areas. The index ranged between -1 and 1, with -1 indicating that all movements occur within the area and *vice versa*. The E-I index was calculated as:

$$E\text{-}I\ Index = \frac{Number\ of\ external\ ties - number\ of\ internal\ ties}{total\ number\ of\ ties} \quad (1)$$

RESULTS

Annual data

In 2002, approximately 60,000 holdings had at least one animal move onto their premises, and approximately 84,000 had at least one animal moving off. The number of animals moving onto premises was considerably lower than that moving off. Much of this difference was due to animals moving off AHs to slaughter (2.3M). Approximately 2.7M births were recorded in 2002. The majority of movements were recorded by animal keepers, although a proportion were generated by the British Cattle Movement Service (which controls movement recording) in order to account for data discrepancies, such as where an animal is recorded as arriving on a farm, but no record of it having left its previous location existed. The distribution of the number of movements per holding was highly skewed, with the majority of holdings having few movements on or off, and a small number having considerable numbers (Fig. 2). This figure describes the total number of animals arriving on, or leaving, an AH rather than the number of movement events. Hence, these include the simultaneous movement of multiple animals between two AHs.

Similar temporal trends were evident for both on and off movements, with peaks occurring in spring and autumn (data not shown). There was clear evidence of missing data for late December 2002. An effect of day of week was also evident. Relatively few movements occurred on Sundays throughout the year. Somewhat more movements occurred on Saturdays, but unlike Sundays, the number of animals moving on Saturday increased during the spring and autumn peaks. Reduced movement on Public Holidays was also clearly evident.

Detailed study of a 4-week period

During the 4-week study period in February 2002, the majority of farms contacted few or no other farms, as illustrated by the large number of weak components which included only 1 AH (Fig. 3). Approximately 35,000 AHs formed a single giant weak component (i.e. connected to the component by at least one movement). The next largest weak component included less than 10 AHs. The giant strong component included almost 2000 AHs (illustrated in Fig. 4), whilst the next largest included fewer than 20. This component formed a "small-world network" with substantially greater clustering (~100 fold increase) but similar average path length compared to the equivalent random network. Similar to the entire year, the distribution of the number of movements for the 4-week period was highly skewed, with one holding directly contacting over 20% of holdings in the strong component, and representing an important hub in the sub-network. Although direct contacts of this holding (i.e. those within 1 step) were largely confined to

Scotland, considerable movement to all of Great Britain was evident within 3 steps (data not shown). The distribution of the degree of each AH (i.e. the number of other AHs each was linked to) was similarly skewed (Fig. 5). The distribution of in- and out-degree for the largest strong component (P(k)) approximated a power law, with P(k)~$k^{-\gamma}$, where $\gamma = 2.1$ for both the on and off-degree.

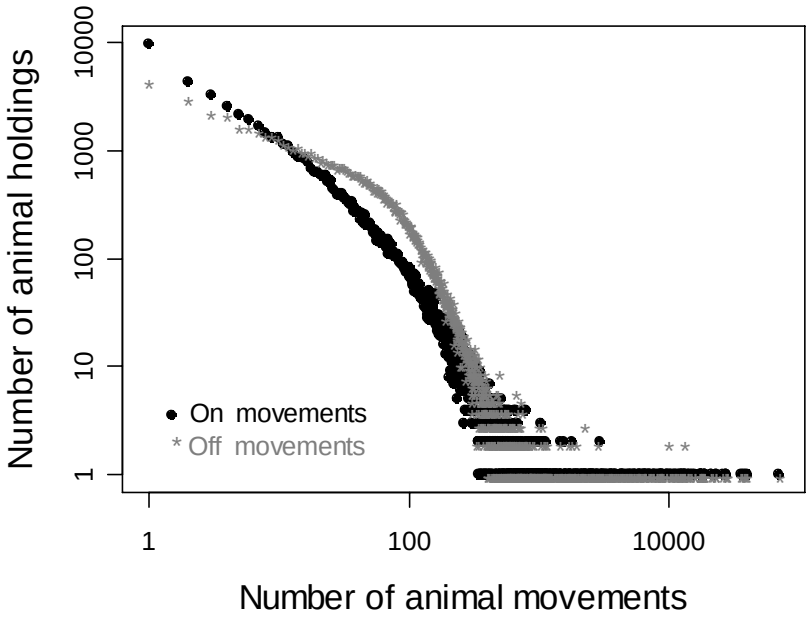


Fig. 2 The distribution of movement counts per animal holding in 2002

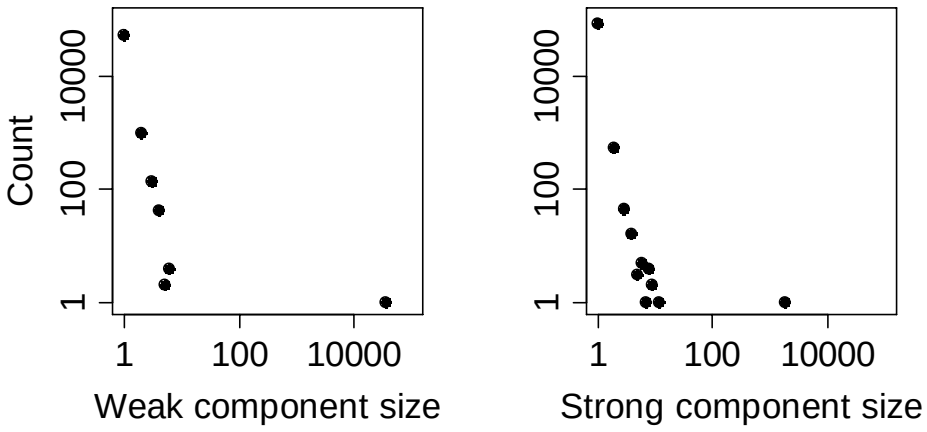


Fig. 3 The distribution of the size of weak and strong farm components arising from movement of cattle during February 2002

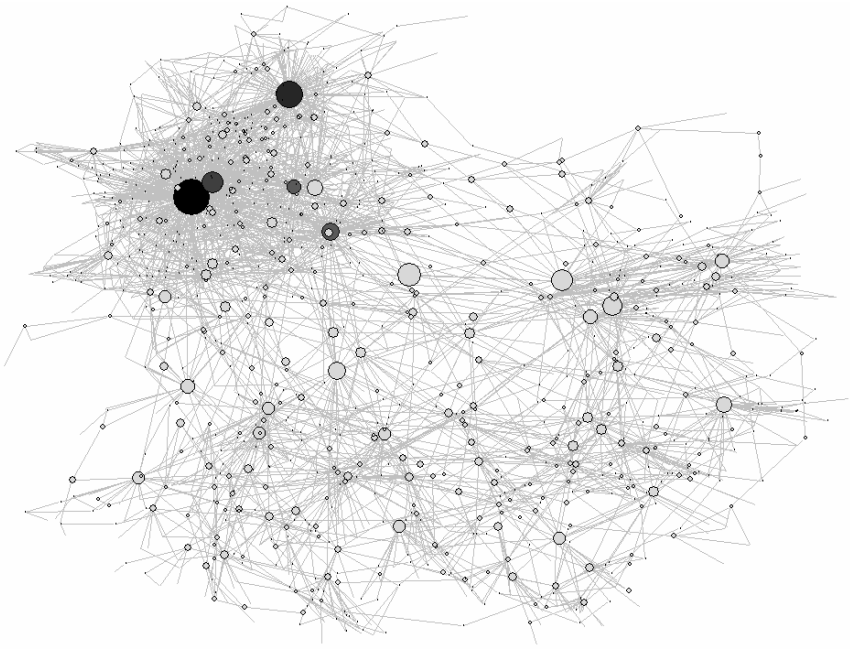


Fig. 4 The giant strong component in February 2002. The nodes represent animal holdings, the lines represent connections due to animal movements. The shade of the nodes is proportional to their degree, with the darkest nodes having the highest degree. The size of the nodes is proportional to their betweenness, with the largest nodes having the highest betweenness.

The network structure of the GSC in February 2002 is illustrated in Figure 4. The distributions of node in- and out degree and betweenness were highly skewed (Fig. 5), with the majority of nodes having a low degree (Interquartile range 2-5, maximum 414) and normalised betweenness (IQR 0-0.16, maximum 44.3). The distribution of in- and out-farness was also somewhat skewed (Fig. 6).

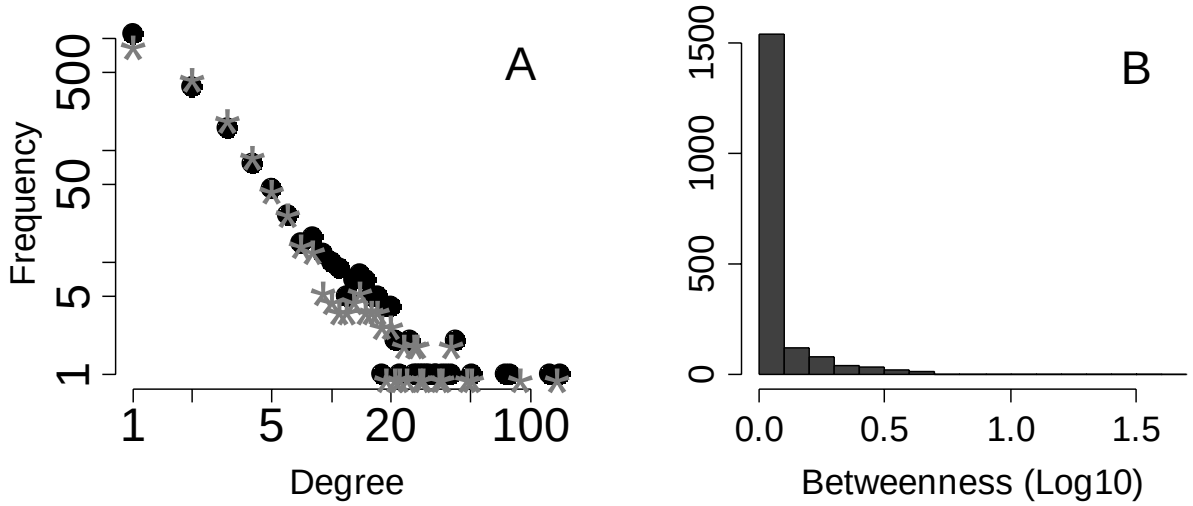


Fig. 5 The (A) distribution of in- and out-degree (black circles and grey stars, respectively) for the giant strong component in February 2002 and (B) frequency histogram of betweenness of nodes in the February giant strong component

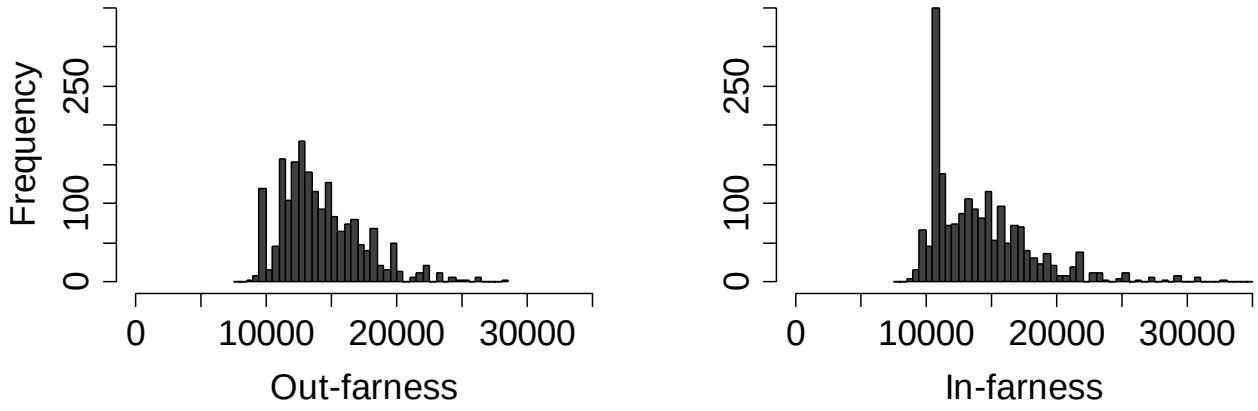


Fig. 6 Frequency histogram of out- and in-farness of nodes in the giant strong component in the February 2002 network

The distribution of distances over which cattle movements occurred during February 2002 was highly skewed, with the majority of movements occurring over a relatively short distance (Fig. 7). The minimum distance recorded was 0.1km, whilst the maximum was 1000km; from Orkney to the Southeast of England. The median distance was 39 km (IQR 17 to 98 km).

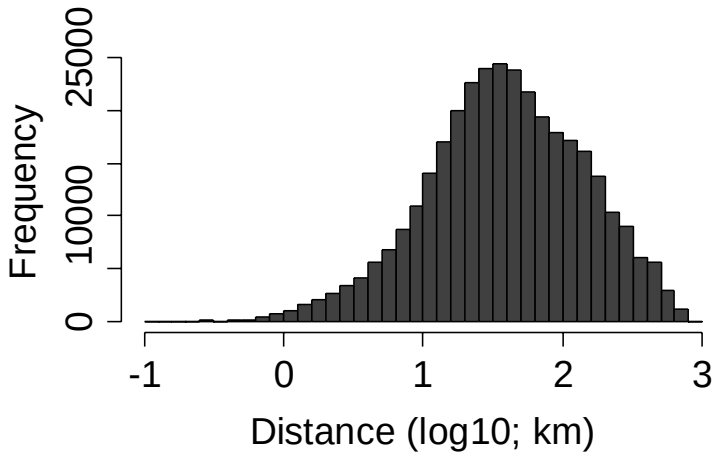


Fig. 7 Distribution of distances over which cattle were moved in February 2002

The United Kingdom is divided into 123 postcode areas representing contiguous geographical regions, based on the first part of the postcode. The farms in the February GSC were located in 79 different postcode areas. Due to errors in the address data for 141 farms, the location of these was not determined. The overall E-I index for the February GSC was 0.284, which was significantly different ($p<0.001$) to the expected value (0.931), suggesting more movements occurred within postcode areas than would be expected if cattle movement was random. Many farms moved animals either entirely within or outside their postcode area (Individual E-I index of -1 and 1, respectively; Fig. 8). However, when considered at the level of the postcode area, the E-I indices tended to be positive, suggesting most animals were moved outside their area of origin.

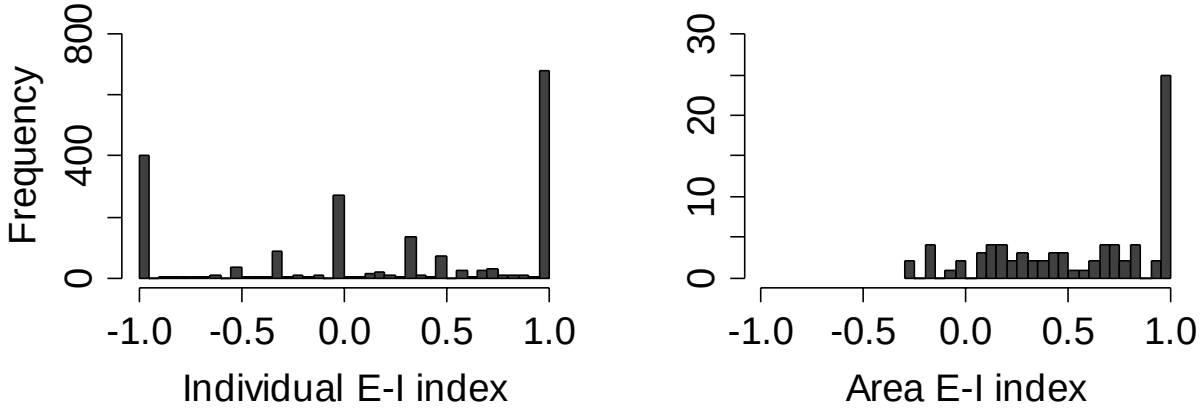


Fig. 8 Frequency histogram of the E-I index for individual animal holdings and for postcode areas in the giant strong component in the February 2002 network

DISCUSSION

These data highlight some features of the UK cattle industry that may have important implications for the dynamics of infectious agents. Many spatially explicit epidemiological models assume that contact between farms is simply a function of the Euclidean (straight-line) distance between locations. Whilst this contact definition is appropriate in some circumstances, these results demonstrate that transmission between farms occurring via movement of livestock (as well as equipment, people and vehicles) is more complex, and geographically remote locations may be closely linked by animal movements. Networks of contact between sheep (Webb & Sauter-Louis, 2002), between racehorse trainers (Christley & French, 2003) and between cattle and swine farms (Bigras-Poulin et al., 2004) have demonstrated similar complexity.

The number of animal movements per day varied with season and day of the week. Such variation may affect the rate and distance of spread of an agent. The periods of greatest movement occurred at the end and beginning of winter; periods when animals are being moved from summer pasture to winter housing. Hence, this period may also be associated with on-farm mixing of animals, further increasing potential for transmission. Examination of longer time series is necessary to determine the true seasonality of movements.

During 2002, the majority of animal holdings had relatively few animals moving on or off their premises. However, a few had a vast number of movements, up to approximately 70,000 arriving and leaving. These holdings are likely to be markets (although this could not be determined from the available data) and can be considered to be 'hubs' which are likely to play an important role in pathogen dissemination. This effect was also evident in the degree distribution for the 4-week period more thoroughly investigated. This scale free (power-law) distribution has been noted in many real networks (Barabasi & Albert, 1999) and may result in maintenance of an agent in a population, even with very low transmission probabilities. Such heterogeneous mixing increases the basic reproductive number (R_0), compared to that which would be expected in a homogeneously mixing population in which all nodes have *k* contacts. In such heterogeneously mixing networks, $R_0=\rho_0(1+(CV)^2)$, where ρ_0 is the reproductive number expected in a homogenously mixing population, and CV is the coefficient of variation of node degree (May & Lloyd, 2001). Hence, for the largest strong component in February 2002 reported here, the R_0 was 6 to 10 times higher that ρ_0. Hence, agents with considerably lower

transmission coefficients could be maintained, or even become epidemics, in such a network structure compared to equivalent homogeneously mixing networks. However, such estimations assume random mixing, within the constraints of a power-law degree distribution. The networks observed here (and most other real world networks) were small-world networks, which demonstrated considerably more clustering than would be expected in a random network (Watts & Strogatz, 1998). In such networks agents tend to spread more rapidly, but ultimately infect fewer individuals, compared to randomly missing networks (Christley et al., 2004; Newman, 2003).

Calculation of centrality measures, such as degree, betweenness and farness, may provide useful information to help guide surveillance and disease control procedures. Several studies, using simulated or simple real, small populations have found considerable association between these parameters and risk of infection (Bell et al., 1999; Christley et al., 2004). Whether such relationships hold in more complex populations is unknown.

Animal movements most commonly occur over distances of 10 to 100 km but long-range movements (up to the length of the British Isles) could result in rapid dissemination of agents over a wide area (as was observed during the 2001 FMD outbreak). At a regional level, animal movements tended to be made to holdings outside a holding's own postcode area. However, in some areas most or all movements occurred between holdings within that area. Such data may prove valuable, when used in 'real time' in surveillance planning, targeting areas at high risk of acquiring or transmitting infection, or in response to outbreaks in order to quantify the risk of local versus national spread.

Over relatively short periods (4 weeks) farms were linked by animal movements to form large interconnected networks (giant components) that were geographically dispersed. In the example given, a single giant weak component (GWC) formed and incorporated a single giant strong component (GSC) at its *core*. The remaining components (weak and strong) were small. Several small strong components formed giant out-components, i.e. they were reachable from the GSC by a directed path. Hence disease may have moved from the GSC to these holdings, but not *vice versa*. Similarly, a few formed giant in-components, in that disease could transmit from these to the GSC, but not *vice versa*. The few remaining small components were isolated from other components. The formation of giant components has also been noted in a study of connections between sheep farms (Webb & Sauter-Louis, 2002). Identification of such network structures are of considerable importance because if disease enters a giant component, then a considerable proportion of the population may be at risk. However, disease entry into other parts of a network may result in relatively few affected individuals (farms).

It is important to note that these network analyses assume that directed connections between holdings are permanent structures, not brief events. Some of this effect has been addressed by analysing networks formed over relatively short periods. Such simplifications enable network models to be relatively easily interpreted, but may reduce their validity. Future analyses will investigate the effect of such dynamic processes.

The quality of the data used in this study has been extensively audited (Anon, 2003). Discrepancies exist in approximately 3% of movement records, the lifetime movement records of approximately 1 in 8 cattle are incomplete and the current location of approximately 2% of the national herd is unknown. This is consistent with the absence from our network for February 2002 of approximately 3% of movements recorded in the RADAR database. Furthermore, several methods of identification of holdings were available and results differ (very) slightly

depending on which was used. However, considerable advances have been made improving the quality and speed of data recording and these data provide a valuable resource for future research and for planning of surveillance and control strategies.

ACKNOWLEDGEMENTS

This research is funded by the Department for Environment, Food and Rural Affairs (Defra), UK. The data for this project were provided by Defra and RADAR. The assistance of Giles Paiba and Lisa Smith (Veterinary Surveillance Division, Defra), Professor Martin Everett (University of Westminster) and Mr John Davies (University of Liverpool) is gratefully acknowledged.

REFERENCES

Albert, R., Jeong, H. and Barabasi, A.-L. (2000). Error and attack tolerance of complex networks. Nature 406, 378-382

Anon (2003). Identifying and tracking Livestock in England, National Audit Office, UK.

Barabasi, A.-L. and Albert, R. (1999). Emergence of Scaling in Random Networks. Science 286, 509-512

Bell, D.C., Atkinson, J.S. and Carlson, J.W. (1999). Centrality measures for disease transmission networks. Social Networks 21, 1-21

Bell, D.C., Montoya, J.M., Atkinson, J.S. and Yang, S.-J. (2002). Social networks and forecasting the spread of HIV infection. JAIDS 31, 218-229

Bigras-Poulin, M., Thompson, A., Chriel, M., Mortensen, S. and Greiner, M. (2004). Network anlaysis of the Danish Cattle and swine industry trade patterns as an evaluation of risk potential for disease spread. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, pp 189-199

Christley, R.M. and French, N.P. (2003). Small-world topology of UK racing: the potential for rapid spread of infectious agents. Eq. Vet. J. 35, 585-588

Christley, R.M., Pinchbeck, G.L., Bowers, R.G., French, N.P., R., B. and Turner, J. (2004). Network parameters and risk of infection in isolated populations. In: Society for Veterinary Epidemiology and Preventive Medicine (poster)

Guthrie, A.J., Stevens, K.B. and Bosman, P.P. (1999). The circumstances surrounding the outbreak and spread of equine influenza in South Africa. Rev. sci. tech. Off. int. Epiz. 18, 179-185

Kao, R.R. (2002). The role of mathematical modelling in the control of the 2001 FMD epidemic in the UK. Trends Microbiol. 16, 279-286

Keeling, M.J., Woolhouse, M.E.J., Shaw, D.J., L., M., Chase-Topping, M., Haydon, D.T., Cornell, S.J., Kappey, J., Wilesmith, J.W. and Grenfell, B.T. (2001). Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogenous landscape. Science 294

Krackhardt, D. and Stern, R.N. (1988). Informal networks and organizational crises: an experimental simulation. Soc. Psychol. Q. 51, 123-140

Liljeros, F., Edling, C., Amaral, L.A.N., Stanley, H.E. and Åberg, Y. (2001). The web of human sexual contacts. Nature 411, 907-908

Lloyd, A.L. and May, R.M. (2001). How Viruses Spread Among Computers and People. Science 292, 1316-1317

Mansley, L.M., Dunlop, P.J., Whiteside, S.M. and Smith, R.G.H. (2003). Early dissemination of foot-and-mouth disease virus through sheep marketing in February 2001. Vet. Rec. 153, 43-50

May, R.M., Gupta, S. and McLean, A.R. (2001). Infectious disease dynamics: what characterizes a successful invader? Philos. Trans. Roy. Soc. Lond B 356, 901-910

May, R.M. and Lloyd, A.L. (2001). Infection dynamics on scale-free networks. Phys. Rev. E 64, 066112

Newman, M.E.J. (2003). Properties of highly clustered networks. Cond Mat 0303183

Pastor-Satorras, R. and Vespignani, A. (2001). Epidemic dynamics and endemic states in complex networks. Phys. Rev. E 63, 066117

Sheahan, M., Maher, P. and O'Hagan, G. (2002). Brucellosis in cattle in the Republic of Ireland 1997-2001: Review and outlook. Irish Vet. J. 55, 394-398

Wasserman, S. and Faust, K. (1994). Social Network Analysis: Methods and Applications, Cambridge University Press, Cambridge

Watts, D.J. and Strogatz, S.H. (1998). Collective dynamics of 'small-world' networks. Nature 393, 440-442

Webb, C.R. and Sauter-Louis, C. (2002). Investigations into the contact structure of the British Sheep population. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, pp 10-20

Zanette, D.H. and Kuperman, M. (2002). Effects of immunization in small-world epidemics. Physica A 309, 445-452

PUBLIC HEALTH

COMPARING AND CONTRASTING THE EPIDEMIOLOGY OF SHEDDING OF *ESCHERICHIA COLI* O157 AND *CAMPYLOBACTER* SPP. ON UK DAIRY FARMS

S.E. ROBINSON[1], P.E. BROWN, J. S. DUNCAN, E.J. WRIGHT, H.E. CLOUGH, A.J.H. LEATHERBARROW, P.S.L. KWAN, M. UPTON, A.J. FOX, P.J. DIGGLE, C.A. HART AND N.P. FRENCH

SUMMARY

Targeted interventions at the farm level may be an effective means of reducing human exposure to *Campylobacter* spp. and *E. coli* O157. The epidemiology of shedding of *E. coli* O157 and /or *Campylobacter* spp. from freshly voided cattle faecal samples was compared. The molecular epidemiology and the relationship between multilevel risk factors and isolation of *E. coli* O157 and *Campylobacter* spp. from faecal samples was explored. There were several similarities in the epidemiology of these two pathogens. Within each farm, the weaned management group was associated with the highest levels of shedding and the relationship between age and the risk of shedding appeared to be very similar for both organisms. Cattle on farms that shared similar strains of *E. coli* O157 also shared similar strains of *C. jejuni*. Previous studies have suggested that dietary manipulation may be a potential on-farm intervention for reducing shedding of either *Campylobacter* spp. or *E. coli* O157 in cattle, but factors affecting shedding of both organisms have rarely been explored.

INTRODUCTION

Although several food animal species have been associated with causing foodborne illness, between 1992 and 1999 it was estimated that 16% of human cases of infectious intestinal disease (IID) were related to the consumption of red meat (Smerdon et al., 2001). Evidently, ruminants are important source of human infection with foodborne pathogens.

Escherichia coli O157:H7 and *Campylobacter* spp. are both considered as important causative agents of IID in the UK. Ruminants, in particular cattle, are widely regarded as natural reservoirs of *E. coli* O157, asymptomatically shedding these bacteria into the environment. Humans become exposed to cattle faeces contaminated with *E. coli* O157 through various routes although most commonly through food or water and through direct or indirect animal contact through the environment (Locking et al., 2001). Human infection with *Campylobacter* spp. is considered to be a largely foodborne disease which, up until recently, has been most commonly associated with consumption of poultry food items (Tauxe, 1992). *Campylobacter jejuni* is the species most frequently associated with human illness and there have been several documented outbreaks of *Campylobacter* spp. infection in the UK associated with contaminated milk (Gillespie et al., 2003). Therefore, although the routes by which humans

[1] Susan Robinson, Epidemiology Group, Faculty of Veterinary Science, University of Liverpool, Leahurst, Neston, CH64 7TE, UK. Email: s.e.robinson@liv.ac.uk

become exposed to these two organisms may differ, cattle appear to be an important source of human infection for both *E. coli* O157 and *Campylobacter* spp.

Both organisms are frequently isolated from cattle faeces and the most recent UK studies have reported prevalence estimates of 8.1% (Paiba et al., 2003) and 36% (Brown et al., 2004) for *E. coli* O157 and *C. jejuni* respectively. When comparing studies that describe the epidemiology of shedding of these pathogens in cattle populations, several features appear to be similar. Shedding of *E. coli* O157 is characterised by intermittent shedding (Robinson et al., 2004) and appears to be more prevalent in young stock (Paiba et al., 2003). In comparison to *E. coli* O157, there is a lack of studies of carriage of *Campylobacter* spp. in cattle, although shedding also appears to be characterised by intermittent shedding, and a higher prevalence in young stock (Nielsen, 2002; Stanley et al., 1998;).

The application of pre-harvest interventions to reduce synergistically the entry of pathogens into the food chain has been proposed. However this approach has been largely restricted to comparing the epidemiology of Salmonellae and *E. coli* O157 in cattle populations (Edrington et al., 2004), probably due to initial low estimates of the prevalence of *Campylobacter* spp. in cattle. The epidemiology of shedding of *E. coli* O157 and *Campylobacter* spp. in dairy cattle on 5 farms was compared during two concurrent longitudinal studies conducted over a minimum of 12 months. Furthermore, new molecular techniques for typing were applied to compare the genetic relatedness of strains of *E. coli* O157 and *C. jejuni* respectively, isolated on the different farms.

MATERIALS AND METHODS

Farm selection

Farmers were contacted at random from the list of dairy farmers registered with the Leahurst Farm Animal Veterinary Practice. On an initial sampling visit, fresh faecal samples were taken. If any samples tested positive for *E. coli* O157, the farm was invited to join the study. Eight farms were sampled in total, before 5 positive farms were identified. The farms varied in location, herd size, and type. Farms 1 and 3 had herd sizes of approximately 100 and 200 milking cows respectively, and both were solely dairy enterprises. Farms 2, 4 and 5 had approximately 50 milking cows, with farms 4 and 5 having beef suckler as well as dairy cattle present on the farm. Farms 3 and 5 were in a similar geographical location as were farms 1, 2, and 4. Farms 1 and 4 were contiguous premises. During the study, full sets of sampling equipment, including overalls, measuring and cleaning materials, were retained on each farm premises to minimise the risk of transfer of infected material between farms.

Collection of faecal samples

Each farm was visited approximately every 4 weeks from September 2001 to December 2003 and sampling of all farms was completed over a two-week period in each round. On each visit, the management groups: unweaned, weaned, heifers, lactating and dry cattle, were identified. Five (herds 2, 4, 5) or ten (for herds 1 and 3) samples were taken from each management group dependent on total herd size. If management groups were held in two or more separate pens/fields, stratified sampling with proportional representation was conducted; in all other cases animals were sampled randomly. All samples were taken from fresh faeces after animals were observed defecating. Information on potential risk factors was collected during

each sampling by the first author. These variables were at several different levels including visit, housing, animal and faecal pat. For each pen or field, the total number of animals was recorded together with the ear tag numbers of all animals. This provided information on the location of study animals at each sampling visit.

Bacterial isolation

All samples collected on sampling visits between September 2001 and April 2003 were cultured for *E. coli* O157. From January 2003 until April 2003 each faecal sample was vortexed and split to form two separate samples that were cultured for both *E. coli* O157 and *Campylobacter* spp.. Samples collected after April 2003 were cultured for *Campylobacter* spp. only. Isolation of *E. coli* O157 was conducted at Leahurst whilst samples to be cultured for *Campylobacter* spp. were stored in the fridge overnight and transported to Manchester Health Protection Agency (HPA) for culture and isolation.

E. coli O157: The isolation of *E. coli* O157 was achieved as described previously (Robinson et al., 2004) with some minor modifications. Briefly, 2g faeces were weighed into 18ml buffered peptone water (Lab M, Bury, UK) + vancomycin ((8 mg l^{-1}, Sigma Aldrich, Poole, UK) BPW + V). The remaining sample was then incubated for 18h at 37^{o}C. After selective enrichment, samples were subjected to immuno-magnetic separation (IMS) (using the manufacturer's recommendations) with O157-specific magnetic beads (Dynal, Bromoborough, UK) and the presence of the O157 gene and virulence determinants confirmed as previously described (Robinson et al., 2004).

Campylobacter spp.: A cotton tipped swab of faeces (approximately 1g) was used to inoculate *Campylobacter* enrichment broth (Bolton broth) prepared with selective supplement X-131 (Lab M, Bury, United Kingdom) without the addition of blood in thick walled glass universal bottles with minimum head-space. Enrichment cultures were incubated at 37°C for 24 h and then at 42°C for a further 24 h. After incubation, a loopful of enrichment cultures were sub-cultured onto *Campylobacter* blood-free selective agar plates (CM739; Oxoid, Basingstoke, United Kingdom) containing charcoal cefoperazone-deoxycholate agar (mCCDA) selective supplement (SR155E; Oxoid, Basingstoke, United Kingdom) and were incubated microaerobically at 37°C for 48 h. Identification of presumptive campylobacter isolates was based on colony morphology, Gram stain and the oxidase test. Isolates were then sub-cultured onto Columbia blood agar for 24-48 at 37^{o}C. Identification of *C. jejuni* isolates was performed with the hippurate hydrolysis test and by PCR as described by Best et al., (2003).

Molecular Typing

Verocytotoxigenic *E. coli* isolates were grouped by farm, management group, virulence determinants and serogroup. Sixty-seven O157 isolates were then randomly selected from within these groups (with the number selected from each group proportional to its size) and compared by pulsed-field gel electrophoresis. The method was based on one-day standardized protocol for molecular subtyping of *E. coli* O157 as described by the Centers for Disease Control and Prevention (CDC, 1998). Following electrophoresis and staining, gels were photographed and entered into the Molecular Analyst Software using the Geldoc 2000 system. Genomic analysis was performed using Molecular Analyst® Software Fingerprinting Version 1.6 (Bio-Rad

Laboratories Ltd.). Grouping analysis used Dice coefficients and the unweighted pair group method using average (UPGMA) clustering method (Dice, 1945) using a 2% tolerance window.

Isolates of *C. jejuni* were grouped as for *E. coli* O157 and multilocus sequence typing on a random sample of 100 *C. jejuni* isolates (chosen to represent the five different farms and temporal aspects of the data) was carried out at Manchester HPA according to the method described by Dingle et al., (2001).

Statistical analysis

Continuous variables: Multivariable logistic generalised additive models (Hastie & Tibshirani 1990) were used to assess the effect of the age of faecal pats on the risk of isolating *E. coli* O157 or *Campylobacter* spp., and to look for a time trend in the prevalence of the bacteria over the course of the study. This non-parametric modelling technique produces a very flexible estimate of the relationships, and was used as an exploratory tool to determine a more specific functional form for the association. This analysis was implemented using the function 'gam' in S-PLUS (S-PLUS 2000, Mathsoft Inc.).

Multilevel mixed effects models: All variables considered as possible risk factors were initially tested for their association with shedding of either *Campylobacter* spp. or *E. coli* O157 using fixed effects generalised linear models (GLM). We assessed the importance of each covariate using backward elimination procedures such that variables remained in the model if they significantly improved the fit of the model (assessed by a reduction in the AIC) or if removal resulted in substantial change in the effect of other variables. When only significant variables remained, we implemented a mixed effects model that included variables judged to be significant from the GLM. The data collected has a hierarchical structure, with each sample (level 1) nested within cow (level 2), within pen (level 3), within management group and farm. As there were only 5 farms and 5 management groups these levels were fitted as fixed effects. There were insufficient animals re-sampled on separate visits to warrant an animal level random effect and therefore a random effect for animals within different management groups on different visits was included in the model. The effect of biologically plausible interactions between variables was also tested for in the model.

Chi-squared test was used to test for any association between a sample testing positive for *E. coli* O157 and *Campylobacter* spp.. All models were fitted in the statistical package R (www.r-project.org).

RESULTS

Descriptive results

Table 1 shows the comparison of the frequency with which *E. coli* O157 and *Campylobacter* spp. were isolated from cattle faecal samples from different management groups. *Escherichia coli* O157 was isolated from 209/2580 (8.1%) samples collected between September 2001 and April 2003. *Campylobacter* spp. were isolated from 390/1203 (32.4%) of samples collected between January 2003 and December 2003. Although not all of the isolates of *Campylobacter* have been assigned to species, approximately 85% of a subset that have been tested were *C. jejuni*. Although *Campylobacter* spp. was more frequently isolated from faecal

samples than *E. coli* O157 in all management groups, the proportion of samples positive followed a similar trend for both pathogens, with the weaned group associated with highest levels of isolation and adult cattle (lactating and dry groups) associated with the lowest levels of isolation (Table 1).

Table 1. Frequency of isolation of *E. coli* O157 and *Campylobacter* spp. in different management groups on five UK dairy farms

Management group	No. samples positive for *E. coli* O157	No. samples collected	**% samples positive**	No. samples positive for *Campylobacter* spp.	No. samples collected	**% samples positive**
Unweaned	39	386	**10.1**	47	163	**28.8**
Weaned	108	693	**15.6**	158	326	**48.5**
Heifers	21	422	**5.0**	81	226	**35.8**
Lactating	29	648	**4.5**	62	300	**20.7**
Dry	12	385	**3.1**	42	188	**22.3**
Total	209	2534[a]	**8.1**	390	1203	**32.4**

[a] For 46 samples the management group that the animal belonged to was unknown.

The prevalence of both pathogens varied considerably between the five farms, and ranged from 1% to 15% for *E. coli* O157 and, 28% to 40%, for *Campylobacter* spp.. There appeared to be no obvious consistency between farms that had a high or low prevalence of shedding of *E. coli* O157 and those with a high or low prevalence of shedding of *Campylobacter* spp..

Generalised additive models

The fitted relationship between age and the risk of isolating *E. coli* O157 and *Campylobacter* spp. from faeces from a multivariable GAM are shown in Fig. 1.

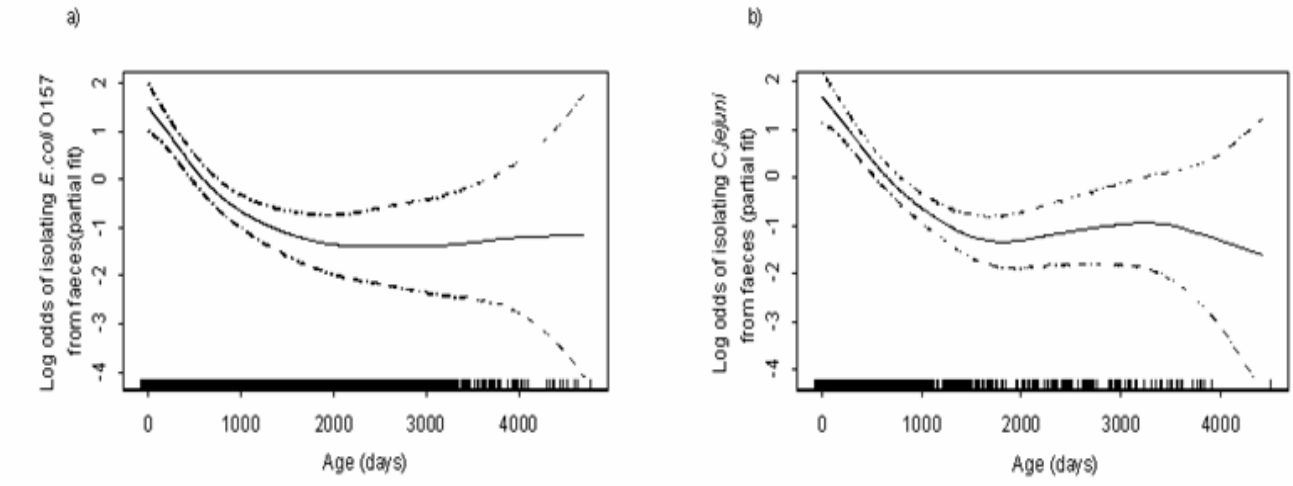


Fig. 1 Fitted relationships between samples testing positive for a) *E. coli* O157 and b) *Campylobacter* spp. after adjusting for the other confounders: farm, management group and month of year. The relationship was fitted non-parametrically using penalised least squares smoothing. The plots show the fitted curves (—) with 95% confidence intervals (- - -). The rug plots along the x-axis represent the number of data points.

After allowing for the confounders management group, farm and month the risk of shedding *E. coli* O157 and *Campylobacter* spp. declined monotonically with age although a slight increase in the proportion of cattle shedding was evident in cows aged over 3000 days. However the wide confidence intervals for cattle aged over 3000 days indicates the scarcity of cattle on farms over 3000 days. Although the risk of isolating each pathogen is different for cattle aged over 2000 days, for cattle aged less than 2000 days the relationship between age and risk of isolating each pathogen was remarkably similar.

To explore any seasonal effects on the risk of isolating *E. coli* O157 and *Campylobacter* spp. from cattle faeces, the continuous variable 'day of year' and was also explored using GAMs. When fitting 'day of year' in a univariable GAM, the isolation of both *E. coli* O157 and *Campylobacter* spp. from cattle faeces appeared to be associated with increased risk in the spring (March) and autumn (October) and summer (June) and autumn (October), months respectively. However, in a multivariable GAM, after adjusting for farm, management group and type of housing, there appeared to be an increased risk of isolates associated with the summer months for both *Campylobacter* spp. and *E. coli* O157. The effect of any temporal trends in shedding was determined in the subsequent multivariable generalised linear mixed models described below.

Comparison of the risk factors for isolating *E. coli* O157 and *Campylobacter* spp. from faeces

The results from the final multivariable models (fit using Penalised Quasi-likelihood) of risk factors associated with isolating *E. coli* O157 and *Campylobacter* spp. from faeces are shown in Table 2. Confirming the descriptive findings stated above, cattle on different farms and in different management groups were associated with a different levels of risk in terms of isolating either *Campylobacter* spp. and /or *E. coli* O157 from cattle faeces. Farm 4 was associated with the highest level of risk of in both models (OR = 2.91 and 2.27) although there were no further consistencies between different farms and the risk of isolating both pathogens from cattle. For instance, Farm 3 was associated with a significantly reduced risk of isolating *E. coli* O157 but a significantly increased risk of isolating *Campylobacter* spp. from cattle faeces than for Farm 1. The weaned management group was associated with a significant increased risk of isolating *E. coli* O157 (OR= 2.27) and *Campylobacter* spp. (OR=1.82) when compared to the unweaned group. Adult cattle were associated with a reduced risk of shedding both *E. coli* O157 and *Campylobacter* spp. than cattle in the unweaned, weaned and heifer management groups.

The presence of whole pieces of grain in the faeces of cattle was found to be associated with an increase in both the risk of detecting *E. coli* O157 and *Campylobacter* spp., although the highest level of risk was associated with different scores of grain intensity. The relationship between the risk of detecting these pathogens and the relative intensity of grain within faeces was similar, with large numbers of whole pieces grain associated with the presence of multiple pieces of grain (score 3) associated with a marked reduction in the probability of detecting *E. coli* O157 from faeces. However, the collection of fewer faeces samples containing these high levels of grain may have also affected the association at this level of grain intensity. There was a significantly decreased risk of isolating *E. coli* O157 from the faeces of cattle out at pasture compared to cattle housed indoors, even after allowing for the effect of season in the model. This effect was not significant for *Campylobacter* spp., although a similar tendency was found. Other possible confounders such as diet, although included, did not remain in the model.

Table 2. Multivariable logistic regression models of the risk factors associated with faecal samples testing positive for *E. coli* O157 and *Campylobacter* spp.

	***E. coli* O157**			***Campylobacter* spp.**		
Variable	Estimates	Odds Ratio	95% CI	Estimates	Odds Ratio	95% CI
Random Effect	Variance estimate			Variance estimate		
Samples from animals in same management group at each visit	0.36			0.08		
	Coefficients			Coefficients		
Fixed Effects						
Farm						
1	0	1 [a]	-	0	1 [a]	-
2	**-2.28**	**0.10**	**0.03, 0.32**	0.15	1.16	0.71, 1.90
3	**-0.95**	**0.39**	**0.22, 0.68**	**0.68**	**1.97**	**1.33, 2.92**
4	**1.07**	**2.91**	**1.79, 4.76**	**0.82**	**2.27**	**1.13, 4.55**
5	0.18	1.20	0.69, 2.06	**0.54**	**1.72**	**1.03, 2.86**
Management group						
Unweaned	0	1 [a]	-	0	1 [a]	-
Weaned	**0.82**	**2.27**	**1.09, 4.72**	**0.60**	**1.82**	**1.01, 3.28**
Heifers	0.35	1.42	0.58, 3.47	0.46	1.58	0.84, 2.98
Lactating	-0.05	0.95	0.41, 2.23	-0.39	0.68	0.35, 1.30
Dry	-0.07	0.93	0.37, 2.37	-0.17	0.84	0.44, 1.62
Type of Housing						
Indoor	0	1 [a]	-	0	1 [a]	-
At pasture	**-0.71**	**0.49**	**0.29, 0.84**	-0.24	0.79	0.52. 1.20
Both	-1.30	0.27	0.06, 1.22	-0.10	0.90	0.35, 2.32
Season						
Spring	0	1 [a]	-	0	1 [a]	-
Summer	0.12	1.13	0.57, 2.25	**0.99**	**2.69**	**1.57, 4.62**
Autumn	0.009	1.00	0.51, 1.98	0.001	1.00	0.57, 1.77
Winter	-0.30	0.74	0.37, 1.47	-0.32	0.73	0.47, 1.13
Presence of whole pieces of grain in faeces						
0 (no grain)	0	1 [a]	-	0	1 [a]	-
1	**0.96**	**2.61**	**1.59, 4.30**	0.42	1.52	0.94, 2.46
2	0.53	1.70	0.91, 3.18	**1.06**	**2.89**	**1.58, 5.28**
3 (>100 pieces)	-1.31	0.27	0.04, 1.92	0.37	1.45	0.56, 3.70

[a] Indicates reference category
Numbers in bold represent significant risk factors
Spring = March-May , Summer = June-August, Autumn = September-November, Winter = December to February

In both models the parameter estimates associated with the seasons spring, autumn and winter were remarkably similar considering the differences in the size of the data sets and the different time-period over which the studies were conducted. Winter was associated with lower risk of shedding than were the spring and autumn months, which were associated with similar risk. Although the summer months (June-Aug) were associated with an increased risk of isolating both pathogens from faeces the effect was more pronounced for the isolation of *Campylobacter* spp. This effect was significant even though type of housing, an important confounder with housing, was also kept in the model.

Test for correlated excretion

Between January 2003 and April 2003, when the two studies ran concurrently, six hundred and eight samples collected were screened for the presence both *E. coli* O157 and *Campylobacter* spp.. As illustrated in Table 4, during this period, 199 and 43 samples were tested positive for *Campylobacter* spp. and *E. coli* O157 respectively. Using the chi-squared test, there was no evidence of any correlated excretion of these two pathogens (χ^2 value = 1.75, df =1, P-value = 0.19).

Table 4. The proportion of faecal samples from which *Campylobacter* spp. and /or *E. coli* O157 was isolated during the four month period that two separate longitudinal studies of *E. coli* O157 and *Campylobacter* spp. on five UK dairy farms ran concurrently.

	No. samples positive for *Campylobacter* spp.	No. samples negative for *Campylobacter* spp.	Total
No. samples positive for *E. coli* O157	18	25	**43**
No. samples negative for *E. coli* O157	181	384	**565**
Total	**199**	**409**	**608**

Comparison of the molecular epidemiology of isolates of *E. coli* O157 and *C. jejuni*

Nine unique restriction profiles (RP) were identified from the 67 *E. coli* O157 isolates typed by *XbaI* PFGE. Among the 100 isolates of *C. jejuni* typed by MLST, the most common clonal complexes represented (CC) were: 21, 22, 42, 49, 61 and 48. Figure 2 illustrates the RPs of *E. coli* O157 and CCs of *C. jejuni* identified by farm. There was striking similarity between the farms that shared similar RPs of *E. coli* O157 and those that shared similar CCs of *C. jejuni*. For instance, the predominant sequence types of *C. jejuni* isolated on Farms 3 and 5 belonged to CC 61, farms that also shared the same RP of *E. coli* O157. On Farms 3 and 5 the dominant strain of *E. coli* O157 (% of isolates) belonged to RP1 with the virulence profile *eae*A and *vt*2. On Farms 2 and 4 the dominant strain of *E. coli* O157 belonged to RP2 with the virulence profile *eae*A, *vt*1 and *vt*2. Clonal complexes C 21, 22 and 42 were among the most common clonal complexes representing the sequence types of *C. jejuni* on these farms. The CC 22 and 42 were uniquely associated with these three farms. Multiple isolates from the same samples tended to be of indistinguishable PFGE pattern and CC for *E. coli* O157 and *C. jejuni* respectively.

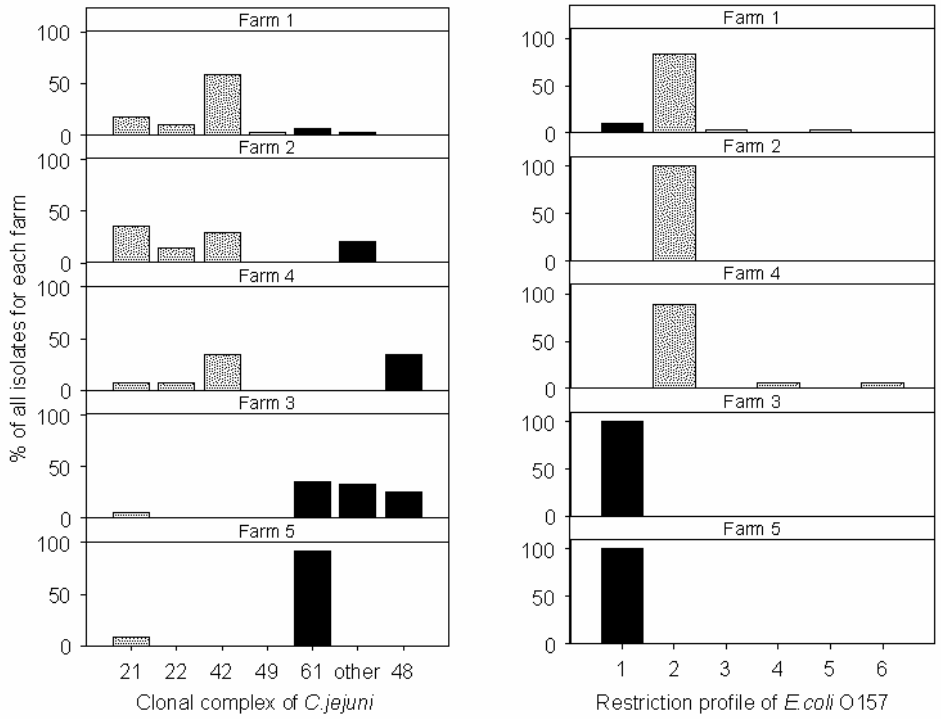


Fig. 2 The relative contribution of each clonal complex and restriction profile for isolates of *C. jejuni* and *E. coli* O157 respectively for each farm.

DISCUSSION

In this study we have demonstrated that in the same population of cattle, albeit at different time points, several features of the epidemiology of shedding of both *E. coli* O157 and *Campylobacter* spp. in cattle are similar. In particular, the functional relationship between age and the risk of isolating *E. coli* O157 or *Campylobacter* spp. from faeces was similar. In addition, factors associated with increasing the risk of isolating *E. coli* O157 and *Campylobacter* spp. from cattle faeces appeared to be consistent. Furthermore, the molecular epidemiology governing the presence and dissemination of strains of *E. coli* O157 and clonal complexes of *C. jejuni* between and within farms also appear to suggest that these pathogens may share similar transmission routes. As both of these pathogens are inhabitants of the cattle gastrointestinal (GI) tract, similarities in the epidemiology of shedding of these two organisms are not necessarily surprising. However, the lack of any statistical evidence of correlated excretion of these two pathogens also indicates the complexity of the population dynamics of foodborne pathogens in cattle GI tracts.

In both models the presence of whole pieces of grain in the faeces of cattle was positively associated with an increase in the risk of isolating either pathogen from faeces of cattle. Furthermore the non-linear relationship between the relative intensity of whole pieces of grain in faeces and the risk of isolating either pathogen from faeces was similar and may reflect the complex ecology of these pathogens within the bovine GI tract. Diet and, in particular, the presence of grain in the diet of cattle has been previously found to be associated with increasing the risk of isolating *E. coli* O157 and *Campylobacter* spp. in separate studies (Buchko et al., 2000, Busato et al., 1999). It has been suggested that high-grain diets change concentrations of VFA and other gut metabolites, promoting either survival or proliferation of *E. coli* O157.

Although several confounders with presence of grain in the faeces could explain the increased risk of isolating these pathogens, the presence of higher quantities of undigested grain in the faeces of cattle may be associated with sub-acute ruminal acidosis, indicating animals experiencing dietary stress. Stress at the mucosal surface has been shown to increase the adhesion of *E. coli* O157 to the murine gut mucosa (Chen et al., 2003) and interventions based on reducing the degree of dietary stress in cattle, such as more gradual introduction of grain into the diet of cattle, may be considered.

Longitudinal studies have the potential to identify temporal patterns in shedding. There was an increased risk of isolating *E. coli* O157 and *Campylobacter* spp. in the summer months, although the effect was only significant for *Campylobacter* spp. This effect was also clear after allowing for the type of housing and diet. In terms of the management of cattle, season is confounded by several factors including housing, diet and stocking densities. Previous risk factor studies have identified changing effects of season dependent on inclusion of confounders with season in statistical models (Synge et al., 2003). The model for *Campylobacter* spp. contained fewer data points and was conducted over a shorter time period than for *E. coli* O157. Therefore, although there is evidence that shedding of *Campylobacter* spp. may vary by season, further work, over a longer sampling time frame, and detailed data regarding several risk factors, are required before the effects of season can properly be evaluated.

Cattle on farms that shared similar strains of *E. coli* O157 also shared similar clonal complexes of *C. jejuni*. The only consistent difference that was noted in this study between farms that shared the same RP and clonal complexes of *E. coli* O157 and *C. jejuni* respectively, was the geographical location of the farms. At some points during the study, cattle on Farms 1 and 4 were in nose-to-nose contact. Farm 2 was also in close proximity (<1km) to Farm 4, and as well as frequent visits made by farm personnel between premises, stock were also shared between farms. Farms 3 and 5, which shared a common genotype, were in close proximity to each other (<2km). This may indicate transmission between farms via the local environment. However, it is interesting that the risk associated with each farm was different for *Campylobacter* spp. and *E. coli* O157. Although in this study the molecular epidemiology has been carried out on the species *C. jejuni*, the majority (approximately 85%) of *Campylobacter* spp. isolated have so far been determined as *C. jejuni*. It could be considered that whilst transmission of strains between farms may be governed by factors associated with the survival/ transmission of these strains within each, the farm environment may be related to strain specific characteristics, with different characteristics important for transmission and dissemination within farms. Interestingly, each farm was associated with a different level of risk for the different pathogens, possibly indicating differences in within farm transmission dynamics. These differences in shedding may be related to differences in the within pen transmission between cattle of *Campylobacter* spp. and *E. coli* O157. For example, variation in survival of the organism in the environment may account for some of the observed variation.

The relative contribution of the cattle reservoir to human cases of Campylobacter infection is unknown at this time. The clonal complexes identified in this study are similar to those reported in other studies of cattle populations in the UK, and indeed those associated with human disease (Colles et al., 2003). Although the epidemiology of human infection caused by *Campylobacter* spp. and *E. coli* O157 appear to be quite different in terms of seasonal and temporal trends, this may be as a result of the different routes by which humans become exposed to each pathogen and not necessarily different sources. How humans become exposed to these pathogens may merely reflect differences in the transmission and survival of each pathogen once in the environment.

A reduction in the prevalence of shedding of these pathogens on the farm is likely to lead to a reduction in the levels of human gastrointestinal disease. Previous studies reporting on the shedding of either *C. jejuni* or *E. coli* O157 conducted on different cattle populations have reported similar findings to those reported here. This study has provided evidence that whilst animals may not co-excrete *E. coli* O157 and *Campylobacter* spp., of which the majority were *C. jejuni*, several features of the epidemiology of their shedding in cattle are similar. Generic on-farm control strategies aimed at reducing both shedding of *E. coli* O157 and *C. jejuni* may provide a cost effective strategy for reducing overall cases of human IID.

ACKNOWLEDGEMENTS

This work was funded by the Department for Environment, Food and Rural Affairs (DEFRA). We also thank all of the farmers involved in the study and co-workers at the University of Liverpool Veterinary Field station, in particular Mrs. J. Sutherst, Dr. P. Cripps, Miss E. Brook and Mrs L. Newbold for contributions to both the field and laboratory work.

REFERENCES

Best, E.L., Powell, E.J., Swift, C., Grant, K.A. and Frost J.A. (2003). Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. FEMS Microbiol. Lett. 229, 237-241

Brown, P.E., Christensen, O.F., Clough, H.E., Diggle, P.J., Hart, C.A., Hazel, S., Kemp, R., Leatherbarrow, A.J., Moore, A., Sutherst, J., Turner, J., Williams, N.J., Wright, E.J. and French, N.P. (2004). Frequency and spatial distribution of environmental *Campylobacter* spp.. Appl. Environ. Microbiol. 70, 6501-6511

Buchko, S.J., Holley, R.A., Olson, W.O., Gannon, V.P. and Veira, D.M. (2000). The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. J. Food Protect. 63, 1467-1474

Busato, A., Hofer, D., Lentze, T., Gaillard, C. and Burnens, A. (1999). Prevalence and infection risks of zoonotic enteropathogenic bacteria in Swiss cow-calf farms. Vet. Microbiol. 69, 251-263

Centers for Disease Control and Prevention. Standardized molecular subtyping of foodborne bacterial pathogens by pulsed-filed gel electrophoresis. Atlanta: CDC, 1998

Chen, C., Brown, D.R., Xie, Y., Green, B.T. and Lyte, M. (2003). Catecholamines modulate *Escherichia coli* O157:H7 adherence to murine cecal mucosa. Shock 20, 183-188

Colles, F.M., Jones, K., Harding, R.M. and Maiden, M.C. (2003). Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl. Environ. Microbiol. 69, 7409-7913

Dice, L.R., (1945). Measures of the amount of ecological association between species. Ecology 26, 297-302

Dingle, K.E., Colles, F.M., Wareing, D.R., Ure, R., Fox, A.J., Bolton, F.E., Bootsma, H.J., Willems, R.J., Urwin, R. and Maiden, M.C. (2001). Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol. 39, 14-23

Edrington, T.S., Hume, M.E., Looper, M.L., Schultz, C.L., Fitzgerald, A.C., Callaway, T.R., Genovese, K.J., Bischoff, K.M., McReynolds, J.L., Anderson, R.C.and Nisbet, D.J. (2004). Variation in the faecal shedding of Salmonella and *E. coli* O157:H7 in lactating dairy cattle and examination of Salmonella genotypes using pulsed-field gel electrophoresis. Lett. Appl. Microbiol. 38, 366-372

Gillespie, I.A., Adak, G.K., O'Brien, S.J. and Bolton, F.J. (2003). Milk-borne general outbreaks of infectious intestinal disease, England and Wales, 1992-2000. Epidemiol. Infect. 130, 461-468

Hastie, T. and Tibshirani, R. (1990). Generalized Additive Models. Chapman and Hall, London.

Locking, M. E., O'Brien, S.J. Reilly, W.J., Wright, E.M., Campbell, D.M., Coia, J.E., Browning L.M. and Ramsay C.N. (2001). Risk factors for sporadic cases of *Escherichia coli* O157 infection: the importance of contact with animal excreta. Epidemiol. Infect. 127, 215-220

Nielsen, E.M. (2002). Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. Lett Appl Microbiol 35, 85-89

Paiba, G.A., Wilesmith, J.W., Evans, S.J., Pascoe, S.J., Smith, R.P., Kidd, S.A., Ryan, J.B., McLaren, I.M., Chappell, S.A., Willshaw, G.A., Cheasty, T., French, N.P., Jones, T.W., Buchanan, H.F., Challoner, D.J., Colloff, A.D., Cranwell, M.P., Daniel, R.G., Davies, I.H., Duff, J.P., Hogg, R.A., Kirby, F.D., Millar, M.F., Monies, R.J., Nicholls, M.J. and Payne, J.H. (2003). Prevalence of faecal excretion of verocytotoxigenic *Escherichia coli* O157 in England and Wales. Vet. Rec. 153, 347-353

Robinson, S.E., Wright, E.J., Hart, C.A., Bennett, M. and French, N.P. (2004). Intermittent and persistent shedding of *Escherichia coli* O157 in cohorts of naturally infected calves. J. Appl. Microbiol. 97, 1045-1053

Smerdon, W.J., Adak, G.K., O'Brien, S.J., Gillespie, I.A. and Reacher, M. (2001). General Outbreaks of Infectious Intestinal Disease Linked With Red Meat, England and Wales, 1992-1999. Commun. Dis. Pub. Health 4, 259-267

Stanley, K.N., Wallace, J.S., Currie, J.E., Diggle, P.J. and Jones K. (1998). The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. J. Appl. Microbiol. 85, 472-480

Synge, B.A., Chase-Topping, M.E., Hopkins, G.F., McKendrick, I.J., Thomson-Carter, F., Gray, D., Rusbridge, S.M., Munro, F.I., Foster, G. and Gunn, G.J. (2003). Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows. Epidemiol. Infect. 130, 301-312

Tauxe, R.V. (1992). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In *Campylobacter jejuni*: *Current Status and Future Trends* (I. Nachamkin, M.J. Blaser and L.S. Tompkins, Eds.), pp. 9-19. American Society for Microbiology, Washington, DC.

CAMPYLOBACTER TRANSMISSION IN EXPERIMENTAL BROILER FLOCKS

T. J.W.M. VAN GERWE[1], A. BOUMA, W.F. JACOBS-REITSMA, J. VAN DEN BROEK, D. KLINKENBERG, J.A. STEGEMAN AND J.A.P. HEESTERBEEK

SUMMARY

Control of *Campylobacter* spp. infections in broilers is an important way of reducing the exposure of humans to *Campylobacter* spp. In order to develop measures to reduce the number of infected broilers, quantitative knowledge of transmission of *Campylobacter* spp. in broiler flocks is necessary. The aim of this study was to determine the transmission rate parameter in broiler flocks under experimental conditions.

Four experiments were performed, each with 400 chicks. A mathematical model was used to quantify the transmission rate, which was determined to be 1.04 new cases per colonized chick per day. This implies that a flock of 20,000 broilers will be fully colonized within 20 days after introduction, and an increase in prevalence from 5% to 95% will be reached within 6 days.

The model and the estimated transmission rate parameter can be used to develop a suitable sampling scheme to determine transmission in commercial broiler flocks, to estimate which control measures reduce the transmission rate, or to estimate when *Campylobacter* spp. was introduced into a flock.

INTRODUCTION

Campylobacter species are frequently identified bacterial causes of human gastroenteritis throughout the world (Allos, 2001). An important source of human infections is the handling and consumption of contaminated poultry meat (Saleha et al., 1998), thus a reduction in poultry meat contamination might reduce human exposure, and consequently the risk of campylobacteriosis.

Several control measures have been implemented to reduce the exposure of humans to *Campylobacter* spp., either by reducing the incidence of *Campylobacter* spp. infections in broiler flocks by biosecurity measures at farms or by improving slaughterhouse hygiene. However, these measures are apparently not sufficiently effective, because many broiler flocks still become colonized with *Campylobacter* spp. (Anon, 2000). Therefore, intervention strategies should be improved or alternatives developed.

Current intervention strategies are based on risk factors identified in field surveys (e.g. Bouwknegt et al., 2004; Evans & Sayers, 2000; van de Giessen et al., 1998). An important

[1] Twan van Gerwe, Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands. Email: t.j.w.m.vangerwe@vet.uu.nl

disadvantage of these field surveys is that they used associative static models to determine an association between risk factors and the presence of *Campylobacter* spp. in a flock and are based on qualitative data on the infection status of the flocks at the end of the fattening period. These studies did not take the dynamic aspects of a *Campylobacter* spp. infection in a flock into account, whereas knowledge of the transmission characteristics is important in order to control the rate of transmission (e.g. Koopman, 2004). This is important because the rate of transmission determines the change in prevalence of *Campylobacter* spp. with time and hence the probability of detection, which in turn affects the extent to which humans are exposed to *Campylobacter* spp. via poultry products. Such information may also help to identify the source of contamination by making it possible to determine the moment of introduction of *Campylobacter* spp. into a flock (e.g. Shanker et al., 1990), and to determine which control measures can reduce transmission (De Jong, 1995).

Clear quantitative information on *Campylobacter* spp. transmission is still lacking, although some transmission experiments have been carried out (Shanker et al., 1990; Stern et al., 1988). Unfortunately, transmission in these studies was only determined qualitatively.

In the current study, data from the study of Jacobs-Reitsma (1996) were available for further analysis. Four experiments were conducted by these authors to determine whether groups of 400 broilers could be colonized after introduction of a few *Campylobacter* spp.-inoculated seeder birds. This experimental set-up, with four seeder birds per group, a high sampling frequency scheme, and relatively large sample sizes, offered the opportunity to quantify transmission. Here, results of a further quantitative analysis of these data (Jacobs-Reitsma, 1996) are presented. Furthermore, how the transmission parameter can be used to estimate the moment of *Campylobacter* spp. introduction in the field situation, and how the precision of this estimation is affected by the sampling scheme and sample size, are also presented.

MATERIALS AND METHODS

Animals

In four experiments (1-4), the horizontal spread of *Campylobacter* spp. among broilers (type Ross) was studied. Day-old chicks, used in experiments 1 and 2, were obtained from a *Campylobacter* spp.-free parent flock housed at the Centre for Poultry Research and Information Services 'het Spelderholt' (Beekbergen, The Netherlands). This flock was tested for the presence of *Campylobacter* spp. in fourteen pooled samples (four caecal droppings per pooled sample) at day 7 after egg collection. No *Campylobacter* spp.-positive samples were found after 48 hours of culture (method described in Jacobs-Reitsma et al. 1995a). The chicks used in experiments 3 and 4 originated from a commercial parent flock, which was colonized with *Campylobacter* spp.

Housing

In each experiment, 400 broilers were accommodated in a separate shed at a density of 20 broilers per m^2, which is similar to the housing density under commercial conditions. The broilers were fed on commercial broiler feed. They were housed on wood shavings and the drinking water was supplied by means of a nipple drinking system. Before the start of the experiments, samples were taken from water, feed, and wood shavings in the broiler sheds, and tested for *Campylobacter* spp.

Inoculation

The Campylobacter strains and inoculation doses are listed in Table 1. *Campylobacter coli* strain C136 was isolated from a pig farm in March 1990 (Heres et al., 2004). *Campylobacter jejuni* strain C356 was isolated in 1990 from broilers (Penner serotype O2) (Jacobs-Reitsma et al., 1995b). The strains were stored in glycerol at -80°C and have often been used by the Animal Sciences Group in Lelystad for infection experiments and as reference control strains (e.g. Boer et al., 2000). *Campylobacter jejuni* strain C4021 (experiment 4) originated from the parent flock of the chicks.

Table 1. Challenge strains and inoculation dose

Experiment	Campylobacter strain	Campylobacter strain	Dose (c.f.u./broiler)
1	*C. coli* 136	O:46	6.5×10^8
2	*C. coli* 136	O:46	6.5×10^8
3	*C. jejuni* 356	O:2	6.5×10^8
4	*C. jejuni* 4021	not determined	1.1×10^5

The strains were freshly cultured in Heart Infusion Broth (micro-aerobically, 37°C, overnight) and diluted in saline to obtain the intended inoculation dose. The actual concentration (colony forming units) of *Campylobacter* spp. in the administered suspensions was determined by plating on Cephoperazone Desoxycholate Agar (CCDA).

Experimental design

In experiments 1 and 2, four chicks per group were orally inoculated with 0.1 ml of the *Campylobacter* spp. inoculation suspension at day of hatching. In experiments 3 and 4, four chicks per group were orally inoculated with 0.1 ml of the *Campylobacter* spp. inoculation suspension 1 day after hatching. The inoculated chicks (seeders) were marked on the head with a black spot, given an identification wing number and were returned to their shed. All experiments lasted 42 days.

Sampling

The chicks were sampled at fixed time points (Table 2). In experiments 1 and 2, the 4 seeders and 50 chicks, chosen at random, were removed from the groups for sampling for *Campylobacter* spp. by cloacal swabbing. After sampling, the broilers were put back into their groups. In experiments 3 and 4, the seeders were removed from the groups for the time necessary to obtain a fresh (caecal) dropping. A swab was taken from these droppings. Fifty samples of soft, fresh, wet, and homogeneous caecal droppings were collected from the boiler sheds, which were divided into five sectors (1 x 4 m each). Defaecation was stimulated by turning on the lights and making a noise, which ensured the samples were fresh. When all samples appeared to be *Campylobacter* spp.-positive, the sample size was reduced in all four experiments to 10 or 12 per group.

Samples were collected with sterile swabs and transported to the laboratory in modified Amies transport medium without charcoal. Swabs were directly streaked on CCDA (Oxoid

CM739 + SR155) and incubated micro-aerobically at 42°C for 2 days and examined for the presence of *Campylobacter* spp. To exclude the possibility of infection from another source, the isolates were Penner serotyped as described by Jacobs-Reitsma et al. (1995b).

Table 2. Number of contact infections in each experiment

Age (d)	Number of *Campylobacter* spp.-positive broilers							
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Seeders (4)	Contacts	Seeders (4)	Contacts	Seeders (4)	Contacts	Seeders (4)	Contacts
2	0	nd[a]	0	nd				
3					0	nd	0	nd
4	3	0/50	3	0/50	0	0/50	2	0/50
5	4	0/50	4	0/50	1	1/50	2	0/50
6					2	0/50	2	0/50
7	4	9/50	3	8/50	4	0/50	2	0/50
9	4	26/50	4	25/50	4	0/50	3	0/50
11					4	1/50	3	0/50
12	4	48/50	4	45/50				
14	4	49/50	4	50/50	nd	38/50	4	20/50
16					nd	47/50	nd	40/50
18					nd	50/50	nd	49/49
23					nd	12/12	nd	12/12
28	nd	10/10	nd	10/10				
29					nd	12/12	nd	12/12
35					nd	10/10	nd	10/10
40	nd	10/10	nd	10/10				
42	nd	10/10	nd	10/10	nd	20/20	nd	20/20

[a] nd = not determined

Quantification of transmission

An *SI*-type model was used to describe the dynamics of transmission with time, with $s(t)$ being the proportion of susceptible birds at time t and $i(t)$ the proportion of infectious birds. The *SI* model assumes that once a bird becomes infected, it will remain infectious during the experimental period (e.g. Heres et al., 2004), and that contacts within the population are random. In addition, both classes S and I are assumed to be homogeneous, and the transmission rate is taken to be constant during the entire infectious period and equal for all infectious broilers.

Susceptible birds are assumed to become infected at rate $\beta s(t)i(t)$. The transmission rate parameter β can be defined as the average number of secondary cases caused by one infectious bird per time unit in a susceptible population (Diekmann & Heesterbeek, 2000). Although transmission between individuals is inherently a chance process, the dynamics in a large enough population can be approximated by a deterministic differential equation. In the case of the *SI*-model we have (Eq. 1):

$$\frac{di(t)}{dt} = \beta s(t) i(t) \tag{1}$$

of which the solution is the logistic curve (Eq. 2)

$$i(t) = \frac{ce^{\beta t}}{1 + ce^{\beta t}} \tag{2}$$

with $c = i(0)/(1 - i(0))$, $i(0)$ being the proportion of infectious birds at $t = 0$. The curve is shown in Fig. 1.

Following production of the logistic $i(t)$-curve, a logistic regression analysis was conducted to model the change in $i(t)$ over time. A delay time τ was added to the model to account for a possible time shift in the start-up of the epidemic process. This resulted in the following model for the log-odds of $i(t)$ (Eq. 3):

$$\ln\left[\frac{i(t)}{1-i(t)}\right] = \ln\left(\frac{i(0)}{1-i(0)}\right) + \beta(t-\tau) = \ln\left(\frac{i(0)}{1-i(0)}\right) + \beta t + a \tag{3}$$

The model was fitted by standard logistic regression with $\ln(i(0)/(1\text{-}i(0)))$ as offset, t as a covariate, and a as intercept. The fit resulted in an estimate for β and for a, from which the delay τ was calculated as $\tau = -a/\beta$.

Separate models were fitted for each experiment, resulting in four β values and four a values, and shared models were fitted in all possible combinations of these experiments, resulting in common β values for the different experiments. Akaike's Information Criterion (AIC) (Burnham and Anderson, 1998) was used to decide which model had the best fit, and to decide whether different values of β should be adopted for different (sets of) experiments.

As an example of how β can be used, the precision with which the moment of *Campylobacter* spp. introduction can be estimated by regularly sampling flocks was

investigated. Ten thousand outbreaks in flocks of 20,000 chicks were simulated with $\beta = 1.04$ (the estimated value) and starting at time $t = 0$. Simulations were carried out using the so-called Sellke construction (Anderson and Britton, 2000): First, for each bird j, a value Q_j is drawn from an exponential distribution with mean 1; then the epidemic is reconstructed by supposing bird j becomes infected when the cumulative infectiousness $\beta \int_0^t i(u)du$ reaches Q_j, with $i(u)$ being the fraction of infected birds at time u. In this simulation, 10, 20 or 60 birds were sampled every 1, 3, 7, or 14 days, the time of the first sample having been randomly selected from the appropriate uniform distribution, and the number of infected birds at time t was recorded. The resulting proportion of infected birds at each sampling time was then used to carry out a logistic regression analysis, as described above, in which β was fixed at 1.04 and a was estimated (and consequently τ). Because every simulation started at $t = 0$, the estimated τ is the error made in estimating the time of *Campylobacter* spp. introduction. Thus, the 10,000 simulations yielded estimation errors for each combination of sample size (10, 20, or 60) and sampling interval (1, 3, 7, or 14 days).

RESULTS

Course of the infection

Campylobacter spp. were not detected in samples of water, feed, or wood shavings at the start of the experiments. In experiments 1 and 2, contact broilers became *Campylobacter* spp.-positive between days 5 and 7 (see Table 2). In experiments 3 and 4, the first contact broilers became *Campylobacter* spp.-positive between days 9 and 11, and days 11 and 14, respectively.

Quantification of transmission

The logistic regression model was used to estimate β for each experiment separately, and to estimate shared β values in all possible combinations. As the simplest model with a single β for all four experiments had one of the lowest AIC scores, there was no evidence that a more complex model was needed. The joint β was estimated at 1.04 per day with a standard error of 0.06, which means that after introduction of *Campylobacter* spp. in a flock, each broiler will infect on average 1.04 new broilers per day. In a broiler flock of 20,000 birds, it takes 5.5 to 9.5 days (90% confidence interval) to reach a prevalence of 5% from the moment of introduction. Then, an increase in prevalence from 5 to 95% takes approximately 6 days and finally another 5.5 to 9.5 days is required to reach 100% prevalence. In a population of 400 broilers, only the first and final part of the epidemic curve differs, as can be seen in Fig. 1.

The estimated intercepts a were -2.57 (s.e. 0.47), -2.77 (s.e. 0.48), -6.06 (s.e. 0.71), and -7.416 (s.e. 0.78) for experiments 1, 2, 3, and 4, respectively. This resulted in estimated delay times of 2.4, 2.7, 5.8, and 7.1 days, respectively.

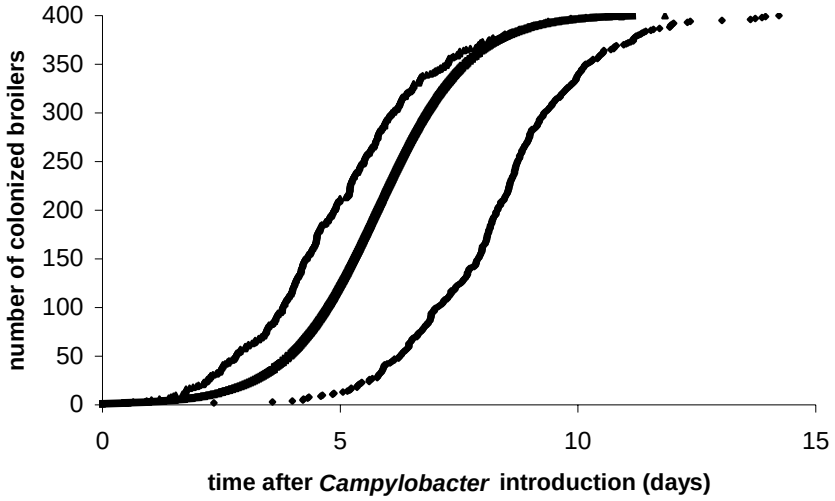


Fig. 1 Simulated course of *Campylobacter* infection in a population of 400 broilers, starting at $t = 0$. The middle curve is the deterministic (logistic) curve; the other two are random simulations. As can be seen, the deterministic and stochastic curves are similar, except for a time shift due to random effects in the initial phase of the outbreak.

Table 3 shows the 90% intervals of the estimation errors when flocks of 20,000 chicks are regularly sampled to estimate the time of *Campylobacter* introduction. If β is assumed to be 1.04, then 1-day, 3-day and 7-day sampling intervals had comparable errors, irrespective of whether 10, 20, or 60 samples were taken.

Table 3. Precision of determination of the time of *Campylobacter* introduction into a flock of 20,000 broilers, with different sample sizes and sampling intervals. Denoted are the 90% intervals of the estimated introduction times of 10,000 simulated outbreaks starting on day 0.

Sample size	β =1.04 Sampling interval			
	1 day	3 days	7 days	14 days
10	-1.13 - 2.93	-1.31 - 3.05	-1.72 - 3.25	-3.71 - 4.84
20	-1.08 - 2.88	-1.19 - 2.94	-1.43 - 3.11	-3.08 - 4.34
60	-1.05 - 2.87	-1.08 - 2.89	-1.17 - 2.91	-2.17 - 3.68

DISCUSSION

The aim of this study was to quantify the transmission of *Campylobacter* spp. among broiler in experimental conditions. β was estimated from four experiments previously carried out by Jacobs-Reitsma et al. (1996). The estimated β value was 1.04 day^{-1}. The model used assumed that the birds mixed randomly, which seems reasonable given the observations of Preston and Murphy (1989). However, Hartnett et al. (2001) interpreted the same data differently and assumed that broilers stay within a cluster and that clusters move. However, since our simpler

mathematical model fitted the experimental data well, there seems no reason to introduce a more complicated model for contact structure.

The overall estimates of β did not differ significantly between the experiments 1-4, indicating that despite variations in other circumstances the infection processes run a similar course. The transmission rate parameter β can be used in further studies to evaluate control measures for their reduction of the transmission of Sample size or to determine the within-flock prevalence over time in the field.

As with all laboratory studies, there is the question of to what extent findings can be extrapolated to the field situation. However, in this instance, the problem with extrapolation is only relevant for the start of the epidemic, when the first birds become colonized, and when the prevalence is still low. In this phase of the epidemic, chance processes play an important role. When the delay times found in these experiments were analyses, it was concluded that they could not be explained by chance processes alone. Further investigation is needed to unravel this phenomenon. However, the results suggest that once the infection is spreading, the time taken to for the prevalence to increase from 5% to 95% will be approximately the same in most situations, allowing extrapolation to the field, and this is substantiated by other observations in the field (e.g. Bouwknegt et al., 2004; Evans & Sayers, 2000).

Knowledge of epidemiological mechanisms and parameters underlying Sample size transmission in broiler flocks is important for the evaluation and development of control strategies. This knowledge allows identification of measures that can reduce transmission. Furthermore, this knowledge can be used to determine whether the magnitude of the effect is sufficient to reduce transmission or to postpone introduction, which in turn decreases the prevalence in a flock, and subsequently the exposure of humans to *Campylobacter* spp. via contaminated poultry products.

REFERENCES

Allos, B.M. (2001). Campylobacter jejuni Infections: Update on Emerging Issues and Trends. Clin. Infect. Dis. 32, 1201-1206

Andersson, H. and Britton T. (2000). Stochastic epidemic models and their statistical analysis. Lecture notes in statistics 151. Springer-Verlag New York, Inc., New York

Anon (2000). www. europa.eu.int/comm/dgs/health_consumer/ newsletter

Boer, P. De, Duim, B., Rigter, A., Vander Plas, J., Jacobs-Reitsma, W. F., and Wagenaar, J.A. (2000). Computer-Assisted Analysis and Epidemiological Value of Genotyping Methods for Campylobacter jejuni and Campylobacter coli. J. Clin. Microbiol. 38, 1940-1946

Bouwknegt, M., Van de Giessen, A.W., Dam-Deisz, W.D., Havelaar, A.H., Nagelkerke, N.J., and Henken, A.M. (2004). Risk factors for the presence of Campylobacter spp. in Dutch broiler flocks. Prev. Vet. Med. 62, 35-49

Burnham, K.P. and Anderson, D.R. (1998). Model Selection and Interferende: A Practical Information - Theoretic Approach. Springer Verlag, New York

De Jong, M.C.M. (1995). Mathematical modeling in veterinary epidemiology: why model building is important. Prev. Vet. Med. 12:183-193

Diekmann O. and Heesterbeek, J.A.P.. (2000). Mathematical Epidemiology of Infectious Diseases: model building, analysis and interpretation. J.Wiley and Sons, Chichester

Evans, S.J. and Sayers, A.R. (2000). A longitudinal study of campylobacter infection of broiler flocks in Great Britain. Prev. Vet. Med. 46:209-223

Hartnett E., Kelly, L., Newell, D., Wooldridge, M. and Gettinby, G. (2001). A quantitative risk assessment for the occurrence of campylobacter in chickens at the point of slaughter. Epidemiol. Infect. 127,195-206

Heres, L., Engel, B., Urlings, H.A., Wagenaar, J.A. and Van Knapen, F. (2004). Effect of acidified feed on susceptibility of broiler chickens to intestinal infection by Campylobacter and Salmonella. Vet Microbiol. 99, 259-267

Jacobs-Reitsma, W.F., Van de Giessen, A.W., Bolder, N.M. and Mulder, R.W.A.W. (1995a). Epidemiology of Campylobacter at two Dutch broiler farms. Epidemiol. Infect. 114, 413-421

Jacobs-Reitsma, W.F., Maas, H.M.E. and Jansen, W.H. (1995b). Penner Serotyping of Campylobacter Isolates from Poultry, with Absorbed Pooled Antisera, Journal of Appl. Bacteriol. 79, 286-291

Jacobs-Reitsma, W.F. (1996). Experimental horizontal spread of Campylobacter amongst one-day-old broilers. In: report Project 5038: Huisvesting en transportstress als factoren in de Campylobacter besmetting bij pluimvee, Lelystad, The Netherlands

Koopman J. (2004). Modeling infection transmission. Annu. Rev. Public Health. 25, 303-326

Mollison, D. (1995). Epidemic Models: Their Structure and Relation to Data. Cambridge University Press, Cambridge, UK

Preston A.P. and Murphy, L.B. (1989). Movement of broiler chickens reared in commercial conditions. Bri. Poul. Sci. 30,519-532

Saleha, A.A., Mead, G.C., and Ibrahim, A.L. (1998). *Campylobacter jejuni* in poultry production and processing in relation to public health. World's Poultry Science Journal. 54, 49-58

Shanker, S., Lee, A. and Sorrell, T.C. (1990). Horizontal transmission of *Campylobacter jejuni* among broiler chicks experimental studies. Epidemiol. Infect. 104, 101-110

Stern, N.J., Bailey, J.S., Blankenship, L.C., Cox, N.A. and McHan, F. (1988). Colonization characteristics of Campylobacter jejuni in chick ceca. Avian Dis. 32, 330-334

van de Giessen, A.W., Tilburg, J.J.H.C., Ritmeester, W.S. and van der Plas, J. (1998). Reduction of campylobacter infections in broiler flocks by application of hygiene measures. Epidemiol. Infect. 121, 57-66

VARIATIONS IN ANTIMICROBIAL RESISTANCE: A LONGITUDINAL STUDY OF *E. COLI* POPULATIONS FROM CATTLE

R.W. HUMPHRY[1], G.J. GUNN, D. FENLON AND J.C. LOW

SUMMARY

A short-term longitudinal study was carried out to measure the proportion of ampicillin resistant *E. coli* in faeces from three adult cattle from the same group. The aim was to estimate the temporal correlations in resistance for samples taken from the same animal so allowing the estimation of the viable time scales for predicting changes in resistant populations.

Mean ampicillin MIC values were estimated and these showed significant differences between animals. Time trends were observed in two of the three animals and, once the data were detrended, temporal associations were identified for samples taken within a few days of one another (from the same animal).

The short time period for association (≤ 7 days) may be an artefact of unknown confounding environmental factors. Other evidence suggested otherwise and therefore it is concluded that the data are in accord with previous work that suggests very rapid rate of change in antimicrobial resistance. Study-designs for future work should allow for this rapid change in phenotype.

INTRODUCTION

In order to understand better the ecology, at the population level, of antimicrobial resistance in commensal bacteria it is necessary to be able to describe resistance in a quantitative and biological meaningful manner. Therefore, there is considerable interest, *inter alia*, in the rate of change of resistance at the bacterial population level.

The time-horizon over which populations from the same habitat (animal) are associated is indicative of the rate of change of resistance. Estimates of this time-horizon inform the design of future surveys and studies and help answer questions of how 'typical' a measurement is of the population found in a particular animal.

This study aims to estimate the time-horizon for which populations remain associated.

[1] SAC Epidemiology Research Unit, Animal Health Team, Drummondhill, Stratherrick Road, Inverness, IV2 4JZ, UK. Email: roger.humphry@sac.ac.uk

MATERIALS AND METHODS

Sampling

Faecal samples were taken from three adult cows from the same group from a low-ground beef-suckler herd near Inverness, UK during September and October of 2001. Two replicate samples were taken from each animal at four times (generally on Monday morning, Monday afternoon, Wednesday morning, Wednesday afternoon) on each of five consecutive weeks from 24th September 2001 until 25th October 2001 (see Appendix I for further details).

The replicates were transported separately in two polystyrene cool boxes overnight for analysis in the microbiology laboratory, SAC, Craibstone.

A control isolate of a sensitive strain (*E. coli*, 10418) was also tested at various time points (Appendix I) to act as a reference. The strain was cultured in nutrient broth (Oxoid Ltd.) overnight at 37° C to give *ca*. 10^8 - 10^9 cfu ml^{-1}. From the resulting broth a 1 in 1000 dilution was prepared in maximum recovery diluent (Oxoid Ltd.) to give a culture of *ca*. 10^5 - 10^6 cfu ml^{-1}.

Enumeration of bacterial density

Samples were diluted both ten-fold and 1000-fold. A spiral plater was used to estimate the density of *E. coli* within each sample dilution that would grow on Tryptone Bile X-glucuronide agar plates at 12 different concentrations of ampicillin using the method of Humphry et al. (2002). This provided a set of dose response data of bacterial density against concentration of antibiotic. Two replicate batches of media were made for each week of the trial.

Statistical analysis

Analysis of data was in two main parts. First, the data were analysed using non-linear regression to estimate whether there were one or two phenotypes (in terms of ampicillin resistance). The parameters gained from the non-linear regression were then used as descriptors of each sample and were treated as coming from a time-series.

Stage 1: Non-linear regression of raw data: The method described by Humphry et al. (2002) was modified to include all data for a particular sample using an appropriate weighting (as opposed to the discarding of data described in Humphry et al. (2002). A cumulative density function from the normal curve was used to describe the dose-response curve.

$$Y = T.\left(1 - b.\Phi\left(\frac{[X - \mu_1]}{\sigma_1}\right) - (1 - b).\Phi\left(\frac{[X - \mu_2]}{\sigma_2}\right)\right) \quad (1)$$

Where

Y = the number of *E. coli* cfu g^{-1} of sample growing on a plate containing antibiotic

T = the total number of *E. coli* cfu g^{-1} of sample growing on a control plate without antibiotic

$\Phi(z)$ = the cumulative density function of the standard normal distribution

X= the $\log_2$(concentration of antibiotic in the media)

μ_1 = the population 1 sensitivity (the mean $\log_2$(concentration) at which *E. coli* in population 1 show sensitivity and do not grow)

σ_1 = the standard deviation of the population 1 sensitivity

b = the proportion of the bacteria that are in population 1

μ_2 = the population 2 sensitivity

σ_2 = the standard deviation of the population 2 sensitivity

The observations were given their own weighting based on either the predicted or actual count of bacteria, the volume of the sample and the concentration of the sample (see Appendix II for more details). An alternative, without weighting but using a log transformation was also used.

Equation 1 was modified to make it simpler for two situations:

a) Where there appeared to be only 1 phenotype of bacteria with respect to antimicrobial resistance, the value of b was fixed at one, thus simplifying Eq. (1) to model a one phenotypic population (Eq. (2))

$$Y = T.\left(1 - \Phi\left(\frac{[X - \mu_1]}{\sigma_1}\right)\right) \tag{2}$$

b) Where it appeared that the highest concentrations of ampicillin used were not high enough to kill all bacteria, it appeared that there were 2 phenotypes but that the 2nd phenotype could not be described with the data collected, the equation used was:

$$Y = T.\left(1 - b.\Phi\left(\frac{[X - \mu_1]}{\sigma_1}\right)\right) \tag{3}$$

A selection hierarchy was then used

i) If Eq. (1) or Eq. (2) were successful in terms of convergence and visual appearance of residuals then the following rules determined whether a 1 or 2 population model was chosen, and which of the 3 weighting methods was chosen:

1. For any weighting where both models (1 population and 2 populations) are successful, this takes precedent over any other weighting where only 1 model is successful;

2. The hierarchy of weightings is weight using predicted count > weight using actual count > log transformation (called weighting classes);

3. Where, in each of the weighting classes, only the 1 population model OR the 2 population was successful then 1 population model (higher weighting class) > 2 population model (lower weighting class);

4. Where, in all weighting classes, only the 1 population model OR the 2 population was successful then 2 population model (higher weighting class) Versus 1 population model (lower weighting class) is undefined and left to the operator's judgement.

ii) If Eq. (1) and Eq. (2) were unsuccessful then they were repeated with the standard deviation sigma1 given a fixed value. If this worked for either 1 or 2 populations then the above rules were applied.

iii) If Eq. (1) and Eq. (2) failed even with the standard deviation fixed then Equation 3 was used.

Stage 2: Statistical analysis of the longitudinal data arising from the non-linear regression: The values for T (an estimate of the total density of *E. coli* in the sample) and μ_1, (the mean sensitivity of the first population of *E. coli*) estimated from the process of non-linear regression for each sample collected, were examined separately. First, evidence of simple trends was explored by visual examination and by linear regression of both first and second order polynomials and an exponential model. Where there was convincing evidence of a time-trend, the data were detrended by using the residuals from the chosen regression. Further analyses to examine autocorrelation were then applied to the detrended data so as to avoid autocorrelation caused by this time-trend (Chatfield, 1996).

The detrended data were then examined by pairing each sample with future samples taken from the same animal and looking at the difference and the association (using Pearson's correlation coefficient) between samples within each pairing. Replicates from the same animal at the same time allowed pairings for which the time difference was zero. These differences in log(T) and μ_1 (over time) were transformed (square root of the absolute difference) to give the most normally distributed set of data (assessed visually).

RESULTS

There were 137 samples [4 halfdays*5weeks*2replicates*3animals (=120) + 17 Controls at various time points] (Appendix I). There was successful fit of a model using non-linear regression to all 137 samples, thereby giving estimates for μ_1 and T for all 137 samples.

Mean log MIC, E(μ_1)

There were differences between animals for the estimated value for μ_1 . Animal X was different from the other two animals. There was a significant difference between animals X&Y, X&Z but not Y&Z: Mean(μ_1 X) = 0.86 (SEM = 0.1); Mean(μ_1 Y)= 1.26 (SEM=0.06); Mean(μ_1 Z)=1.27 (SEM=0.06).

There was evidence of linear time trends in μ_1 for animals X and Y but not for Z or the control samples (see Fig. 1).

Having detrended the data, there was some evidence for higher association and lower differences between samples over time differences of less than 3 days (which is equivalent to a batch of laboratory media) compared to longer time differences (Fig. 2). Sample-pairs with a time lag of less than 3 days had a higher level of correlation than sample-pairs with a time lag of 3 or more days (P=0.011, Satterthwaite T-test (unequal variances) on the Pearson Correlation coefficients (no transformation of the correlation coefficients was deemed necessary based upon a Shapiro-Wilk test, P>0.3, and a probability plot)).

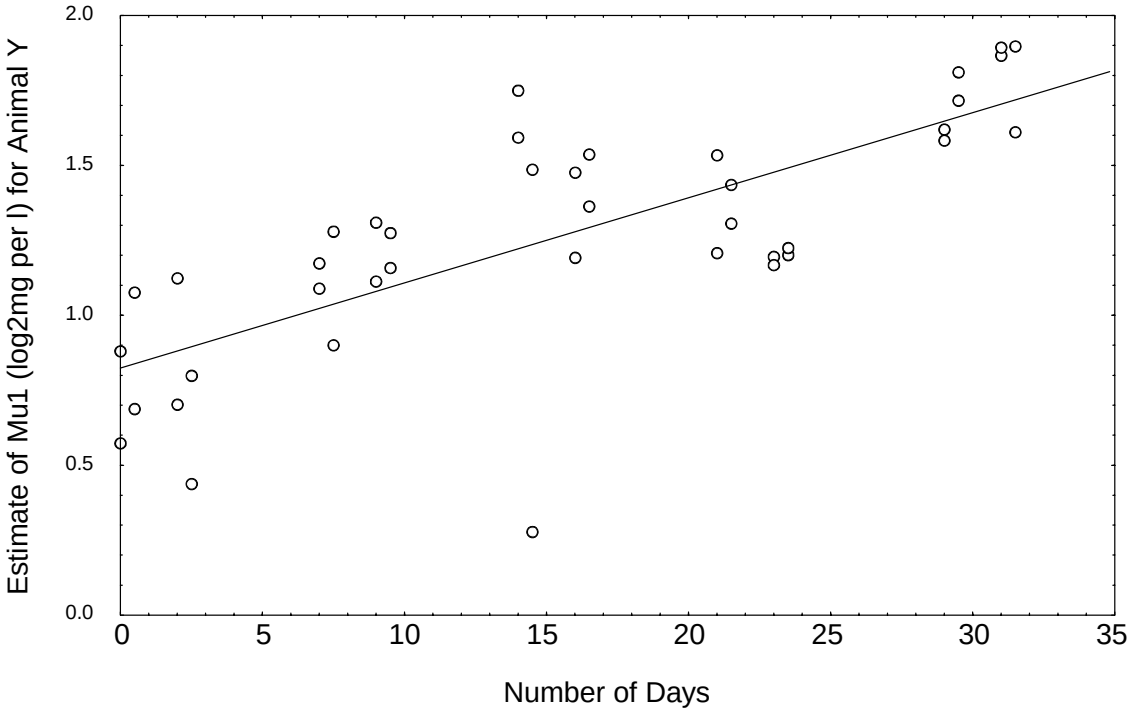


Fig. 1 Example of a time trend seen for Estimated μ_1 in samples from animal Y. Of the models tested, the most parsimonious which gave a good fit was a linear regression as indicated.

There was no evidence of decreasing autocorrelation within the 3 day time lag or for time lags of greater than 3 days (P>0.4 in both cases).

For time differences of up to 7 days (i.e. the same batch of media), there was evidence of increasing difference as the time difference increases from zero to 7 days (Fig. 2).

There was no evidence of associations for detrended μ_1 between the animals although there was a strong correlation between μ_1 (not detrended) for animals X and Y. This is not consistent with a batch effect.

Examination of the correlation between animals based on the mean for each week we found again a correlation between X and Y for μ_1, but also revealed a correlation between Y and C for detrended μ_1 (Table 1). This is not generally consistent with a batch effect.

Log Total bacterial density, log(T)

There were differences between animals for log(T). Animal Z stood out when compared to the other 2 animals.

There was evidence of a trend for X (linear) and C (quadratic) over time.

There was evidence of increasing difference as time difference increases.

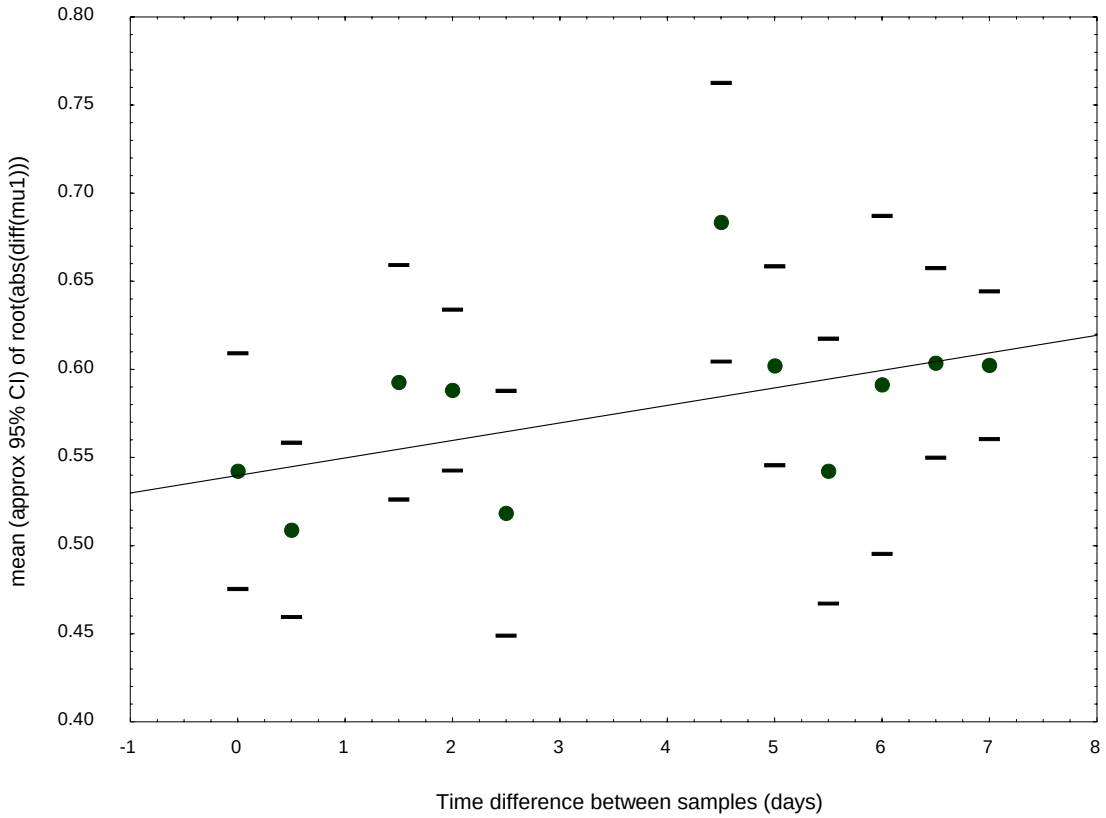


Fig. 2 Mean (with approximate 95% confidence limits) of the square root of the absolute difference between estimates of μ_1 for pairs of samples from the same animal collected up to seven days apart. Over this time scale there was evidence of the transformed difference increasing with increasing time-lag (Fit from linear regression is presented, y = 0.01x+0.54; H_0: m=0, P=0.005).

There was also evidence of increasing difference as time difference increased, for time lags of less than 3 days (thus just WITHIN batches). This was evidence (animals X and Y and all 3 together, but not Z alone) of a temporally-increasing disassociation between samples that is NOT due to the batch effect.

There was little evidence of associations between samples taken at the same time from different animals for log(*T*) or detrended log(*T*). This is consistent with there being no laboratory batch effect.

There was no strong evidence of associations between animals for detrended log(T) or log(T) when examining the means of each animal by week. This is again consistent with there being no laboratory batch effect.

Table 1. The level of association (Pearson's Correlation Coefficient ρ and P-value H_0:ρ =0) between animals in the estimated μ_1 for each animal matched by sampling occasion following detrending of the data. Generally there is little evidence of any association.

Variable	μ_1 X (detrended)	μ_1 Y (detrended)	μ_1 Z (detrended)
μ_1 Y (detrended)	ρ = 0.22 P=0.16 N=40		
μ_1 Z (detrended)	ρ = 0.048 P=0.77 N=40	ρ = -0.067 P=0.68 N=40	
μ_1 Control (detrended)	ρ = 0.020 P=0.94 N=17	ρ = -0.18 P=0.49 N=17	ρ = 0.25 P=0.34 N=17

DISCUSSION

The preliminary analysis of data described suggests a rapid rate of change in antimicrobial resistant populations of commensal *E. coli* in the gut of cattle. The main interest is the changes in resistance, but it is also of interest that similar results were found for the total bacterial density.

Longitudinal or time-series data on antimicrobial resistance have been examined elsewhere using a different approach to ours. Hartley and Richmond (1975) examined the survival of particular strains of *E. coli* and suggested a 'mean residence time' of between 5.5 and 6.5 days. Hoyle et al. (2004) reported a temporal decline over a time-scale of months, associated with increasing calf age, in the proportion of calves with resistant *E. coli* in a fixed quantity of sample. An even longer time-series of data extending over several years was examined by Lopez-Lozon et al. (2000) who used time-series analysis to examine the relationship between hospital prescribing of antimicrobials and resistance. Normand et al. (2000) similarly examined data on resistance over a time-scale of several years and found evidence of increasing resistance.

This study, like that of Hartley and Richmond (1975), considers the bacteria as the population of interest (as opposed to the animal population). For this reason it is important to describe the resistance of the bacterial population(s) and the methodology employed here was an improvement upon an earlier quantitative method (Humphry, et al., 2002). This method provides

estimates of the total density (*E. coli*) and the mean of the log of the MIC for a total population of phenotypically similar bacteria.

Temporal studies always carry the risk of temporal confounders. Methods to minimise this can include random testing of stored samples, although this does not necessarily remove temporal confounding and may introduce greater confounding associated with the storage process. In this work, we included the analysis of a control strain to assess the likelihood of any temporal associations being an artefact of laboratory effects.

The primary result from analyses of these data is that there was evidence of a higher association between samples taken within a few days of one another than samples taken further apart in time but thereafter increasing the time-lag does not appear to be associated with decreased association. In our study, the experimental design was such that any time interval greater than two days must have been five or more days. Given that this may be the interval at which time related association stops decreasing it obviously would have been better to have had more frequent sampling over a shorter overall experimental period.

This primary result may be a laboratory batch effect as a new batch of media was made up each week. However, there was evidence against this conclusion. First, there was no single trend identified amongst the three animals or the control. Second, the control did not show any general trend at all. Third, there was no association between samples (once the data were detrended) from different animals taken during the same week. Finally, the associations between controls from the same week were no higher than those tested between weeks and neither were significantly different from zero ($P>0.1$). Further analyses on the data are still required. However, the above evidence and the estimate of around 6 days for the mean residence time (Hartley & Richmond, 1975) suggest the associations seen between samples taken in the same week are likely to be genuine associations and not merely an artefact of laboratory effects.

Such a short time horizon for the decrease in autocorrelation reaffirms the perception of the dynamic nature of commensal bacteria. This means that knowledge of a bacterial population at any time point generally may not be a good predictor of bacterial populations in the future. This suggests that empirical modelling of the association between antimicrobial resistance and the general state of the environment (e.g. the animal, food-type, treatment regime) may provide better predictions of antimicrobial resistance than a reductionist or mechanistic modelling approach at the bacterial population level. When interpreting survey data, certain results (e.g. the apparent lack of association between prescription and resistance (Gunn, et al., 2003)) may be explained by the speed with which bacterial populations change in their sensitivity to antimicrobials. Future work aims to understand better whether the results seen here are duplicated across herds and to what extent single animal samples can represent the dynamics of antimicrobial resistance at the herd level.

If the dynamic nature of commensal bacteria were also true for pathogenic bacteria, then the possible consequences for practitioners in the field may be an increased desire for speedy analysis of samples when looking for antimicrobial sensitivity and shorter time period between repeat testing. Similarly, there may be a greater desire to respond quickly to the results of such tests whilst they remain relevant. However, this will only be the case if, (i) practitioners choose to use these more quantitative methods for describing antimicrobial sensitivity in bacteria or, (ii) the results presented here are duplicated with simpler and more widespread methods of detecting resistance.

ACKNOWLEDGEMENTS

The authors thank Gail Wilson for help in processing samples. SAC receives financial support from Scottish Executive Environment Rural Affairs Department. Thanks to Malcolm Hall for advice on the manuscript and the statistical analyses.

REFERENCES

Chatfield, C., (1996). The analysis of time series: An introduction. CRC Press, Boca Raton, Florida.

Gunn, G. J., Hall, M. and Low, J. C., (2003). Comparison of antibiotic resistance for *Escherichia coli* populations isolated from groups of diarrhoeic and control calves. Vet. J. 165, 172-174

Hartley, C. L. and Richmond, M. H., (1975). Antibiotic resistance and survival of *E. coli* in the alimentary tract. Brit. Med. J. 4, 71-74

Hoyle, D. V., Shaw, D. J., Knight, H. I., Davison, H. C., Pearce, M. C., Low, J. C., Gunn, G. J. and Woolhouse, M. E. J., (2004). Age-related decline in carriage of ampicillin-resistant *Escherichia coli* in young calves. Appl. Environ. Microb. 70, 6927-6930

Humphry, R. W., Blake, D., Fenlon, D., Horgan, G., Low, J. C. and Gunn, G. J., (2002). The quantitative measurement of antimicrobial resistance in *Escherichia coli* at the meta-population level (meta-population analysis). Lett. Appl. Microbiol. 35, 326-330

Lopez-Lozon, J. M., Monnet, D. L., Yague, A., Burgos, A., Gonzolo, N., Campillos, P. and Saez, M., (2000). Modelling and forecasting microbial resistance and its dynamic relationship to antimicrobial use: a time series analysis. Int. J. Antimicrobial Agents 14, 21-31

Normand, E. H., Gibson, N. R., Taylor, D. J., Carmichael, S. and Reid, S. W. J., (2000). Trends of antimicrobial resistance in bacterial isolates from a small animal referral hospital. Vet. Rec. 146, 151-155

APPENDIX 1

Timetable of sample collection:

Shaded days indicate days on which samples were taken. Superscripts 1 or 2 indicate days for which the control strain was also tested. A superscript 1 indicates that a single replicate for the control strain was tested. The superscript 2 indicates that the control strain was tested against both laboratory batch replicates.

	Mon	Tues	Wed	Thurs	Fri
Week 1 (Sept 2001)	24	25	26^1	27	28
Week 2 (Oct 2001)	1^2	2	3^2	4	5
Week 3	8^2	9	10^2	11	12
Week 4	15^2	16	17^2	18	19
Week 5	22	23^2	24	25^2	26

APPENDIX 2

Derivation of weighting used in the non-linear regression:

Let the concentration of the sample be *c*
The volume in the sector measured is *v*
The true bacterial density is *b*

The Poisson process would predict that the expected (number of colonies) would be *c*b*v* and the variance of this also *c*b*v.*

The estimated bacterial density, *d*, for one sample is *d = numberofcolonies/(c*v)*

*Var(d) = Var(numcolonies)/((c*v)^2) = b/(c*v)*

Thus, the weighting for an estimated bacterial density, d, would be *1/Var(d)= c*v/b*

In place of *b*, the estimate for *b* is used*, n/c*v*, so that *_weight_ = (c*v)^2/n*

Society for Veterinary Epidemiology and Preventive Medicine

PAST PRESIDENTS

1982-'83	G. Davies
1983-'84	P.R. Ellis
1984-'85	G. Gettinby
1985-'86	R.W.J. Plenderleith
1986-'87	M.V. Thrusfield
1987-'88	K.S. Howe
1988-'89	A.M. Russell
1989-'90	S.G. McIlroy
1990-'91	J.E.T. Jones
1991-'92	J.M. Booth
1992-'93	E.A. Goodall
1993-'94	R.G. Eddy
1994-'95	J.T. Done
1995-'96	G.J. Gunn
1996-'97	M.S. Richards
1997-'98	J.D. Collins
1998-'99	F.D. Menzies
1999-'00	K.L. Morgan
2000-'01	S.W.J. Reid
2001-'02	A.D. Paterson
2002-'03	L.E. Green
2003-'04	J.L.N. Wood

EXECUTIVE COMMITTEE 2004-2005

E.G.M. van Klink (President), J.L.N. Wood (Senior Vice-President), D.J. Mellor (Junior Vice-President), J.R. Newton (Honorary Secretary), L. Kelly (Honorary Treasurer), N. Honhold, E.J. Peeler, K.D.C. Stärk, L. Matthews, G.L. Pinchbeck, G.J. Gunn (co-opted)

Honorary Auditors: J.M. Booth, R.G. Eddy

LIFE MEMBERS

J.M. Booth, M.J. Clarkson, J.D Collins, G. Davies, R.G. Eddy,
P.R. Ellis, E.A. Goodall, A.M. Russell, M.V. Thrusfield

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2005	Bill Reilly: From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More: Towards eradication of bovine tuberculosis in Ireland: A vritical review of progress
2004	Ulrich Kihm: BSE and the stable to table concept	Gary Smith: Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox: The current state of statistical science	Ynte Schukken: Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby: Informatics and epidemiology – the first 400 years	Bryan Grenfell: Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston: Science politics and animal health policy: epidemiology in action	Mart de Jong: Design and analysis of transmission experiments
2000	Jim Scudamore: Surveillance – past, present and future	Dirk Pfeiffer: Spatial analysis – a new challenge for veterinary epidemiologists
1999	Aalt Dijkhuizen: The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse: Understanding the epidemiology of scrapie
1998	Wayne Martin: Art, science and mathematics revisited: the role of epidemiology in promoting animal health	-

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

APPLICATION FOR MEMBERSHIP

Name .....

Address .....

.....

.....

.....

Telephone: .....

Fax: .....

E-mail:.....

Signed ..... Date .....

Please enclose the membership fee (£20 sterling) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's web site, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Louise Kelly
Department of Statistics and Modelling Science
University of Strathclyde
Glasgow
G1 1XH

℅ +44 (0) 141 548 3659
FAX +44 (0) 141 552 2079
Email: louise@stams.strath.ac.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. Statistical/Mathematical Modelling)
- ☐ Herd/Flock Level Disease Control Strategies
- ☐ National/International Disease Control Policy
- ☐ Sero-Epidemiology
- ☐ Herd Health and Productivity Systems
- ☐ Disease Nomenclature and Epidemiological Terminology
- ☐ Economic Effects of Disease on Animal Production
- ☐ Veterinary Public Health and Food Hygiene
- ☐ Computing, including data logging
- ☐ Computer Programming *per se*
- ☐ Population and Animal Disease Databases
- ☐ Information System Design
- ☐ Geographical Information Systems (GIS)
- ☐ Risk Analysis

CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.
4. Membership is conditional on the return to the Secretary of a completed application form and subscription equivalent to the rate for one calendar year. Subsequent subscriptions fall due on the first day of May each year.
5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than four ordinary elected members. However, the Committee will have powers of co-option.

ELECTION

8. The election of office bearers and ordinary committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the annual general meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. He will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or the Honorary Secretary.
11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not be reported either in part or in whole without the written permission of the Executive Committee.
15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.
17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.
18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.
19. In case of an equal division of votes, the Chairman of the meeting will have a second and casting vote.
20. All members on election will be supplied with a copy of this constitution.
21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.
22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

April, 1982
Revised March, 1985; April, 1988; November 1994
Corrected January 1997; April 2002