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SALMONELLA

THE EFFECT OF SOURCE HERD AND ABATTOIR FACTORS ON PIG CARCASE *SALMONELLA* CONTAMINATION EVALUATED BY MULTILEVEL MODELLING

F.M. BAPTISTA*, J. DAHL AND L.R. NIELSEN

SUMMARY

In Denmark, a Surveillance-and-Control Programme for *Salmonella* in pigs has been in place for several years. This study investigated factors associated with *Salmonella* pig carcase prevalence, measured in pools of five carcase swab samples. A total of 20,128 pooled carcase swabs collected in 22 Danish abattoirs, from 2002 to 2008, were included in a multilevel logistic regression model.

Study results indicate that the probability of *Salmonella* positive carcases is mainly influenced by weekday, estimated daily number of *Salmonella* seropositive pigs delivered to slaughter and average *Salmonella* seroprevalence of the source herds that delivered each of the five pigs contributing to the pool that was sampled on the day of slaughter. Model results suggest that risk-mitigation actions at the abattoir such as improved practices including hygiene and staff training, logistic slaughter and decontamination might result in a further reduction in carcase pool *Salmonella* prevalence.

INTRODUCTION

In 1993, Denmark implemented a National Surveillance-and-Control Programme for *Salmonella* in pigs, which has since then undergone several adjustments (Mousing et al., 1997; Alban et al., 2002).

In Denmark, a target has been set to reduce the individual carcase prevalence of *Salmonella* in pork to below 1%. According to Sørensen et al. (2007) a conversion factor of 3 can be applied to calculate the individual prevalence from a pooled prevalence. Hence, the pooled carcase prevalence should be kept below 3%. Carcase contamination might result from intestinal carriage of the pig itself, but also from contact with other infected or contaminated pigs, carcases or the environment in the abattoir (Botteldoorn et al., 2003). Carcase prevalence is an indicator of public health risk, since it reflects the *Salmonella* load at the end of the slaughter process. Consequently, abattoir hygiene and management practices are expected to have an important impact on carcase contamination.

*Filipa Matos Baptista, Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Grønnegårdsvej 8, DK-1870 Frederiksberg C, Denmark; CIISA, Faculdade de Medicina Veterinária, TULisbon, Av. da Universidade Técnica, 1300-477 Lisboa, Portugal. E-mail: baptista@life.ku.dk

In the last few years, post-harvest decontamination methods have been investigated including hot-water decontamination, steam cleaning and application of organic acids (Huffman, 2002; Lawson et al., 2009). Since 2001, pigs from high seroprevalence herds have been decontaminated with hot-water in one abattoir in Denmark. Prior to chilling, carcases are treated with 80ºC water for 15 seconds. Published results show that hot-water decontamination can reduce *Salmonella* on pig carcase up to two log units, as well as other pathogens of human concern (Jensen & Christensen, 2001).

This study aimed to investigate herd and abattoir factors associated with *Salmonella* pig carcase contamination.

MATERIALS AND METHODS

The Danish *Salmonella* Surveillance-and-Control Programme in finisher pigs includes both herd and fresh meat surveillance. Surveillance data covering a period from 2002 to 2008 were obtained from the Danish Agricultural & Food Council. Herd surveillance is based on the serological level in meat-juice samples collected at slaughter which enables the monthly classification of herds into three levels, based on a calculated *Salmonella* index. Fresh meat surveillance consists of bacteriological testing of five pooled carcase swabs collected daily at slaughter, after chilling (Sørensen et al., 2007). Animal movement data were also used to collect information on herds and number of pigs delivered to the abattoirs on each day of sampling.

A multilevel logistic regression model was used taking into account the hierarchical data structure and adjusting the parameter estimates to the random variation at the abattoir level. Data analysis was performed using the glimmix procedure in the software SAS® v. 9.1.3. (SAS Inst., Inc., Cary, NC). The following variables were evaluated: estimated daily number of *Salmonella* seropositive pigs delivered to slaughter, average *Salmonella* seroprevalence of the source herds that delivered each of the five pigs contributing to the pool, weekday (Monday, Tuesday, Wednesday, Thursday, Friday, Weekend), year, season and abattoir size (small, medium, large abattoirs). Samples collected on Saturday and Sunday were merged as “Weekend” due to the small number of observations compared to the working days. Abattoir size was based on the average number of pigs slaughtered per day: small <1,000 pigs; medium 1,001-6,000 pigs; large >6,000 pigs). Samples collected in one abattoir were excluded since no positive samples were found (N = 68). Different patterns of seasonality were tested including a sine-cosine function. In total, 20,128 pooled carcase swabs collected in 22 Danish abattoirs were included in the analyses.

RESULTS

Convergence criteria were satisfied and residual plots did not indicate departures from model assumptions. Only weekday, the estimated daily number of *Salmonella* seropositive pigs delivered to slaughter and average *Salmonella* seroprevalence of the source herds that delivered each of the five pigs contributing to the pools were significantly associated with the probability of *Salmonella* pool positivity. No other factors were found to be significant ($p > 0.05$) (Table 1). No significant random effects for slopes were found, indicating that the effects of the explanatory variables were the same for all abattoirs. No seasonal variation was found to be significant.

The effect of two different interventions was evaluated. Figure 1 presents the effect of 1) improved underlying practices at the abattoir and 2) applying hot-water decontamination compared to the probability of *Salmonella* pool positivity for an average abattoir. Effect of improved underlying practices at the abattoir was simulated by using the calculated average intercept of the five abattoirs with the lowest probabilities of *Salmonella* pool positivity in the final multilevel model. A 90% reduction was assumed for the hot-water decontamination effect (Jensen & Christensen, 2001).

Table 1. Multilevel logistic model of the probability of *Salmonella* positive carcase pools after chilling in 22 Danish abattoirs from October 2002 to December 2008

VARIABLES	VARIANCE	S.E.
RANDOM EFFECT		
Abattoir	0.20	0.09
FIXED EFFECTS	ESTIMATE	P – VALUE
Constant	-3.7	
Estimated number of seropositive pigs delivered to slaughter	0.13	P <0.01
Average seroprevalence of the source herds that delivered pigs contributing to the pool	3.8	P <0.01
Weekday		P <0.01
Monday	-0.33	
Tuesday	-0.33	
Wednesday	-0.44	
Thursday	-6.3	
Friday	-6.2	
Weekend	0	

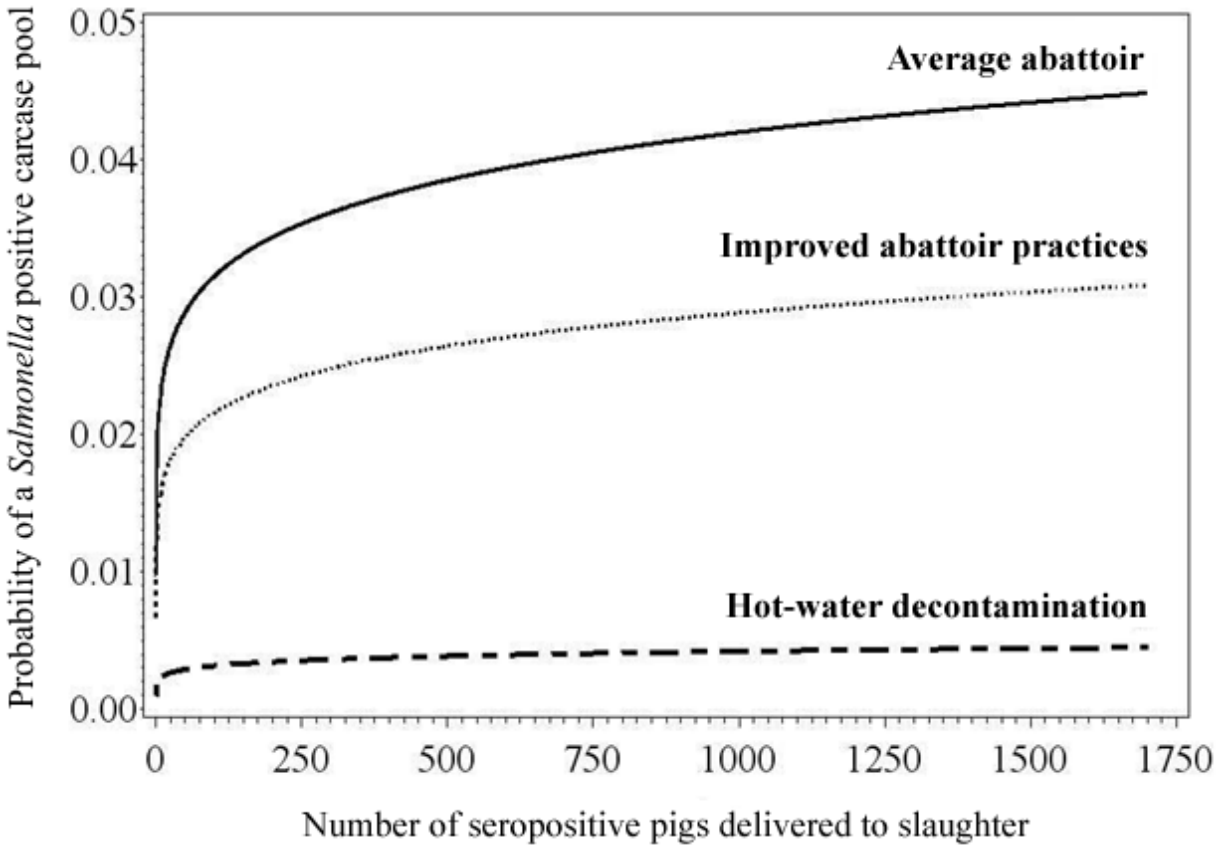

Fig. 1 Plot of the predicted probability of a *Salmonella* positive carcase pool versus the number of seropositive pigs delivered to slaughter in a day for three scenarios: an average abattoir (Average), underlying abattoir practices improved to the level of the five best abattoirs (Improved abattoir practices), or subjecting all carcases to hot-water decontamination (Hot-water decontamination)

DISCUSSION

To our knowledge this was the first study taking into account multiple factors influencing *Salmonella* carcase contamination in finisher pigs, including the overall *Salmonella* input to the abattoir, in a large number of abattoirs, over a long period of time.

Salmonella herd seroprevalence was significantly associated with *Salmonella* pool positivity indicating the importance of the source herds. Pools collected during the weekend presented higher odds of being positive compared to working days suggesting compromised hygiene practices or a high *Salmonella* input to the abattoir over the weekend. This higher input could be explained by longer periods in lairage which has been show to increase *Salmonella* shedding (Lo Fo Wong et al., 2002). Even though *Salmonella* infections in humans are significantly correlated to season (Hald & Andersen, 2001), some studies have already failed to prove a seasonal trend in *Salmonella* seroprevalence in pigs (Lo Fo Wong et al., 2004; Benschop et al., 2008).

Variation between abattoirs might be related to underlying practices at the abattoir, including hygiene conditions and staff training. This study indicates that if abattoir practices can be improved, carcase contamination might be reduced resulting in lower carcase prevalence. However, if a very low prevalence is aimed for, other risk-mitigating actions should be applied.

Hot-water decontamination has shown to be an efficient tool to significantly reduce the proportion of *Salmonella* positive carcase pools. However, for a small abattoir the costs per pig are much higher compared to large abattoirs (1.07 euros versus 0.17 euros). Hence, in small abattoirs this might not be a cost-effective strategy. Overall, if the number of seropositive pigs delivered in a day can be kept low (<50) in small abattoirs, a 3% target for the pool prevalence might be achieved. Pre-harvest interventions are costly and research has shown that a reduction in the number of *Salmonella* seropositive pigs in individual herds have a minimum impact in the number of positive carcases found in an abattoir (Hurd et al., 2008). In medium-to-large abattoirs where the *Salmonella* input to the abattoir is high on average, interventions conducted at the abattoir level, including decontamination might have a higher value than pre-harvest interventions.

In conclusion, the results of the study suggest that improved practices at the abattoir might lower the risk of *Salmonella* carcase contamination. This study shows that different strategies should be in place depending on herd and abattoir characteristics. Hence, targets aiming for protection of public health should be set at carcase level. Cost-effectiveness analysis should be conducted in the future, taking into account country/regional differences, i.e., herd *Salmonella* seroprevalence and herd and abattoir structure. Other decontamination techniques should also be investigated.

REFERENCES

Alban, L., Stege, H. and Dahl, J. (2002). The new classification system for slaughter-pig herds in the Danish *Salmonella* surveillance-and-control program. Prev. Vet. Med. 53, 133-146.

Benschop, J., Stevenson, M.A., Dahl, J., Morris, R.S. and French, N.P. (2008). Temporal and longitudinal analysis of Danish Swine Salmonellosis Control Programme data: implications for surveillance. Epidemiol. Infect. 136, 1511-1520.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K. and Herman, L. (2003). *Salmonella* on pig carcasses: positive pigs and cross contamination in the slaughterhouse. J. Appl. Microbiol. 95, 891-903.

Hald, T. and Andersen, J.S. (2001). Trends and seasonal variations in the occurrence of *Salmonella* in pigs, pork and humans in Denmark, 1995-2000. Berl. Munch. Tierarztl. Wochenschr. 114, 346-349.

Huffman, R.D. (2002). Current and future technologies for the decontamination of carcasses and fresh meat. Meat Science 62, 285-294.

Hurd, H.S., Enøe, C., Sorensen, L., Wachman, H., Corns, S.M., Bryden, K.M. and Grenier, M. (2008). Risk-based analysis of the Danish pork *Salmonella* program: past and future. Risk Anal. 28, 341-351.

Jensen, T. and Christensen, H. (2001). Decontamination of pig carcasses with hot water. Proceedings of the 4th international symposium on the epidemiology and control of *Salmonella* and other food borne pathogens in pork. 127-129.

Lawson, L.G., Jensen, J.D., Christiansen, P. and Lund, M. (2009). Cost-effectiveness of *Salmonella* reduction in Danish abattoirs. Int. J. Food Microbiol. 136, 126-132.

Lo Fo Wong, D.M.A., Hald, T., van der Wolf, P.J. and Swanenburg, M. (2002). Epidemiology and control measures for *Salmonella* in pigs and pork. Livestock Production Science 76, 215-222.

Lo Fo Wong, D.M., Dahl, J., Wingstrand, A., van der Wolf, P.J., von, A.A. and Thorberg, B.M. (2004). A European longitudinal study in *Salmonella* seronegative- and seropositive-classified finishing pig herds. Epidemiol. Infect. 132, 903-914.

Mousing, J., Jensen, P.T., Halgaard, C., Bager, F., Feld, N., Nielsen, B., Nielsen, J.P. and Bech-Nielsen, S. (1997). Nation-wide *Salmonella enterica* surveillance and control in Danish slaughter swine herds. Prev. Vet. Med. 29, 247-261.

Sørensen, L.L., Wachmann, H. and Alban, L. (2007). Estimation of *Salmonella* prevalence on individual-level based upon pooled swab samples from swine carcasses. Vet. Microbiol. 119, 213-220.

EARLY DETECTION OF A *SALMONELLA* DUBLIN OUTBREAK IN CATTLE IN GREAT BRITAIN USING SPATIO-TEMPORAL EPIDEMIOLOGICAL METHODS

C. PAPADOPOULOU*, R.R.L. SIMONS, A. VIDAL-DIEZ, D.F. TWOMEY, M. MILLAR, A.J. COOK AND P. SEWELL

SUMMARY

Salmonella Dublin is an endemic cause of significant morbidity in cattle herds in Great Britain (GB), affecting both adult cattle and calves. The Veterinary Laboratories Agency (VLA) applies an early warning system to surveillance data from clinical cases in GB livestock and three potential outbreaks of *S.* Dublin in cattle were identified between March and June 2009. This system has been in use since 2006 on routine surveillance data in order to detect early changes in *Salmonella* reporting from livestock in GB.

The potential outbreaks were investigated further in a spatio-temporal study conducted using SaTScan software, which identified a statistically significant (P=0.002) cluster of *S.* Dublin cases in cattle in the South West of GB. The outbreak was considered to have taken place between December 2008 and June 2009 and involved mainly immature cattle with gastrointestinal disease.

INTRODUCTION

Salmonellosis due to *Salmonella* Dublin infection in cattle results in high morbidity in both adults and calves (Wray et al., 1989); hence, it can be a cause of economic loss and welfare concern in infected cattle herds (Ersbøll & Nielsen, 2008).

Risk factors for *S.* Dublin infection in cattle herds include: contact with infected animals or their excretions (e.g. faeces and milk), for example through animal trade or contact with neighbouring herds and contaminated equipment; herd size; concurrent bovine viral diarrhea virus (BVDV) infection; certain feeding strategies; climate; and liver fluke infestation (Aitken et al., 1981; Wray & Roeder, 1987; Morisse & Cotte, 1994; Losinger et al., 1997; Vaessen et al., 1998; Van Schaik et al., 2002; Nielsen et al., 2007). Endemic strains of *Salmonella* can persist in calf-rearing units and cause recurrent outbreaks (McLaren & Wray, 1991).

Salmonella Dublin infection in cattle can be latent with no or few clinical signs (Wray & Davies, 2000) and long-term carriers can be produced. Carriers can contribute to the spread of infection within herds by either continuously or intermittently shedding *S.* Dublin into the environment through faeces or milk (Veling et al., 2000; Nielsen et al., 2004). Herds with the

*Christina Papadopoulou, Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, UK. E-mail: c.papadopoulou@vla.defra.gsi.gov.uk

highest risk of carrier development are those with clinical disease outbreaks (Nielsen et al., 2004). Carriers may start to shed bacteria during periods of stress such as calving (Counter & Gibson, 1980) and transportation (Wray et al., 1991).

Salmonella Dublin, which is considered to be a host-adapted serovar in cattle (Pullinger et al., 2007), is endemic in cattle herds in Great Britain (GB) and it has been the predominant serovar reported from cattle since 1999 (Papadopoulou & Kidd, 2008; VLA, 2008). *S*. Dublin in cattle has been associated with severe gastrointestinal disease, particularly in adult animals, and abortion, and clinical cases usually peak in the late autumn and winter months (Papadopoulou & Kidd, 2008). This has been associated with increased faecal contact during the housing period, housing stress and stress associated with an increased number of calving and abortions seen at that time. Although *S*. Dublin is not a strain commonly associated with human infections, it has been reported to be very invasive and to have a relatively high mortality rate when infections occur, usually after consumption of milk products that have not been pasteurised properly or of insufficiently cooked meat (Humphrey et al., 2000; Helms et al., 2003; Foley & Lynne, 2007).

MATERIALS AND METHODS

An overview of the *Salmonella* Early Detection System

An Early Detection System (EDS) for *Salmonella* outbreaks in livestock production in GB has been in use at the Veterinary Laboratories Agency (VLA) since September 2006. The system was developed in order to detect early changes in *Salmonella* reporting from livestock for a specific time period, and in particular for serovars associated primarily with human infections and public health. The EDS does not predict future outbreaks, but alerts to contemporary aberrations in expected reporting so that timely consideration may be given to the investigation of potential outbreaks. Data are derived from various sources, including incidents of *Salmonella* originating from clinically ill livestock species. This EDS has been described in detail previously (Kosmider et al, 2006). Briefly, it is a generalised linear model (GLM), based on a Poisson regression model (Farrington et al., 1996) which, after inputting the previous 5 years' and 12 years' data into the detection algorithm, compares the number of observed incidents with an expected value, accounting for seasonality and past outbreaks.

The model is used to estimate the expected count for the current month, $\hat{\mu}_0$. Upper and lower confidence limits (such that the interval contains the expected count with 95% probability) are also calculated. A value above the upper limit can be considered statistically different from the expected count. Thus, the upper limit is defined as the threshold value, *U*. These are compared to the current observed count, y_0, to derive an exceedance score, *X:*

$$X = \frac{y_0 - \hat{\mu}_0}{U - \hat{\mu}_0},$$

If the exceedance score is greater than 1 then this indicates that the current count exceeds the threshold value and a warning is triggered, suggesting that a potential outbreak may be occurring in the field.

Follow-up action is taken as appropriate, e.g. examination of raw data, consultation with experts, on-farm epidemiological investigations, etc. to assess whether the 'flags' triggered are

due to emerging outbreaks, i.e. the occurrence of cases of illness clearly in excess of expectancy over a defined period of time. Further investigation may indicate that the warnings are not associated with a field outbreak but could be related to changes in reporting procedures or increased reports resulting from extensive investigations of a previous incident.

Statistical analysis for disease clustering investigation

The potential outbreaks indicated by the EDS model were investigated further in a spatio-temporal study of disease clustering conducted using the SaTScan software (http://www.satscan.org/). A prospective space-time permutation method (Kulldorff et al., 1998) was carried out. This analysis assumes a constant population across time as it does not require population data.

Statistical analysis of disease clustering is important in three main situations (Lawson & Kulldorff, 1999):

i) In epidemiological research to study the aetiology of disease;
ii) In public (or veterinary) health as part of geographical disease surveillance;
iii) In response to disease cluster alarms to evaluate whether thorough epidemiological investigations are warranted.

A definition of clustering given by Knox (1989) is: 'a geographically bounded group of occurrences of sufficient size and concentration to be unlikely to have occurred by chance'.

Various statistical tests have been developed for hypothesis testing of spatio-temporal clustering. Some of these are non-specific and do not seek to estimate the spatial locations of clusters but simply to assess whether clustering is apparent in a study region. In contrast, specific methods seek to assess the locational structure of clusters (Lawson & Kulldorff, 1999). This study used the spatial scan statistic which is a specific method. It was proposed by Kulldorff and Nagarwalla (1995) and Kulldorff (1997) and employs a likelihood ratio test for the comparison of the number of cases found in the study region population (the null hypothesis) to a model that has different disease risk depending on being inside or outside a circular zone.

Study population and data

The target population for the spatio-temporal analysis included all cattle holdings in GB from which there had been a clinical case of *S.* Dublin reported between January 2005 and June 2009.

Salmonella infection is a reportable zoonosis in livestock in Great Britain under the Zoonoses Order 1989. All laboratory isolations are reported to and collated by the VLA. Through this reporting system, data are made available on the isolation of *Salmonella* serovars from domestic livestock. Data may originate from different sources, for example from investigation of clinical disease or from voluntary surveillance activities. This scanning surveillance system records the clinical data from cattle holdings which were used in this study.

RESULTS

Salmonella EDS 'flags'

Three potential outbreaks of *S.* Dublin in cattle in GB occurring in March 2009, May 2009 and June 2009 (Table 1, Fig. 1) were indicated by the EDS model.

Table 1. Description of three potential outbreaks of *S.* Dublin in GB cattle, identified by VLA's Early Detection System in 2009

Month	Observed Value[a]	Expected Value[b]	Threshold Value[c]	Exceedance Score[d]
March	27	16.61	25.21	1.21
May	31	15.48	23.8	1.87
June	35	17.57	26.39	1.98

[a] Observed value is the actual number of reported *Salmonella* incidents each month.

[b] Expected value is the number of *Salmonella* incidents calculated by the EDS model that would be expected to be reported each month accounting for seasonality and past outbreaks.

[c] Threshold value is the upper 95% confidence limit of the expected value calculated by the EDS model.

[d] Exceedance score is a value derived by the EDS model by comparing the expected value and the threshold value to the observed value. An exceedance score of greater than 1 indicates that the current count exceeds the threshold value and a warning is triggered, suggesting that a potential outbreak may be occurring in the field.

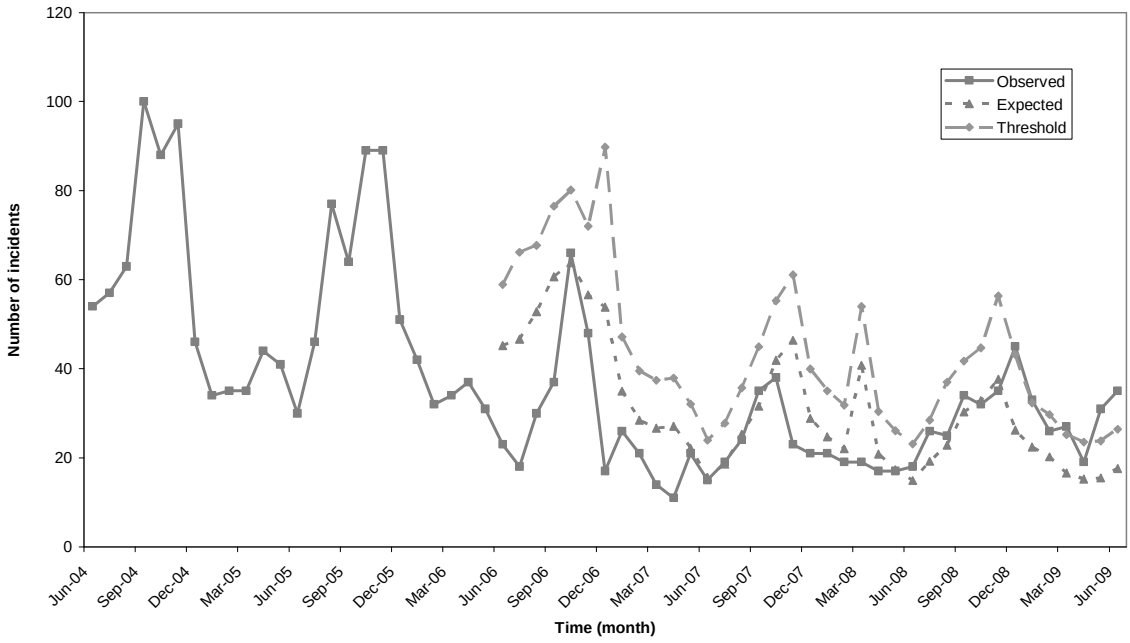

Fig. 1 Number of *S.* Dublin incidents reported from clinically ill cattle in GB from June 2004 to June 2009 and the expected and threshold values estimated by the Early Detection System model from June 2006 and onwards

Spatio-temporal analysis results

The spatio-temporal analysis using the SaTScan software identified a statistically significant (P=0.002) cluster of *S.* Dublin cases in cattle in the South West of GB occurring between December 2008 and June 2009. The spatio-temporal characteristics of the cluster are presented in Table 2 and its location is presented in Fig. 2.

Table 2. Description of cluster identified by the SaTScan procedure for the *S.* Dublin outbreak in cattle in Great Britain (Cartesian coordinates are used)

	Radius (km)	Centre Coordinate X	Centre Coordinate Y	Time frame		Number of cases	Number of locations	P-value
Cluster	32.94	324928	128660	01/12/08	30/06/09	29	23	0.022

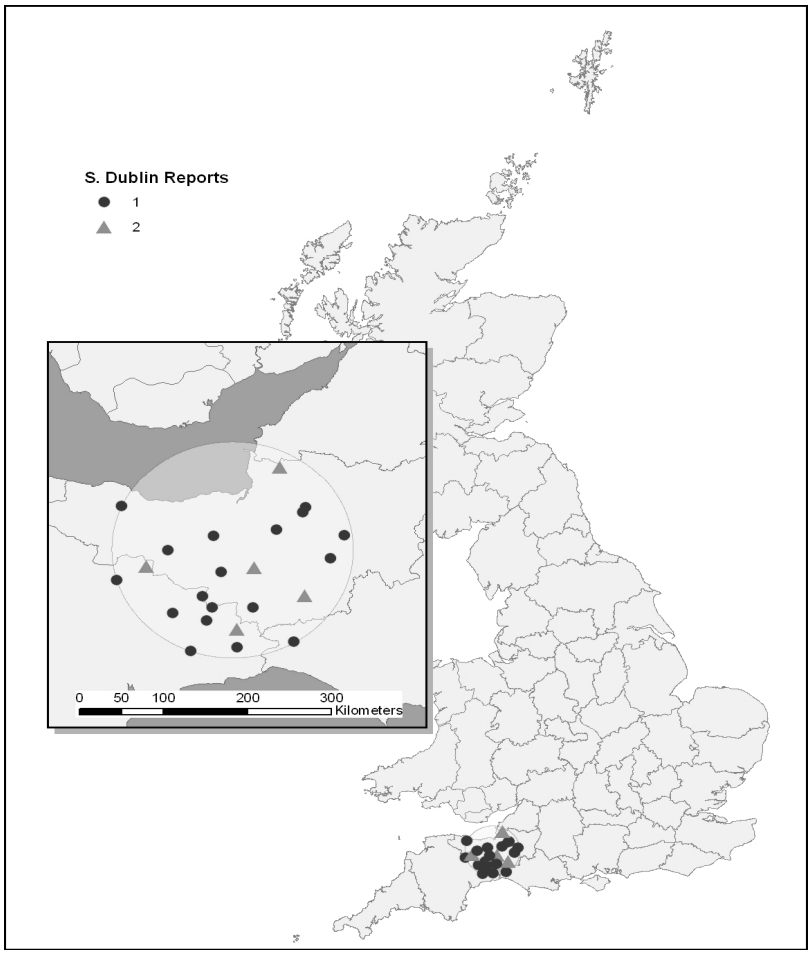

Fig. 2 Location of cluster of *S.* Dublin cases in GB cattle holdings identified by the SaTScan procedure; maximum spatial window was set to 10% spatial coverage and maximum temporal window was 20% of the study period. The map presents the holdings at random within the yellow circle, therefore anonymising the holdings where the incidents occurred)

Descriptive analysis

The cluster comprised of 28 incidents. Twenty-three cattle holdings were included in the cluster. Fourteen (61%) of these premises kept dairy cattle, eight (35%) kept beef cattle, and for one (4%) the type of enterprise was unknown. Eighteen (78%) of the holdings reported one incident each, while five (22%) reported two incidents each. Seventeen (61%) of the reports involved immature cattle, of which 12 (71%) had experienced gastrointestinal disease, nine (32%) of the reports involved adult cattle, of which six (67%) had also experienced gastrointestinal disease, one (4%) involved both adult and immature cattle, and one (4%) involved cattle of unspecified age. Twenty-six (92%) of the reports were of *S.* Dublin strains fully sensitive to all the antimicrobials tested in a standard panel (VLA, 2008), one (4%) was resistant to streptomycin, and one (4%) showed the penta-resistant phenotype ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamide compounds, tetracycline). The diagnostic material had been submitted by 14 different veterinary practices and there was no obvious link to a single private veterinary surgeon.

Assessment of the epidemiological situation

This information was reviewed by VLA's Veterinary Investigation Officers and disease experts, in particular in relation to changes at the national level in the submission rates of diagnostic material and diagnoses of fascioliasis, which is a disease historically known to be associated with bovine salmonellosis. The number of diagnoses of *Fasciola hepatica* increased markedly during 2008, followed by an increase in bovine *S.* Dublin diagnoses at the end of 2008 and in the first few months of 2009 (data not shown). The increase in bovine *S.* Dublin infections diagnosed by VLA in the South West of GB was communicated to the farmers and private practitioners via VLA's routine monthly surveillance reports to raise awareness and facilitate clinical diagnosis.

DISCUSSION

The aim of this study was to conduct an initial investigation in response to the aberrations in reporting of *S.* Dublin in clinically ill cattle indicated by VLA's veterinary *Salmonella* EDS model and to evaluate whether thorough epidemiological investigations were warranted.

VLA's veterinary *Salmonella* EDS model was developed based on a statistical algorithm previously applied to public health data (Farrington et al., 1996). Even though this model has been applied since September 2006 to a selection of scanning surveillance data, the first aberration in reporting ('flag') of *S.* Dublin from clinically ill cattle was in December 2008, followed by a second 'flag' in January 2009 (data not shown). Initial investigation conducted at that time included a descriptive analysis of the raw data e.g. assessment for unusual age of the affected groups of animals, unusual clinical sings, unusual antimicrobial resistance profiles, and examination of morbidity and mortality rates. Nothing unusual was found, hence a decision was taken that no further action was needed at that time. Subsequently, there were three further 'flags' triggered by the EDS model, from March 2009 to May 2009. These results were consistent with spatio-temporal analysis using the SaTScan software which also indicated a cluster of cases occurring in the field between December 2008 and June 2009.

The EDS demands a sufficient amount of observed data for an important change in occurrence to be detected. VLA routinely utilizes both a 5 year and a 12 year baseline with our EDS which only triggered 'flags' when using the last five years of historical data as a baseline.

This could be explained by the lower number of observed cases in recent years as shown in Fig. 1. When only five years of historical data were considered, more emphasis was placed on the lower number of cases reported in recent years and thus a lower threshold value was estimated than when 12 years of historical data were used.

The spatial scan statistic is a robust technique for exploratory analysis of spatial clustering. Its advantages include examination of a potentially infinite range of zone sizes that it accounts for the multiple testing inherent in such a procedure and that it relies on a formal model of null and alternative hypotheses. Its limitations relate to the use of circular regions, which tend to emphasize compact clusters, and so the method has low power against other alternatives such as a long and narrow cluster along a river or main road, or a large number of very small clusters at very different locations (Lawson & Kulldorff, 1999). Another potential problem identified by Ward and Carpenter (2000) is the definition of the scanning window that should be used in the analysis, which ideally should be defined on the basis of the biology of the disease, but subjectivity in defining this window can affect the validity of the results. Because *S.* Dublin is an endemic infection in the cattle population of GB, it was decided to set the maximum size of the spatial cluster size to 10% of the population at risk and to set the maximum size of the temporal cluster size to 20% of the study period to avoid inappropriate SaTScan reporting of large outbreaks which may reflect endemic levels of infection.

Although the *S.* Dublin clusters may have been attributable to an increased incidence of fascioliasis, an assessment of risk factors for the increased reported incidence of *S.* Dublin was outside of the scope of this study. Fascioliasis should therefore not be implicated without consideration of other potential risk factors.

This study demonstrates the value of routine surveillance data and how simple epidemiological analytical tools can assist in early identification of animal disease outbreaks. However, further studies should be conducted to investigate in more detail the aberrations in reporting and the clusters indicated by the *Salmonella* EDS and spatio-temporal analysis, including identification of risk factors and sources of infection as well as analysis of data on cattle movements from/to the holdings in the affected area.

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REFERENCES

Aitken M.M., Jones, P.W., Hall, G.A., Hughes, D.L. and Brown, G.T. (1981). Effects of experimental Responses of fluke-infected and fluke-free cattle to experimental re-infection with *Salmonella* Dublin. Res. Vet. Sci. 31, 120-126.

Counter, D.E. and Gibson, E.A. (1980). Salmonella Dublin infection in self-contained dairy herds in East Anglia: excretion at calving. Vet. Rec. 107, 191–193.

Ersbøll, A.K. and Nielsen, L.R. (2008). The range of influence between cattle herds is of importance for the local spread of *Salmonella* Dublin in Denmark. Prev. Vet. Med. 84, 277–290.

Farrington, C.P., Andrews, N.J., Beale, A.D. and Catchpole, M.A. (1996). A Statistical Algorithm for the Early Detection of Outbreaks of Infectious Disease. J. R. Stat. Soc. Ser. A 159, 547-563.

Foley, S.L., and Lynne, A.M. (2008). Food animal-associated *Salmonella* challenges: Pathogenicity and antimicrobial resistance. J.Anim. Sci. 86 (E. Suppl.):E173–E187.

Helms, M., Vastrup, P., Gerner-Smidt, P. and Mølbak, K. (2003). Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. Br. Med. J. 326, 357–361.

Humphrey, T., Wray, C. and Wray, A. (2000). Public-health aspects of Salmonella infection. In: Wray, C., Wray, A. (Eds.), Salmonella in Domestic Animals. CABI Publishing, New York, 245–263.

Knox, E.G. (1989). Detection of clusters. In: P. Elliott, (ed.), Methodology of Enquiries into Disease Clustering. Small Area Health Statistics Unit, London. 17-20.

Kosmider, R., Kelly, L., Evans, S. and Gettinby, G. (2006). A statistical system for detecting *Salmonella* outbreaks in British livestock. Epid. Infect. 134, 952-960.

Kulldorff, M. and Nagarwalla, N. (1995). Spatial disease clusters: Detection and inference. Stat. Med. 14, 799-810.

Kulldorff, M. (1997). A spatial scan statistic. Comm. Statist. Theory Methods 26, 1481-1496.

Kulldorff, M., Athas, W., Feuer, E., Miller, B. and Key, C. (1998). Evaluating cluster alarms: a space-time scan statistic and brain cancer in Los Alamos. Am. J. Public Health 88, 1377-1380.

Lawson, A.B. and Kulldorff, M. (1999). A review of cluster detection methods. In: A.B. Lawson, A. Biggeri, D. Bohning, E. Lesaffre, J.F. Viel, and R. Bertollini (Eds.), Disease Mapping and Risk Assessment for Public Health, John Wiley & Sons Ltd, New York. pp 102, 103, 105, 109.

Losinger, W.C., Garber, L.P., Smith, M.A., Hurd, H.S., Biehl, L.G., Fedorka-Cray, P.J., Thomas, L.A. and Ferris, K. (1997). Management and nutritional factors associated with the detection of *Salmonella* sp. from cattle fecal specimens from feedlot operations in the United States. Prev. Vet. Med. 31, 231–244.

McLaren, I.M. and Wray, C. (1991). Epidemioiogy of *Salmonella typhimurium* infection in calves: Persistence of salmonellae on calf units. Vet. Rec. 129, 461-462.

Morisse, J.P. and Cotte, J.P. (1994). Evaluation of some risks factors in bovine salmonellosis. Vet. Res. 25, 185–191.

Nielsen, L.R., Schukken, Y.H., Grohn, Y.T. and Ersbøll, A.K. (2004). *Salmonella* Dublin infection in dairy cattle: risk factors for becoming a carrier. Prev. Vet. Med. 65, 47–62.

Nielsen, L.R., Warnick, L.D. and Greiner, M. (2007). Risk factors for changing test classification in the Danish surveillance program for *Salmonella* in dairy herds. J. Dairy Sci. 90, 2815–2825.

Papadopoulou, C. and Kidd, S. (2008). Trends in salmonellosis in cattle, sheep and pigs in Great Britain: 1995-2004. Gov. Vet. J. 18, 32-36.

Pullinger, G.D., Paulin, S.M., Charleston, B., Watson, P.R., Bowen, A.J., Dziva, F., Morgan, E., Villarreal-Ramos, B., Wallis, T.S. and Stevens, M.P. (2007). Systemic translocation of *Salmonella* enterica serovar Dublin in cattle occurs predominantly via efferent lymphatics in a cell-free niche and requires type III secretion system 1 (T3SS-1) but not T3SS-2. Infect. Immun. 75, 5191-5199.

Vaessen, M.A., Veling, J., Frankena, K., Graat, E.A. and Klunder, T. (1998). Risk factors for *Salmonella* Dublin infection on dairy farms. Vet. Q. 20, 97–99.

Van Schaik, G., Schukken, Y.H., Nielen, M., Dijkhuizen, A.A., Barkema, H.W. and Benedictus, G. (2002). Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. Prev. Vet. Med. 54, 279–289.

Veling, J., Van Zijderveld, F.G., Van Zijderveld-van Bemmel, A.M., Barkema, H.W. and Schukken, Y.H. (2000). Evaluation of three newly developed enzyme-linked immunosorbent assays and two agglutination tests for detecting *Salmonella* enterica subsp. enterica serovar Dublin infections in dairy cattle. J. Clin. Microbiol. 38, 4402–4407.

VLA - Veterinary Laboratories Agency, 2008. Salmonella in livestock production in GB 2007.

Ward, M.P. and Carpenter, T.E. (2000). Techniques for analysis of disease clustering in space and time in veterinary epidemiology. Prev. Vet. Med. 45, 257-284.

Wray, C. and Roeder, P.L. (1987). Effect of bovine virus diarrhoea-mucosal disease virus infection on *Salmonella* infection in calves. Res. Vet. Sci. 42, 213–218.

Wray, C., Wadsworth, Q.C., Richards, D.W. and Morgan, J.H. (1989). A three year study of *Salmonella Dublin* infection in a closed dairy herd. Vet. Rec. 124, 532-537.

Wray, C., Todd, N., McLaren, I.M. and Beedell, Y.E. (1991). The epidemiology of *Salmonella* in calves: the role of markets and vehicles. Epid. Infect. 107, 521–525.

Wray, C. and Davies, R.H. (2000). *Salmonella* infections in cattle. In: C. Wray and A. Wray (Eds.), Salmonella in Domestic Animals, CABI Publishing, New York. 169–190.

SPATIAL MODELLING

PREDICTIVE MODELLING OF THE WIND SPREAD OF *CULICOIDES* SPECIES - AN INNOVATIVE APPROACH WHICH MAY CONTRIBUTE TO IMPROVED VACCINATION SCHEMES

GUY HENDRICKX*, YVES VAN DER STEDE, ESTELLE MEROC, BENOIT DURAND AND ELS DUCHEYNE

SUMMARY

Culicoides biting midges can be passively dispersed over long distances by prevailing winds leading to rapid spread of the diseases that they carry. In this paper we describe, based on two previously developed descriptive models, the development of a third generation predictive wind model to predict the short distance local spread and medium distance wind spread of bluetongue. The BTV1 epidemic of 2008 in South-West France is used as an example. First the known BTV1 2008 outbreak was modelled, then the potential spread of an outbreak in Brittany and Normandy was simulated, and finally the further northward spread of BTV1 in 2009 was predicted taking into consideration the known vaccination coverage in France in May 2009. Given the information available in June 2009 and assuming that vaccination induced full immunity in herds, it was predicted that Benelux and Germany were not at risk from short and medium-range wind-induced spread of BTV1. Nevertheless it was also suggested to remain extremely vigilant regarding the possible long-range introduction of BTV1 positive animals (through transport) with the potential to cause new outbreaks in regions beyond the predicted risk-zone and/or where no vaccination was performed. This work was conducted as part of a study to assess the risk of wind driven northern spread of BTV1 in general and the introduction of BTV1 in Belgium in particular funded by CODA/CERVA/VAR (Brussels, Belgium).

INTRODUCTION

Culicoides biting midges can be passively dispersed over long distances by prevailing winds (Sellers, 1992; Braverman and Chechik, 1996) leading to rapid spread of the diseases that they carry. Sellers et al. (1985, 1978) associated *Culicoides* incursions and wind events qualitatively in Cyprus, Turkey and Portugal. Alba et al. (2004) investigated and confirmed the possibility of introduction of infected midges on the Balearic Islands from Sardinia during the 2000 outbreaks using a formal backward trajectory analysis. This approach was further extended and quantified in a first generation model by Ducheyne et al. (2007) for Greece and Bulgaria. They inferred movement patterns of midges indirectly from patterns of the spread of bluetongue outbreaks between farms and then matched these to concurrent wind patterns.

* Guy Hendrickx, Avia-GIS, Risschotlei 33, 2980 Zoersel Belgium, Email: ghendrickx@avia-gis.be

During the BTV8 epidemic of 2006 Hendrickx et al. (2008) developed a second-generation wind density model based on the previous work in Greece and Bulgaria. Six-hourly forward wind trajectories were computed at a pressure level of 850 hPa (typically 1450m altitude) for each infected farm as from the recorded onset of symptoms. The trajectories were filtered to remove wind events that do not contribute to possible spread of the vector. The suitable wind events were rastered and aggregated on a weekly basis to obtain weekly wind density maps. Next to this, cumulated wind density maps were calculated to assess the overall impact of wind dispersal of vectors. A strong positive correlation was established between wind density data and the horizontal asymmetrical spread pattern of the 2006 BTV8 epidemic in temperate Europe. It was shown that short, medium and long distance spread have a different impact on disease dispersal. Computed wind densities were linked to medium/long distance spread whilst short range spread was mainly driven by active *Culicoides* flight.

In this paper we describe the development of a third generation predictive wind model to predict the short distance local spread and medium distance wind spread of bluetongue, taking the BTV1 epidemic of 2008 in South-West France as an example. First the known BTV1 2008 outbreak was modelled, then the potential spread of an outbreak in Brittany and Normandy was simulated and finally the potential northward spread of BTV1 in 2009 was predicted taking into consideration the known vaccination coverage in France. This work was conducted as part of a study to assess the risk of wind driven northern spread of BTV1 in general and the introduction of BTV1 in Belgium in particular.

MATERIALS AND METHODS

The developed third generation predictive BTV spread model is described in detail in Ducheyne et al. (submitted), and summarised below.

Model input data include:

- Epidemiological data for the 2008 BTV1 epidemic in France which were provided by AFSSA at the municipality level and were used to derive the epidemiological curve.
- Denominator data for France which were provided by AFSSA at the municipality level: i.e. number of susceptible farms per municipality. Farm coordinates were modelled within Land Cover classes suitable for livestock (Corine Land Cover data, JRC, 2000).
- Percent vaccination coverage per department for 2009 which were provided by AFSSA.
- Entomological data on the flight behaviour of *Culicoides* which were obtained from two main sources: data collected between 150m and 100m altitude using an airborne trapping device (Goossens et al., submitted), and data collected at ground level with OVI traps and at 12m using a Rothamsted suction trap (Fassotte et al., 2008).
- Wind data which were obtained from the European Centre for Medium term Weather Forecast (ECMWF).

The model was defined in terms of a number of parameters:

- The weekly incidence was calculated from the epidemiological curve and fitted using a least square estimator to the Verhulst-Pearl growth function (Verhulst, 1845).
- As in Hendrickx et al. (2008) a distinction was made between local mainly midge-flight driven, and medium range mainly wind driven spread: 50% of the weekly incidence is observed within a range of 4km (local distance spread) and an additional 45% of the weekly incidence is observed within a range of 22km (medium distance spread). The remaining 5% longer distance spread cases are not accounted for.
- The slowing impact of slope on medium distance wind spread (Bishop et al., 2000, 2005; Ducheyne et al., 2007; Hendrickx et al., 2008) was taken into consideration by reducing the wind magnitude relative to the slope.
- Based on available entomological data on *Culicoides* flight the following wind parameters were included to restrict medium distance wind spread: (a) highest *Culicoides* activity during the period prior to sunset (Fassotte et al., 2008) and (b) highest density of airborne *Culicoides* at an altitude of 150m (Goossens et al., submitted).
- To identify the model seed cases, *i.e.* introduced cases starting the epidemic, first the different spatio-temporal clusters in the epidemic under study were identified using a retrospective space-time permutation model (S_1, S_2,… S_n). Based on this, the location and starting time of the seed cases in each of the spatio-temporal clusters (t_{S1}, t_{S2},… t_{Sn}) were determined.

The model is described in Fig. 1 and progresses through the following steps

- *M1*: Model cases in the first week starting with the seed cases in the first cluster ($t= t_{S1}=0$):
- Calculate farm infection probability for first week:
    - Local infection probability (LDS_{prob}) for all farms within 4km around each seed case.
    - Wind infection probability (WDS_{prob}) for all farms within 22km around each seed case including slowing effect slope where applicable.
- Randomly select (weighted according to probability) infected farms according to fitted incidence first week:
    - 50% from farms with $LDS_{prob} > 0$ ($50\% \ INC_{t=tS1}$).
    - 45% from farms with $WDS_{prob} > 0$ ($45\% \ INC_{t=tS1}$).
- Add randomly selected infected farms to seed cases = *W1*.
- *M2*: Model cases next week starting with *W1*.
- *M2* is repeated for each subsequent week *t* according to INC_t until the starting date of the next cluster is reached. At that time the related seed cases are added to the modelled output of the previous week and *M2* is again repeated on a weekly basis until the next cluster starting date is reached or until the end of the epidemic.

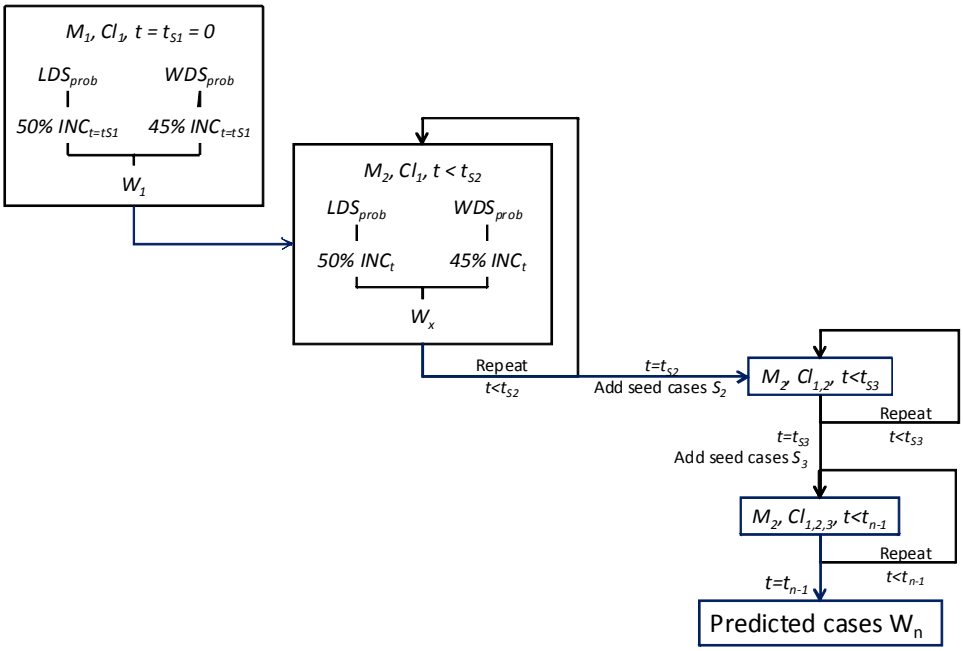


Fig. 1 Model flowchart

In this paper first the known BTV1 outbreak in Southern France was modelled, then the potential spread of an outbreak in Brittany and Normandy was simulated and finally the potential northwards spread of BTV1 in 2009 was predicted taking into consideration the known vaccination coverage in France.

RESULTS

Modelling the 2008 epidemic

South-West France

Three distinct spatio-temporal clusters were identified within the dataset (Fig. 2). The initial cluster (p=0.001) starting on July 17 and finishing on August 20, 2008 had a relative risk ratio (RR) of 5.02. The second cluster (p=0.001), which started on August 14 and ended on September 3, had a RR of 1.88. The final cluster commenced on September 11th and ended on December 3rd and had a RR of 1.73. While the epidemic curves in the first and second cluster follow a near-Gaussian distribution, the third cluster has a peak in the beginning of September.

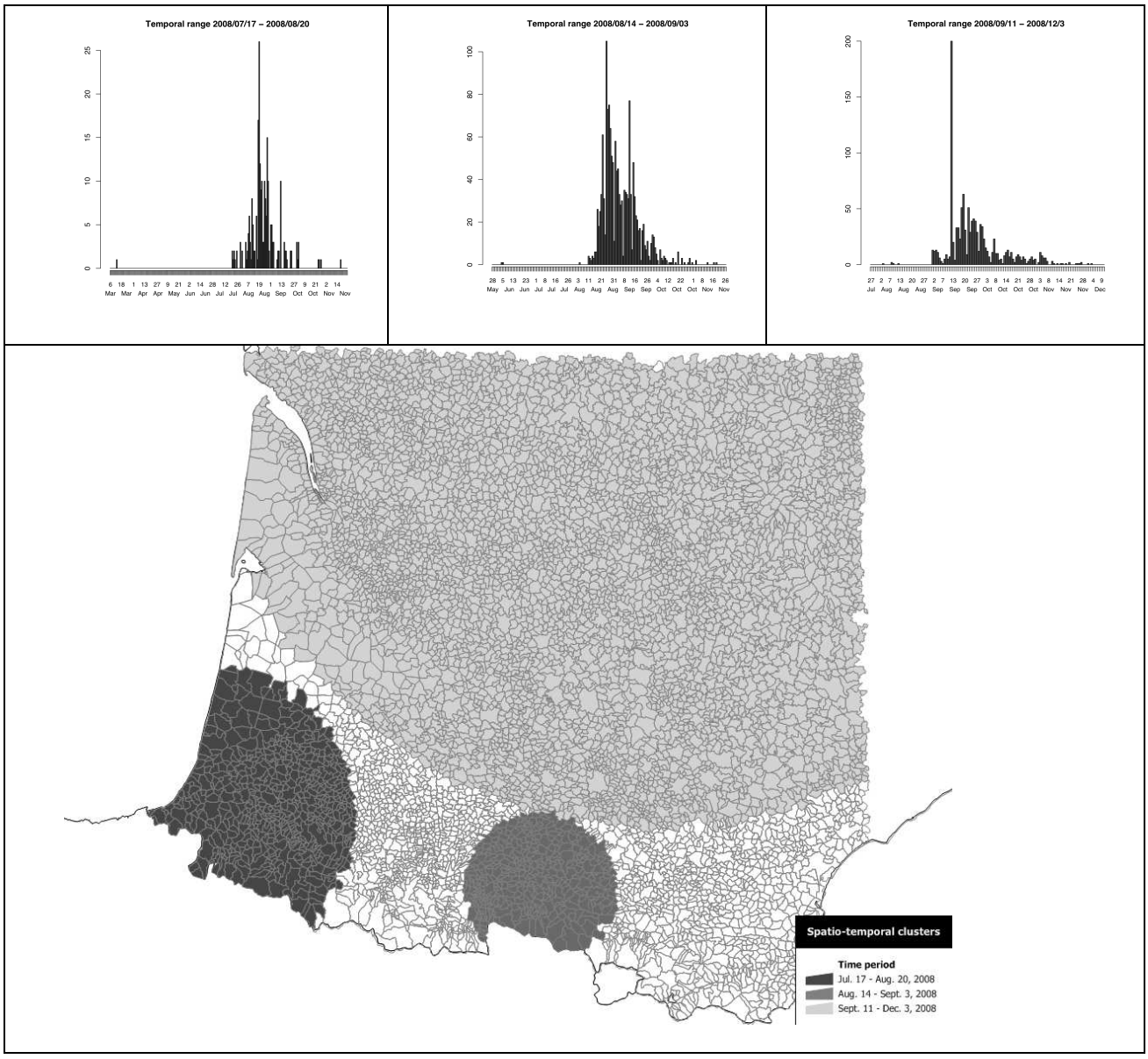
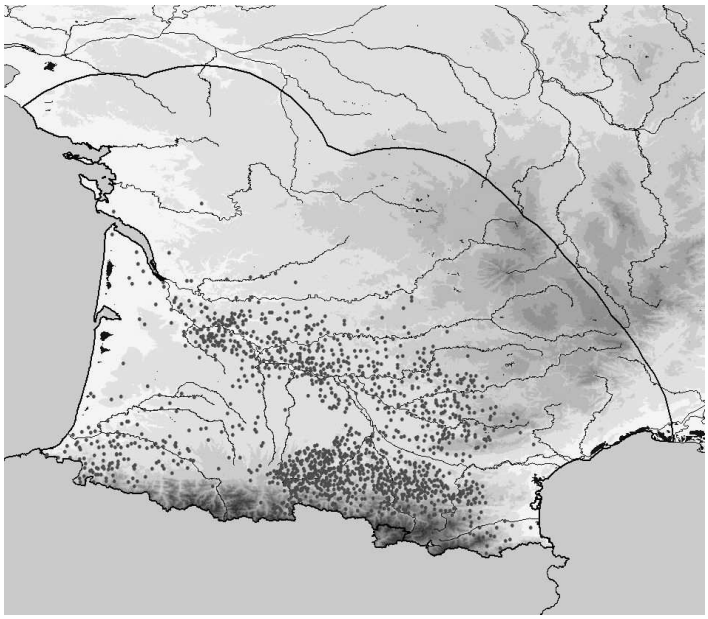
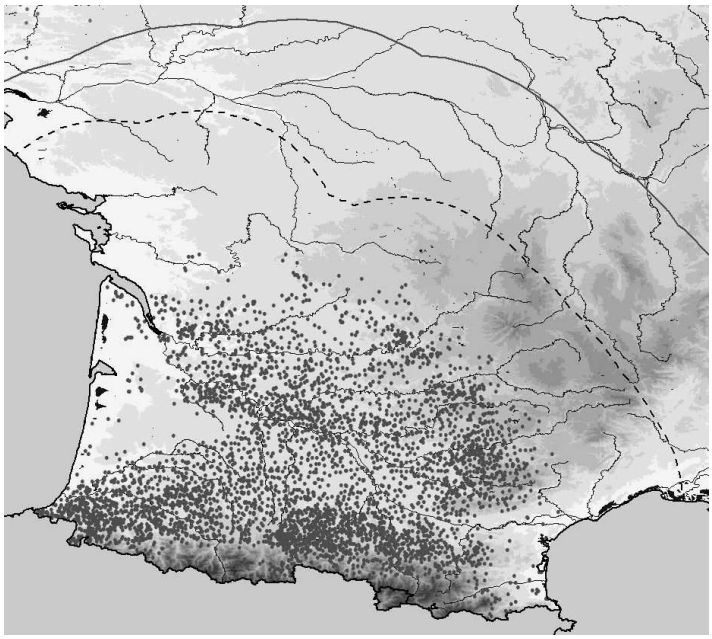


Fig. 2 Spatio-temporal clusters during 2008 BTV1 epidemic in France. In the top row the temporal histograms for each cluster are depicted, the map shows the spatial extent of the clusters.

The model output shows a good overall match with the observed spread (Fig. 3a). In the predicted pattern three high case density areas are observed (Fig. 3b): first in the initial zone of introduction, second along the Pyrenees, and third South of the Massif Central. Predicted case densities are higher than during the observed epidemic (Fig. 3a), mainly near the initial zone of introduction (counties of Haute-Pyrénées and Gers). In the Landes a large area with low case densities was observed. The spatial extent of the predicted epidemic is also slightly larger than the reported cases observed epidemic. This is reflected by the predicted surveillance zone extending about 100km more northwards (solid line as opposed to dotted black line in Fig. 3b).



(a)



(b)

Fig. 3 Observed (a) versus predicted (b) BTV1 epidemic in France in 2008. The dotted line shows the actual restriction zone limit; the solid line indicates the predicted limit.

Brittany and Normandy

In Brittany, three cases were observed during the autumn of 2008. Because they arose later in the year, *i.e.* toward the end of the *Culicoides* activity season, these presumably did not give rise to a recorded spread. Nevertheless non-reported other clinical cases have been observed in the same area (Lancelot, personal communication). To simulate the potential spread given that the initial case had occurred at the beginning of the 'bluetongue season', the model was run from the last week of July. Results show (Fig. 4a) that in this case the entire peninsula of Bretagne would have been covered. To assess the risk of spread to Belgium, the simulation was also repeated in Normandy starting with an arbitrary selected seed case near the border with Brittany. Obtained results show (Fig. 4b) a predicted spread mainly along the coast, consistent with the dominant wind patterns. Importantly the predicted restriction zone also covers part of Belgium, thus suggesting that Belgian farms near the French border would then be at risk.

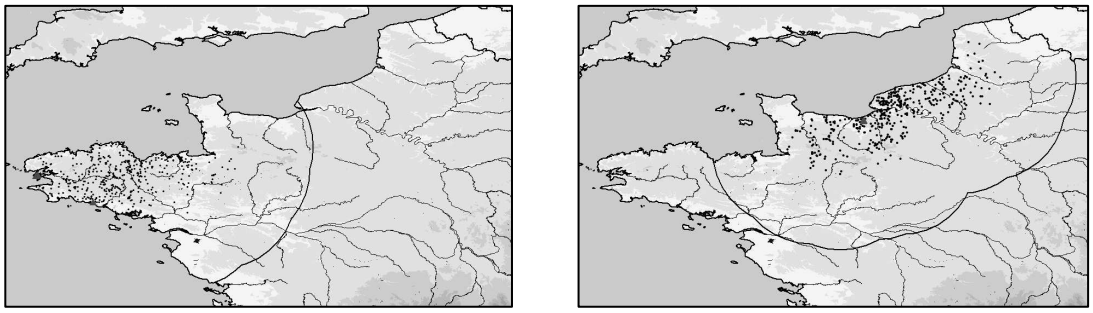


Fig. 4 Simulated BTV1 outbreaks in 2008 in Brittany (a) and Normandy (b).

Predicting the spread of BTV1 in 2009

The predicted spatial extent of a possible 2009 epidemic (August-November), taking into consideration the vaccination status in France as was known in May 2009, and using as seed cases 8% of the positive herds reported at the end of the 2008 epidemic, is shown in Fig. 5. In May 2009 a maximum of 2500 cases were predicted. These cases were anticipated to cause a wind-blown extension of maximum 300km northwards of the total area covered by BTV1 in France in 2008, suggesting Belgium would not be directly at risk of wind-blown introduction of BTV1.

DISCUSSION

This model first attempts the prediction of the further spread of bluetongue during the next 'bluetongue season'. Other models such as the atmospheric dispersion models by Gloster et al. (2007) are used to analyse in retrospect the possible introduction of the disease within a previously disease-free region. However, these are not used operationally for modelling the spread of disease after the initial introduction.

Whilst further investigation of the various factors (epidemiological parameters, entomological parameters) which may affect the quality of the model outputs is ongoing, it may at this stage be concluded that the current model predictions are a major step forward as compared to the previously proposed first generation and second generation model (Ducheyne et al., 2007; Hendrickx et al., 2008). A Health Department having this tool available at the start of

the 2008 epidemic would have been capable of predicting the spatial extent of the epidemic and would have been able to use it for planning and testing emergency vaccination strategies to prevent disease spread optimally. Further scenario-testing is still part of ongoing research.

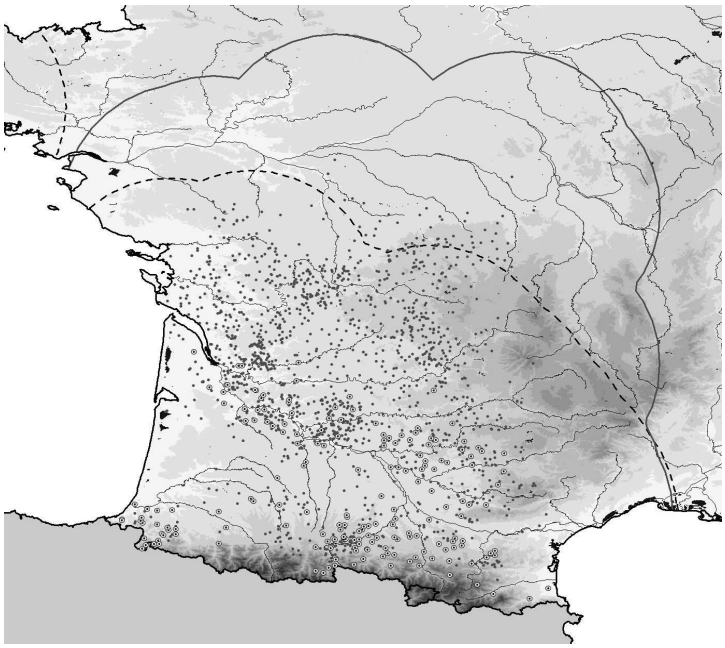


Fig. 5 Predicted extension of the infected BTV1 zone in France in 2009. Circled dots are selected seed cases (relicts from 2008).

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In the first simulation exercise in Brittany, we simulated a scenario where the disease started in July instead of in late autumn. It can be seen from the obtained output that under these conditions a uniform spread across the peninsula can be expected. The developed Normandy scenario also clearly shows that starting from this region and given prevailing wind patterns, the Benelux is at risk.

In all cases it is important to note that dissemination of cases over longer distances, possibly initiated by the transport of infected animals, is not included in the proposed model. Therefore it remains essential that, next to the decision whether or not to vaccinate a certain region or country given the model results, imported animals are under strict control.

Since this is ongoing research, comparing the predicted further spread of BTV1 in France with observed data on the field is still ongoing. At this stage of our analysis it seems that the model is overestimating the actual number of cases in 2009. This may be due to a variety of reasons the most important being that the actual achieved vaccination coverage was higher than as reported in May 2009. It seems that the achieved levels of vaccination may have prevented the build up of an epidemic starting from residual cases from 2008. This may be consistent with an earlier analysis of the effect of vaccination in the BTV8 epidemic (Ducheyne, personal communication) indicated that in order to significantly reduce the geographical extent of bluetongue the level of vaccination should be at least 80%.

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REFERENCES

Alba, A., Casal, J. and Domingo, M. (2004). Possible introduction of bluetongue into the Balearic Islands, Spain, in 2000, via air streams. Vet. Rec. 155, 460-461.

Bishop, A. L., Barchia, I. M. and Spohr, L. J. (2000). Models for the dispersal in Australia of the arbovirus vector, *Culicoides brevitarsis* Kieffer (*Diptera: Ceratopogonidae).* Prev. Vet. Med. 47, 243-254 .

Bishop, A. L., Spohr, L. J. and Barchia, I. M. (2005). Effects of altitude, distance and waves of movement of *Culicoides brevitarsis* Kieffer (*Diptera: Cerotopogonidae).* Prev. Vet. Med. 65, 135-145.

Braverman, Y. and Chechik, F. (1996). Air streams and the introduction of animal diseases borne on *Culicoides* (*Diptera, Ceratopogonidae*) into Israel. Rev. Sci. Techn. OIE 15, 1037-1052 .

Ducheyne, E., De Clercq, E. M., Goossens, E. and Hendrickx G., (submitted). A stochastic predictive model to predict neighbourhood and wind spread of BTV1 from Southern France Vet. Res.

Ducheyne, E., De Deken, R., Becu, S., Codina, B., Nomikou, K., Mangana-Vougiaki, O., Georgiev, G., Purse, B.V. and Hendrickx, G. (2007). Quantifying the wind dispersal of Culicoides species in Greece and Bulgaria. Geospatial Health 2, 177-189.

Fassotte, C., Delécolle, J.C., Cors, R., Defrance, T., De Deken, R., Haubruge, E., Losson, B., 2008, Culicoides trapping with Rothamsted suction traps before and during the bluetongue epidemic of 2006 in Belgium. Prev. Vet. Med 87, 74-83.

Gloster, J., Mellor, P. S., Manning, A. J., Webster, H. N. and Hort, M. C. (2007). Assessing the risk of windborne spread of bluetongue in the 2006 outbreak of disease in northern Europe. Vet. Rec. 160, 54-56.

Gloster, J., Mellor, P.S., Burgin, L. Sanders, C. and Carpenter, S. (2006). Will bluetongue come on the wind to the United Kingdom in 2007? Vet. Rec. 160, 422-4261

Goossens, E.M.C., De Clercq, E.M., Verstappen, N., Fassotte, C. and Hendrickx, G. (submitted) A novel tool and strategy to sample insect populations in the lower troposphere. Vet. Res.

Hendrickx, G., Gilbert, M., Staubach, C., Elbers, A., Mintiens, K. and Ducheyne, E. (2008). A wind density model to quantify the airborne spread of *Culicoides* species. Prev. Vet. Med. 87, 162-18.

Joint Research Centre, (2000). Corine Land Cover classification.

Kulldorff, M., Heffernan, R, Hartman, J., Assunçao, R. and Mostashari, F. (2005). A space-time permutation scan statistic for disease outbreak detection. PLoS Medicine, 2(3): e59.

Sellers R. F. (1992). Weather, *Culicoides*, and the distribution and spread of bluetongue and African horse sickness viruses. In Walton TE, Osburn BI, eds. 1992. Bluetongue, African Horse Sickness, and Related Orbiviruses. Boca Raton, FL: CRC. 1042 p.

Sellers, R.F. and Pedgley, D.E. (1985). Possible windborne spread to western Turkey of bluetongue virus in 1977 and of Akabane virus in 1979. J Hygiene (Lond) 95, 149-58.

Sellers, R.F., Pedgley, D.E. and Tucker, M.R. (1978). Possible windborne spread of bluetongue to Portugal, June-July 1956. J Hygiene (Lond) 81, 189-96.

Verhulst, P.-F. (1845). Recherches mathématiques sur la loi d'accroissement de la population. Nouv. mém. de l'Academie Royale des Sci. et Belles-Lettres de Bruxelles 18, 1-41.

DIFFERENT GEOGRAPHICAL PATTERNS OF ATYPICAL AND CLASSICAL SCRAPIE IN FRANCE

A. FEDIAEVSKY*, D. ABRIAL, D. CALAVAS AND C. DUCROT

SUMMARY

Atypical scrapie (AS) is a recently discovered form of transmissible spongiform encephalopathy of sheep and goats. It exhibits phenotypic and epidemiological differences from the classical form of scrapie (CS). Whether AS is contagious or not remains an open issue, whereas some arguments favour the hypothesis that it could occur spontaneously, without infectious exposure.

To address this issue the spatial distribution of the two diseases was studied, applying two different methods to the French active surveillance data from 2003 to 2007. First, the Kulldorff scan statistic was applied to detect low and high risk clusters. Second, smoothed relative risks of each disease were mapped according to Bayesian conditional autoregressive hierarchical models.

The two diseases differed by their spatial distribution patterns. The relative risk of AS was diffuse across the whole country except in one area with moderately increased risk. In contrast, several areas with high or low relative risk of CS were identified. The results do not indicate that the two diseases are spatially associated. Moreover, the relatively homogenous distribution of AS is not characteristic of a contagious disease.

INTRODUCTION

Scrapie is a transmissible spongiform encephalopathy (TSE) of sheep and goats that has been recognised since, at least, the eighteenth century (Comber & Morborne, 1772). Surveillance of transmissible spongiform encephalopathies (TSEs) in sheep and goats was intensified in the threat of BSE. This led to the discovery of atypical scrapie (AS) first in Norway, followed by many European countries (Benestad et al., 2008). The disease form showed incompatible diagnostic test results and occured in sheep genetically resistant to classical scrapie (CS) and unusually old animals. Only rarely AS has been described in goats therefore this study focused on scrapie in sheep.

In its classical form, scrapie is a contagious disease that transmits mostly from ewes to lambs (Konold et al., 2008; Lacroux et al., 2008; Race et al., 1998). This rare disease principally spreads between flocks through introduction of infected animals (Detwiler & Baylis, 2003). However, some feed contamination could have occurred before the meat and bone meal ban for

* Alexandre Fediaevsky, Direction Générale de l'Alimentation, Ministère de l'Agriculture, 251 rue de Vaugirard 75017, alexandre.fediaevsky@agriculture.gouv.fr

ruminant feed (Philippe et al., 2005) or possibly through feed recycling of milk products from infected ewes (Lacroux et al., 2008); transmission through a contaminated environment could also be possible (Georgsson et al., 2006).

In contrast to CS, some facts suggest that AS could have a non infectious origin (Benestad et al., 2008). AS is experimentally transmissible to sheep (Simmons et al., 2007) but there are doubts about whether AS is contagious in natural conditions (Benestad et al., 2008; Benestad et al., 2003; Fediaevsky et al., in press; Fediaevsky et al., 2009; Hopp et al., 2006; Simmons et al., 2009). From an epidemiological point of view this is particularly supported by rare additional cases in infected flocks (Fediaevsky, et al., in press, Lühken et al., 2007) and risk factor studies (Benestad et al., 2008; Benestad et al., 2003; Fediaevsky et al., in press; Fediaevsky et al., 2009; Hopp et al., 2006; Simmons et al., 2009).

Usually infectious diseases present heterogeneous distribution in time and space because the pattern of transmission of the disease depends on the relationship between animals which depends at least on flock structure, flock density and flock practices. On the other hand, the presence of a spatial pattern in the distribution of a disease does not necessarily imply an infectious process since other factors can cause heterogeneity such as demographic features (age, genetics, and surveillance pressure) or environmental risk factors.

Therefore the aim of this study was to test if AS cases were randomly distributed given the population at risk and to compare spatial distribution of AS and CS cases with the latter considered as reference disease.

MATERIALS AND METHODS

The study was based on the results of the TSE active surveillance in France for the period 2003 to 2007. The surveillance was based on requirements laid out by European Regulation 999/2001 (European Commission, 2001). Every year a sample of adult sheep (over 18 months old) slaughtered for human consumption (healthy slaughter: HS) or collected in farms as dead animals for rendering (fallen stock: FS) was randomly selected for investigation. Brainstem samples were screened for TSEs using rapid diagnostic tests. Not all used diagnostic tests were recommended for the detection of AS on brainstem samples, so the denominator for AS detection comprised only tests performed with recommended kits which represented 60% of the total tested population (European Food Safety Autority, 2005a, b; Fediaevsky et al., 2008). The denominator for CS detection consisted of all tested animals. Positive cases were confirmed at the national reference laboratory where the type of scrapie was determined using biomolecular techniques (Agence Française de Sécurité Sanitaire des Aliments, Lyon, France).

In addition to TSE screening, approximately 0.3% of the animals tested were randomly selected for genotyping at codons 136, 154 and 171. This was performed using a Taqman probe that did not allow for the distinction of ARQ and ARH alleles.

Animals from flocks where a case was identified were excluded from active surveillance. Only index cases were considered and animals from affected flocks were excluded from the analyses if tested after the detection of the index case. The animals were assigned to the centroid of their municipality of origin based on their identification number.

The spatial distribution of AS and CS were studied by two statistical approaches: the search for clusters through the Kulldorff scan statistic (Kulldorff, 1997) and the mapping of smoothed relative risks based on hierarchical Bayesian model.

For the Kulldorff scan statistic, the distribution of the number of cases in each municipality was assumed to be binomial. High and low risk clusters were searched and the maximum size of clusters was set to 10% of the population tested as larger clusters are assumed not meaningful for scrapie. Estimation of the likelihood of observed case numbers in each circle was based on 999 simulations of the null hypothesis of randomness and only clusters with a p-value lower than 5% were selected.

The hierarchical Bayesian model was conducted similarly to the method presented by Abrial about the distribution of BSE (Abrial et al., 2005). This technique provides smoothed relative risks by taking into account the spatial structure of the geographical units through a spatial prior named Conditional Auto Regressive (CAR) component based on Gaussian distributions (Clayton & Kaldor, 1987). The territory was divided into a grid of hexagonal cells of 40km wide that covered France. The size of the cells was a trade-off between minimizing the number of geographical units including no tested animal and the least spatial scale of possible disease spread.

The number of cases y_i in each geographical unit i, was assumed to follow a Poisson distribution with parameter λ_i (mean number of cases) defined as the product of the expected number of cases e_i and the local relative risk r_i. The relative risk r_i, represented the variation of the risk of disease in unit i compared to a baseline risk evaluated for France. The relative risk was composed of a spatial CAR prior u_i based on the spatial contiguities between geographical units which followed a normal distribution with parameters $\bar{u}_{\partial i}$, mean of the spatial components in the set of geographical units ∂i adjacent (neighbouring) to geographical unit i, and variance τ_{u_i}.

In order to take into account the parameters of the surveillance, the expected number of cases were standardised using the intra strata prevalence so that the total number of expected cases equals the total number of observed cases (Eq.(1)):

$$e_i = \sum_{year=2003}^{2007} \sum_{\substack{stream \\ \in\{HS,FS\}}} \sum_{dentition=1}^{3} \sum_{\substack{production \\ \in\{Dairy,Nondairy\}}} p_{year,stream,dentition,production} \cdot N_{year,stream,dentition,production} \quad (1)$$

Where $p_{year,stream,dentition,production}$ refers to the prevalence in and $N_{year,stream\ dentition\ production}$ to the number of animals tested in the strata defined by the year from 2003 to 2007, the stream of surveillance (HS and FS), the category of dentition (two to four definitive incisors, five to seven definitive incisors and eight definitive incisors) and the category of production (dairy production or not).

In a second step, the genetic structure of the tested population was introduced as an adjustment factor. The sample of animals genotyped during the same period (n=3,459) was used to estimate the proportions of animals carrying the alleles ARR, ARQ/H (noted ARQ hereafter), VRQ and AHQ for each geographical unit. For that, the proportions observed at the municipality level were interpolated on the grid of hexagons by kriging, using a spherical semi-variogramme

(Cressie, 1993). The terciles of the frequencies of each allele in each geographical unit were introduced in the model as linear predictors of λ_i (Lawson et al., 2003) as shown in Eq. (2)

$$\ln \lambda_i = \ln e_i + u_i + \beta_1 ARR_i + \beta_2 ARQ_i + \beta_3 VRQ_i + \beta_4 AHQ_i \quad (2)$$

The improvement in model fit by adjusting to these covariates was assessed by comparing the deviation information criteria (DIC) of the model with and without the variables and we kept the model with the lowest DIC (Besag et al., 1991).

Hierarchical models were fitted by Markov chain Monte Carlo method based on Gibbs sampling (Mollié, 1999); 400,000 iterations were run on three chains and the last 5,000 iterations were sampled to estimate the parameters of the model. The convergence was checked graphically and tested using the Heidelberger-Welch convergence diagnostic (Heidelberger & Welch, 1983).

The relative risks were displayed using chloropleth maps. Categories were defined by classes of same range. The units with 95% credible intervals not including one were outlined.

RESULTS

There were 359 cases of AS out of 648,879 tests and 257 cases of CS out of 1,108,817 tests (Table 1). AS and CS were not randomly distributed in space since significant clusters were detected with p-values lower than 0.01.

Table 1. Significant space-time clusters of AS and CS cases detected by active surveillance programme between 2003 and 2007 in mainland France.

Scrapie	Level	No cases observed	No cases expected	No tests
AS	Baseline	359		648879
	cluster 1	0	14.68	26729
	cluster 2	5	31.29	62302
	cluster 3	46	14.08	25485
CS	Baseline	257		1108817
	cluster 4	21	2.36	12574
	cluster 5	0	15.32	89909
	cluster 6	0	16.46	88406
	cluster 7	0	17.89	87378
	cluster 8	26	3.83	18373
	cluster 9	29	3.65	15746

There were two low risk and one high risk clusters for AS (Fig.1) and there were three low risk and three high risk clusters for CS (Fig.2).

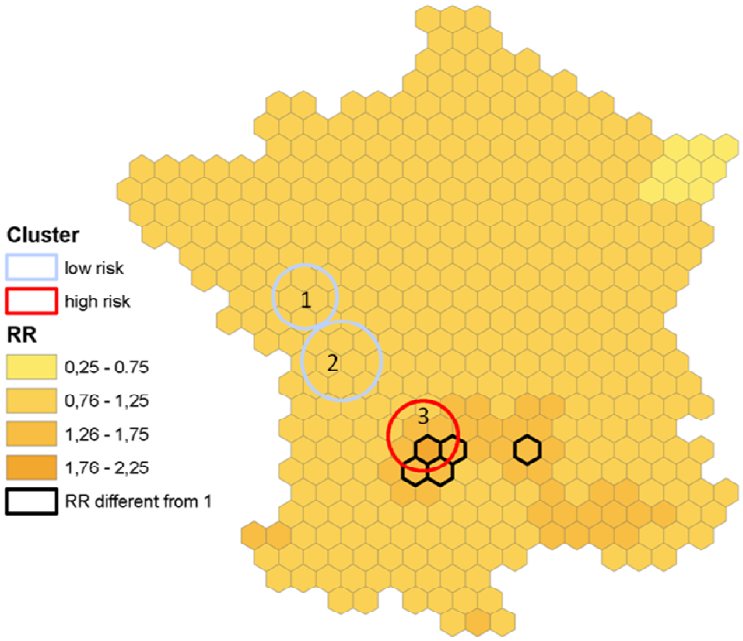

Fig. 1 Distribution of AS clusters and smoothed relative risks detected by active surveillance programme between 2003 and 2007 in mainland France.

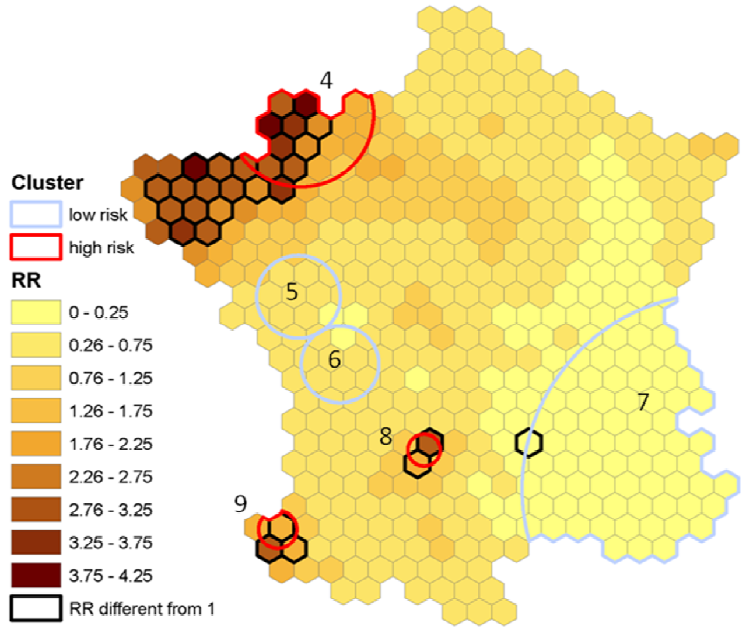

Fig. 2 Distribution of CS clusters and smoothed relative risks detected by active surveillance programme between 2003 and 2007 in mainland France.

The cluster 3 of AS was the only high risk cluster with a relative risk (RR) of 3.3. It was located besides the main dairy sheep production area in France. It overlapped a smaller cluster of CS (cluster 8) with a RR of 6.5. Cluster 9 was a small cluster of CS with a RR of 7.3 that was located in the Basque country, which is the second main dairy sheep production area in France. Cluster 4 of CS was located in the Northwest of the country and was associated with a RR of 10.5.

The low risk clusters (1, 2 for AS and 5, 6 for CS) overlapped and were located in the mid-west side of France, which is an area with intensive meat sheep production. The low risk cluster 7 of CS covered the large region of South East characterised by uphill extensive sheep grazing systems.

The Bayesian model for AS did not have a better fit after adjustment on the genetics, so the simplest model was selected. The RR of AS presented small variations only (Fig.1). The magnitude of variation of the relative risks was limited since the RR ranged from 0.25 to 0.75 in 9 cells, from 0.75 to 1.25 in 392 cells, from 1.25 to 1.75 in 39 cells and from 1.75 to 2.25 in only one cell. Only five cells had RR significantly higher than one (i.e. more than 95% of simulated values higher than 1). Overall the Bayesian model provided similar results to the cluster analysis.

For CS, the model with the genetic adjustment was selected since it had a better fit. In contrast with AS, CS presented important geographical variations throughout the country (Fig.2). There were several areas of high risk which were in similar locations to those detected in the cluster analysis. There was a large zone of low risk in South-East matching cluster 7 and two regions with moderately reduced risk that matched the low risk clusters 5 and 6. The RR varied from 0 to 0.25 in nine cells, from 0.25 to 0.75 in 222 cells, from 0.75 to 1.25 in 148 cells, from 1.25 to 1.75 in 22 cells, from 1.75 to 2.25 in 20 cells, from 2.25 to 2.75 in eight cells, from 2.75 to 3.25 in six cells, from 3.25 to 3.85 in six cells, from 3.85 to 4.25 in one cell. There were 13 cells that had RR significantly higher than one and 38 cells had a RR significantly lower than one.

DISCUSSION

Spatial analysis revealed differences in the distribution patterns of AS and CS. None of the diseases was randomly distributed and low and high risk clusters were detected for both. However, after standardisation on demographic factors, the spatial structure of the RR of AS was weak and only one larger zone appeared slightly more at risk than the rest of the country. The spatial heterogeneity was not explained by the variations of the proportions of the alleles defined on three codons.

The difference between the spatial distribution of the RR of AS and CS indicated that the two diseases might not have the same determinants. The smaller range of RR of AS compared with CS suggests that AS has weaker spatial determinants than CS and was more evenly distributed. This was also found by Green et al. (Green et al., 2007) in Great Britain and corresponds to prevalence levels found across European countries which were similar for AS but differed for CS (Fediaevsky et al., 2008).

The results from cluster analysis and Bayesian analysis were congruent even though they presented slight differences. These could be explained by standardisation on parameters influencing the results of the active surveillance only for the Bayesian analysis. In addition,

methodological particularities of the two approaches might also contribute to differences, the clusters associated to Kulldorff scan statistic are circular and have mobile centres while the hexagonal grid of the Bayesian analysis is static.

The most important source of spread of CS between flocks is usually considered to be animal trade (Detwiler & Baylis, 2003; Green et al., 2007). Purchase of infected animals between neighbouring flocks should be a major component of local increased risk, resulting in areas with high RR. For AS, trade networks and the purchase of animals have not been found to be risk factors (Fediaevsky et al., 2009; Green et al., 2007; Hopp et al., 2006). It would be surprising that the observed widespread distribution of AS in France would be due to natural transmission without considering that the disease transmits well. If the disease transmitted well, the prevalence could be expected to be higher or at least more heterogeneous as it is for CS.

A widespread infectious exposure to AS could be associated with feeding. Feeding proprietary concentrates was found to be a significant risk factor for CS in a case control study (Philippe et al., 2005). However since the feeding of proprietary concentrates contaminated by BSE agent was evidenced to be heterogeneous (Abrial et al., 2005), one could expect to find the same result for proprietary concentrates contaminated by scrapie agent. Thus, this type of exposure is not very likely to explain the pattern of AS. In addition, two case-control studies carried out on AS (Fediaevsky et al., 2009; Hopp et al., 2006) do not suggest feed contamination as risk factor for the disease. However it is interesting to notice that there was an increased risk of CS in regions where there has been an increased risk of BSE (Abrial et al., 2005). But this should be interpreted with caution since exposure definitively should have stopped in 2000.

Overall the results suggest a non infectious origin for AS. However we should remain prudent as some limited heterogeneity was found. This could be associated to limited contagiousness. Subtle infectious transmission would be difficult to evidence and it would require longer period of observation with more accurate data on the structure of the population. On the other hand this also could be associated to environmental effects or demographic structure of the population. Regarding the first hypothesis, two case control studies detected an association between AS and the feeding of vitamin and mineral intakes (Fediaevsky et al., 2009; Hopp et al., 2006). The demographic structure of the population, especially genetics and age, could influence the distribution of the RR of AS and CS. Although available data were used to account for these factors but might have been not sufficient particularly for AS where information on codon 141 was missing. That could explain why adjustment on genetics did not improve the model for AS whereas it was efficient for CS. In addition, the accuracy of the age categories estimated through the dentition is limited especially to distinguish categories among old animals. For both reasons, it is possible that a better adjustment of the spatial distribution of AS to age and genetics would reduce the spatial heterogeneity of AS. Geographical selection bias in the active surveillance programmes could also interfere, because submission of animals to rendering plant and to healthy slaughter was not homogenous (Cazeau et al., 2008; Fediaevsky et al., 2008). The possible effect of such bias was reduced by standardising on major parameters of the surveillance system.

In this study, CS presented important clusters in contrast to AS. The geographical distribution of AS suggested a non-contagious disease and the absence of a link between AS and CS. Some areas presented a moderately increased risk of AS and would be worth further investigation.

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REFERENCES

Abrial, D., Calavas, D., Jarrige, N. and Ducrot, C. (2005). Spatial heterogeneity of the risk of BSE in France following the ban of meat and bone meal in cattle feed. Prev. Vet. Med. 67, 69-82.

Benestad, S.L., Arsac, J.-N., Goldmann, W. and Nöremark, M. (2008). Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Vet. Res. 39, 19.

Benestad, S.L., Sarradin, P., Thu, B., Schönheit, J., Tranulis, M.A. and Bratberg, B. (2003). Cases of scrapie with unusual features in Norway and designation of a new type Nor98. The Vet. Rec. 153, 202-208.

Besag, J., York, J. and Mollié, A. (1991). Bayesian image restoration, with two applications in spatial statistics. Ann. I. of Stat. Math. 43(1), 1-59.

Cazeau, G., Fediaevsky, A. and Calavas, D. (2008). Prévalence des formes classique et atypique de tremblante chez les petits ruminants en France entre 2002 et 2006. Rev. Vet. Med-Toulouse 6, 348-356.

Clayton, D. and Kaldor, J. (1987). Empirical Bayes Estimates of Age-standardized Relative Risks for use in disease Mapping. Biometrics 43, 671-681.

Comber, T. and Morborne, H. (1772). A letter to Dr Hunter, physician in York, concerning the rickets in sheep. In Real Improvements in Agriculture, Letters to Reade Peacock. (London), pp. 73-83.

Cressie, N. (1993). Statistics for Spatial Data (Wiley Series in Probability and Statistics). Wiley-Interscience 928p

Detwiler, L.A. and Baylis, M. (2003). The epidemiology of scrapie. Rev. Sci Tech. OIE, 22, 121-143.

European Food Safety Autority (2005a). Scientific Report of the European Food Safety Authority on the Evaluation of Rapid post mortem TSE Tests intended for Small Ruminants. The EFSA Scientific Report 31, 1-17.

European Food Safety Autority (2005b). Scientific Report of the European Food Safety Authority on the Evaluation of Rapid post mortem TSE Tests intended for Small Ruminants (2). The EFSA Scientific Report 49, 1-46.

European Commission 2001. Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of

certain transmissible spongiform encephalopathies (Official Journal of European Communities), pp. 1-40.

Fediaevsky, A., Gasqui, P., Calavas, D., Ducrot, C. (in press). Discrepant epidemiological patterns between classical and atypical scrapie in sheep flocks under French TSE control measures. Vet. J.

Fediaevsky, A., Morignat, E., Ducrot, C. and Calavas, D. (2009). A case-control study on the origin of atypical scrapie in sheep, France. Emerg. Inf. Dis. 15, 710-718.

Fediaevsky, A., Tongue, S.C., Nöremark, M., Calavas, D., Ru, G. and Hopp, P. (2008). A descriptive study of the prevalence of atypical and classical scrapie in sheep in 20 European countries. BMC Vet. Res. 4, 19.

Georgsson, G., Sigurdarson, S. and Brown, P. (2006). Infectious agent of sheep scrapie may persist in the environment for at least 16 years. J. Gen. Virol. 87, 3737-3740.

Green, D.M., Del Rio Vilas, V.J., Birch, C.P., Johnson, J., Kiss, I.Z., McCarthy, N.D. and Kao, R.R. (2007). Demographic risk factors for classical and atypical scrapie in Great Britain. J. Gen. Virol. 88, 3486-3492.

Heidelberger, P. and Welch, P.D. (1983). Simulation run length control in the presence of an initial transient. Oper. Res. 31, 1109-1144.

Hopp, P., Omer, M.K. and Heier, B.T. (2006). A case-control study of scrapie Nor98 in Norwegian sheep flocks. J. Gen. Virol. 87, 3729-3736.

Konold, T., Moore, S.J., Bellworthy, S.J. and Simmons, H.A. (2008). Evidence of scrapie transmission via milk. BMC Vet. Res. 4, 14.

Kulldorff, M. (1997). A spatial scan statistic. Theory and Methods 26, 1481-1496.

Lacroux, C., Simon, S., Benestad, S.L., Maillet, S., Mathey, J., Lugan, S., Corbière, F., Cassard, H., Costes, P., Bergonier, D., Weisbecker, J.L., Moldal, T., Simmons, H., Lantier, F., Feraudet-Tarisse, C., Morel, N., Schelcher, F., Grassi, J. and Andréoletti, O. (2008). Prions in milk from ewes incubating natural scrapie. PLoS pathogens 4, e1000238.

Lawson, A.B., Browne, W.J. and Vidal Rodeiro, C.L. (2003). Disease mapping with WinBUGS and MLwiN. Wiley, Aberdeen, p.292.

Lühken, G., Buschmann, A., Brandt, H., Eiden, M., Groschup, M.H. and Erhardt, G. (2007). Epidemiological and genetical differences between classical and atypical scrapie cases. Vet. Res. 38, 65-80.

Mollié, A., 1999, Bayesian and Empirical Bayes Approaches to Disease Mapping, In: Lawson, A.B., Biggeri, A., Böhning, D., Lesaffre, E., Viel, J.-F. and Bertollini, R. (Eds.) Disease Mapping and Risk Assessment for Public Health. John Wiley & Sons Ltd, pp. 15 -29.

Philippe, S., Ducrot, C., Roy, P., Remontet, L., Jarrige, N. and Calavas, D. (2005). Sheep Feed and Scrapie, France. Emerg. Inf. Dis. 11, 1274-1279.

Race, R., Jenny, A. and Sutton, D. (1998). Scrapie Infectivity and Proteinase K-Resistant Prion Protein in Sheep Placenta, Brain, Spleen, and Lymph Node: Implications for Transmission and Antemortem Diagnosis. J. Inf. Dis. 178, 949-953.

Simmons, H.A., Simmons, M.M., Spencer, Y.I., Chaplin, M.J., Povey, G., Davis, A., Ortiz-Pelaez, A., Hunter, N., Matthews, D. and Wrathall, A.E. (2009). Atypical scrapie in sheep from a UK research flock which is free from classical scrapie. BMC Vet. Res. 5, 8.

Simmons, M.M., Konold, T., Simmons, H.A., Spencer, Y.I., Lockey, R., Spiropoulos, J., Everitt, S. and Clifford, D. (2007). Experimental transmission of atypical scrapie to sheep. BMC Vet. Res. 3, 20.

EPIDEMIOLOGICAL TOOLS

APPLICATION OF EPIDEMIOLOGICAL TOOLS TO THE INVESTIGATION OF A WILDLIFE DISEASE

J.A. DREWE*, K.T.D. EAMES, J.R. MADDEN AND G.P. PEARCE

SUMMARY

Wildlife diseases present particular challenges to the epidemiologist. Free-ranging animals are often difficult to locate, catch and sample, and repeated targeted sampling of the same individuals over time is rarely possible. In addition, interpreting the results of diagnostic tests in wildlife can be difficult due to lack of validated reference tests or knowledge of disease prevalence. This paper discusses some applications and limitations of three epidemiological tools for studying wildlife diseases: social network analysis, Bayesian statistics and dynamic network modelling, using a study of bovine tuberculosis (TB) in a population of 300 wild meerkats (*Suricata suricatta*) in the Kalahari Desert in South Africa as an example.

Social network analysis is a tool for examining contact patterns between animals and is particularly suited to the study of infectious disease transmission. Analysis of three types of interaction (aggression, foraging and grooming) revealed meerkat social structure to be stable over time. Clustering (a network measure of localised connectedness) was positively correlated with group size for both aggression (Pearson's correlation, $r = 0.73$, $p = 0.04$, $n = 8$ groups) and grooming interactions ($r = 0.71$, $p = 0.04$). This has important implications for the transmission of infectious disease within meerkat networks, suggesting that infections may spread locally within clusters of interacting individuals but be limited from infecting all members of large groups by an apparent threshold in connections between clusters.

A limitation of TB diagnosis in wildlife is low sensitivity of diagnostic tests in live animals. A Bayesian approach for estimating the accuracy of three TB tests (two serological tests and mycobacterial culture) in the absence of a gold standard was employed using data from 581 sampling events. Tests were individually of limited sensitivity or specificity, but interpreting the results from two tests in parallel achieved a diagnostic sensitivity of 83% (95% CI: 67-93%), high enough to inform the development of a transmission model. Empirical data were used to construct an individual-based weighted network model of TB transmission in a meerkat group. The results indicated that grooming (both giving and receiving) was more likely than aggression to be correlated with TB transmission and that the groomers were at higher risk than the groomed.

* Julian Drewe, Centre for Emerging, Endemic and Exotic Diseases, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA, UK. Email: jdrewe@rvc.ac.uk

INTRODUCTION

Infectious diseases in wild animals may pose a health risk to domestic animals, humans and wildlife as well as threatening global biodiversity (Daszak et al., 2000). Studying and managing diseases in wildlife presents particular challenges. Free-ranging animals are often difficult to locate, catch and sample, and repeated sampling of the same individuals over time is rarely possible. In addition, interpreting the results of diagnostic tests in wildlife can be difficult because both the true infection status of individuals and the prevalence of disease in the population are often unknown. These challenges notwithstanding, standard epidemiological tools may be used to gain insights into disease dynamics in wildlife populations. This paper discusses some applications and limitations of three epidemiological tools for studying wildlife disease using a study of TB in free-ranging wild meerkats in South Africa as an example.

Globally, many wildlife species are infected with *Mycobacterium bovis* and some such as Eurasian badgers (*Meles meles*) are implicated in its transmission to cattle. Control of TB in domestic animals is constrained by the lack of a dependable science base addressing *M. bovis* transmission in both livestock and wildlife (Cross et al., 2004; Defra 2007; Gilbert et al., 2005). The social structure of animal populations considerably influences the transmission dynamics and persistence of infectious diseases, yet the impact of host behaviour on the transmission of infectious diseases is rarely quantified. Consequently, disease transmission models are often limited to a theoretical exploration of the influence of host ecology on disease transmission (e.g., Keeling 2005; Lloyd-Smith *et al.* 2005). This is particularly true for wildlife species due to inherent difficulties with collecting empirical data (e.g., Böhm et al., 2008; Cross et al., 2004). The role of social interaction in infectious disease transmission is an area which is crucial to understand if effective management strategies for diseases such as TB in populations of wild animals are to be developed.

It is often assumed that all animals within a population are equally susceptible and infectious for diseases. However, studies of human diseases show that the distribution of the number of infections caused by an individual is strongly skewed, whereby most individuals do not infect anyone, whilst a few infect many (Anderson & May 1991). Such heterogeneities are also likely to apply to wildlife populations (Cross et al., 2007) and thus need to be considered in any epidemiological analysis. Social network analysis – the study of interactions between individuals or groups within a population – is an emerging tool that currently is underused to aid our understanding of animal sociality and disease transmission (Krause et al., 2007). Network analysis focuses on the relationships amongst animals rather than on their attributes (Wasserman & Faust 1994) and in doing so avoids the erroneous but common assumption of homogenous mixing of individuals. Further, multiple interactions between animals may be studied concurrently and across time. Importantly, the influence of host contact patterns on infectious disease transmission may be examined using a network approach, for example TB in brushtail possums (*Trichosurus vulpecula*; Corner et al., 2003), trypanosomes in bumblebees (*Bombus impatiens*; Otterstatter & Thomson 2007) and devil facial tumour disease in Tasmanian devils (*Sarcophilus harrisii*; Hamede et al., 2009).

Meerkats are small (< 1 kg) mongooses from the semi-arid regions of southern Africa. They live in groups of up to 40 individuals consisting of a dominant female and male and a variable number of subordinates of both sexes that aid in rearing the young (Clutton-Brock et al., 1999). Interactions between individuals may be antagonistic, such as aggressive dominance assertions (Kutsukake & Clutton-Brock 2006a), or placatory, such as grooming (Kutsukake & Clutton-Brock 2006b). A population of wild meerkats living in southern Kalahari Desert has been the

focus of detailed behavioural ecology studies since 1993. Tuberculosis due to infection with a member of the animal-adapted lineage of the *Mycobacterium tuberculosis* complex (probably *M. bovis*) is endemic within this study population (Drewe et al., 2009). Habituation of these meerkats to the presence of humans offers a unique opportunity to apply a range of epidemiological tools to the study of TB transmission in a wild animal population. The results aid our understanding of infectious disease epidemiology in other species of wildlife, domestic animals and humans.

MATERIALS AND METHODS

Study site and population

Data and samples were collected at the Kalahari Meerkat Project in the Northern Cape, South Africa (26°58' S, 21°49' E). A free-ranging population of 300 meerkats in 14 groups has been habituated to close (<1m) observation by researchers allowing individual identification via small marks of hair dye, confirmed when necessary by scanning of subcutaneous microchips. Further details of the study site and population are given in Clutton-Brock et al. (1999).

Behavioural data collection

Detailed behavioural observations of 134 meerkats in eight social groups were made over 24 months from January 2006 to December 2007. Group size ranged from 10 to 32 individuals (mean = 17). Interactions between meerkats were recorded *ad libitum* by a single observer as part of an established data collection protocol (Clutton-Brock et al., 1999). Data were collected on three distinct forms of behaviour: aggressive interactions such as biting, wrestling and hitting; foraging competitions which included squabbles over prey; and grooming interactions between two or more meerkats. The identities of the initiator(s) and receiver(s) were recorded in all cases. For further details of interaction definitions, see Madden et al. (2009). Each group was visited on at least four days each week, with observation periods lasting for at least three hours in the morning after the meerkats emerged from their burrows, and for at least one hour before they re-entered their burrow in the evening. To account for a slightly unequal number of visits to each group (the least visited group received 80% of the number of visits of the most visited group), data were standardised by multiplying with a correction factor (the number of half-days in the study period divided by actual number of half-day visits made to the group) to ensure that comparisons between meerkat groups were based on similar amounts of observation time.

Biological sample collection

Two hundred and forty meerkats were each sampled for evidence of *M. bovis* infection, up to eight times every three months between January 2006 and December 2007 (a total of 581 sampling events). Meerkats were caught by hand and anaesthetised with isoflurane (Isofor; Safe Line Pharmaceuticals, Johannesburg, South Africa) administered by face mask. Blood was collected and subjected to two serological tests to detect presence of mycobacterial antibodies: a lateral flow immunoassay and a multiple antigen print immunoassay (see Drewe et al., 2009 for details of these tests). A tracheal wash was performed and a submandibular lymph node aspirate was collected from each meerkat for mycobacterial culture. Following anaesthesia, meerkats were placed on a layer of sand in a dark plastic box to recover, after which they were immediately returned to their group by releasing them in close vicinity and sight of other group members. Mean (sd) duration of anaesthesia from induction to full recovery was 14.5 (3.5) minutes (range: 6 to 31 minutes, n = 570 sampling events where complete data were available).

Social network analysis

Relationships between networks of the same type of interaction over different timescales were tested using a quadratic assignment procedure (QAP) in UCInet for Windows (Borgatti et al., 2002). QAP involved computation of Pearson's correlation coefficients for corresponding cells of two data matrices (each matrix coding for one network), followed by random permutation of one matrix and re-computation of Pearson's correlation coefficients. This was repeated 50,000 times in order to calculate the proportion of times that coefficients from randomly-arranged networks were larger or equal to the observed Pearson's coefficient, and thus generate an associated probability value that the relationship between the networks was due to chance. This analysis accounted for autocorrelation between data points in the network. Relationships between social network measures at the group level and meerkat group attributes were evaluated using Pearson's correlations calculated in SPSS version 15.0. Group-level network measures for each of the eight meerkat groups were generated from data collected at the same time point. For intra-group interactions, groups were considered independent of each other.

Bayesian analysis

The sensitivity and specificity of each of the three diagnostic tests were estimated in the absence of a gold standard using Bayesian statistics (Branscum et al., 2005). This model was previously described by Drewe et al. (2009). It allowed for dependency between the serologic tests but assumed these were conditionally independent of culture. Data from 110 meerkats were analysed using WinBUGS software (Lunn et al., 2000) to run a Markov chain Monte Carlo model with uniform (0, 1) priors on all parameters. Convergence was monitored using two over-dispersed chains, each initiated by random sampling from the prior for each unknown parameter. Estimates of sensitivity and specificity were generated from 20,000 posterior samples collected after thinning the chains every 50 iterations following a burn-in of 1,000 iterations. Convergence was assessed by visual checking of trace plots of both chains for each parameter.

Transmission model

An individual-level weighted network model was constructed using empirical data from one group of meerkats (n = 37 animals). The model was stochastic and dynamic with a time-step of three months. The study period was divided into eight three-month intervals in an effort to match network construction to the dynamics of the pathogen being studied and because networks were stable over this time frame (Table 1). Four types of contact network were included, all of which were weighted and directed: grooming outdegree (where a focal meerkat groomed another individual); grooming indegree (a focal meerkat was groomed by another); aggression outdegree (a focal meerkat was aggressive towards another); and aggression indegree (a focal meerkat was the subject of aggression from another).

For each of the eight time points, each type of interaction, *X*, was represented as a graph consisting of a set of nodes (individual meerkats) joined by edges (where contact occurred). For each graph, *G*, an adjacency matrix, X_G, was constructed whereby for each pair of meerkats *i* and *j*, the *ij*th entry of the matrix took the value of the number of interactions that occurred between meerkats *i* and *j*. Thus, x_{ij} was >0 where contact occurred and 0 if there was no interaction. For each relation, directionality of interaction was maintained such that each adjacency matrix was asymmetric about the diagonal, that is, x_{ij} was not necessarily equal to x_{ji}. The assumption was made that a meerkat could not infect itself, thus all diagonal values were zero, i.e. $x_{ii} = 0$.

A SEIR compartmental model was developed, where S = susceptible, E = exposed (meerkats infected with *M. bovis*), I = infectious (meerkats shedding *M. bovis*) and R = removed (due to death from TB or other causes). This model design was chosen in order to retain information about the latent period of *M. bovis* (the time interval between infection and onset of shedding of the pathogen). Maximum likelihood procedures were used to estimate the latent period using serologic data, and the infectious period using culture data. The rate at which meerkat *i* became infected is given by:

$$\beta_i = \Sigma_j I_j (T_{Gin} . x^{Gin}_{ij} + T_{Gout} . x^{Gout}_{ij} + T_{Ain} . x^{Ain}_{ij} + T_{Aout} . x^{Aout}_{ij}) \quad (1)$$

where I_j = 1 if meerkat *j* was infectious and 0 otherwise; Gin = grooming indegree; Gout = grooming outdegree; Ain = aggression indegree; Aout = aggression outdegree; and T = rate of transmission per interaction per time-step (rate varies with each type of interaction). The 'true' (i.e. measured) infection status of each meerkat was inputed at the start. The model was run with a range of transmission values, initially from 0 to 4 and subsequently up to 25, to estimate the relative importance of each of these four contact types in the transmission of *M. bovis*. The model was run 800 times for each combination of transmission values, a total of 500,000 iterations each time. Model output accuracy was calculated by comparing the number of meerkats allocated to each compartment of the model to the 'true' infection status of each meerkat at the end of each three-month period. The set of transmission values which produced the most accurate classification of individual meerkats over the entire two-year period provided an indication of the relative importance of initiating and receiving grooming and aggression in acquiring *M. bovis* infection within a social group of wild meerkats.

RESULTS

Social network analysis

Networks constructed from interactions between the same meerkats occurring over 1 day, 1 week, 1 month and 3 months were significantly correlated with each other across all four time intervals for all three social interactions (Table 1).

Table 1. Stability of meerkat social networks over time. Correlations between networks constructed from data collected over four different time intervals are shown, for three types of social interaction. For all correlations, $p < 0.001$, assessed from 50,000 matrix permutations.

		1 week	1 month	3 months
Aggression	1 day	0.81	0.69	0.74
	1 week		0.88	0.87
	1 month			0.93
Foraging competitions	1 day	0.48	0.34	0.58
	1 week		0.76	0.66
	1 month			0.67
Grooming	1 day	0.94	0.80	0.71
	1 week		0.92	0.79
	1 month			0.89

Network density (the proportion of potential edges (ties) between nodes that actually exist) was negatively correlated with size of meerkat group for all three types of interaction (Fig. 1) indicating that the larger the group, the smaller the chance of group members interacting with all others in the group. Clustering coefficients (a measure of the extent to which two neighbours of a focal animal are themselves neighbours) showed a positive correlation with group size in networks of both aggression (Pearson's correlation, $r = 0.73$, $p = 0.04$, $n = 8$ groups) and grooming interactions ($r = 0.71$, $p = 0.04$; Fig. 2). Thus, as groups got larger, group members tended to interact more in localised cliques rather than on a group-wide scale.

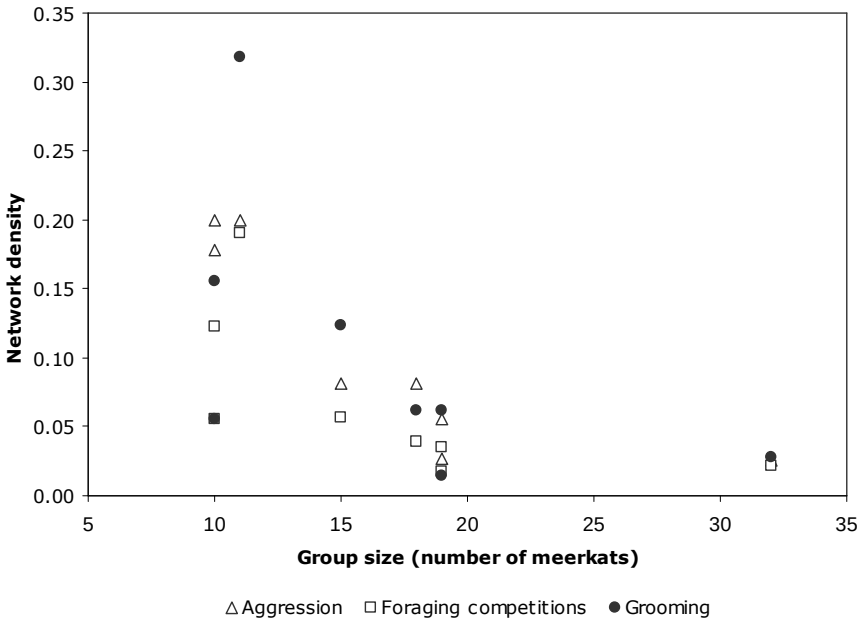

Fig. 1. Relationship between group size and network density for three different types of social interaction in eight meerkat groups.

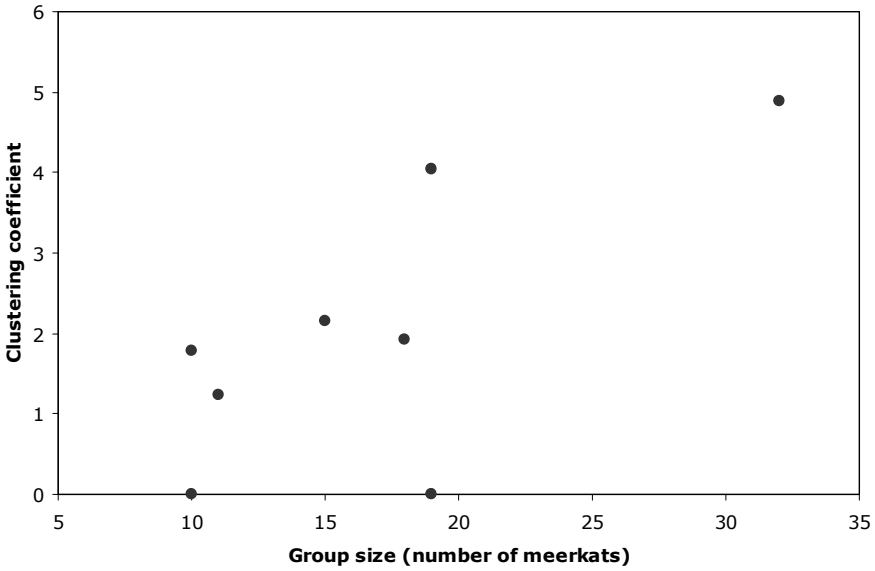

Fig. 2. Relationship between group size and clustering coefficient of grooming interactions in eight meerkat groups.

The tenure of the female dominant meerkat was positively correlated with both clustering coefficient (r = 0.77, p = 0.03) and network distance (a measure of the directness of interactions) (r = 0.69, p = 0.06) in networks of foraging competitions. This implies that the longer a female stays dominant, the more likely that other members of the group will form stable and localised cliques within which they compete against each other.

Diagnostic test performance

All three diagnostic tests for *M. bovis* infection were of limited sensitivity or specificity when used independently. By interpreting the results from the two serological tests in parallel (whereby both tests were run concurrently with a positive diagnosis requiring that only one test result be positive) serological test sensitivity was estimated at 83% (95% CI: 67-93%) and specificity was 0.73 (95% CI: 0.62, 0.82). These equated to a likelihood ratio of a positive test result of 3.1 (95% CI: 1.8, 5.0), and a likelihood ratio of a negative test result of 0.23 (95% CI: 0.09, 0.53) (data from Drewe et al., 2009). Whilst not perfect, this represented a good improvement over use of the tests independently, and it was considered high enough to inform the development of a simple model to examine TB transmission between wild meerkats.

Model output

The best fitting transmission parameters were 25 (T_{Gout}), 9 (T_{Gin}), 4 (T_{Aout}) and 1 (T_{Ain}). This suggests that a grooming encounter was associated with up to 25 times more risk of transmitting TB than was an aggressive encounter. Grooming outdegree (grooming of others) was the type of social contact most likely to be associated with transmission of *M. bovis*. Model performance was consistent when the model was run 100 times or more. The accuracy of the model in allocating individual meerkats to the correct infection status declined linearly with time until the last time point, when accuracy increased (Fig. 3). The best performing model had a mean accuracy across all time points of 58% (range: 46-67%).

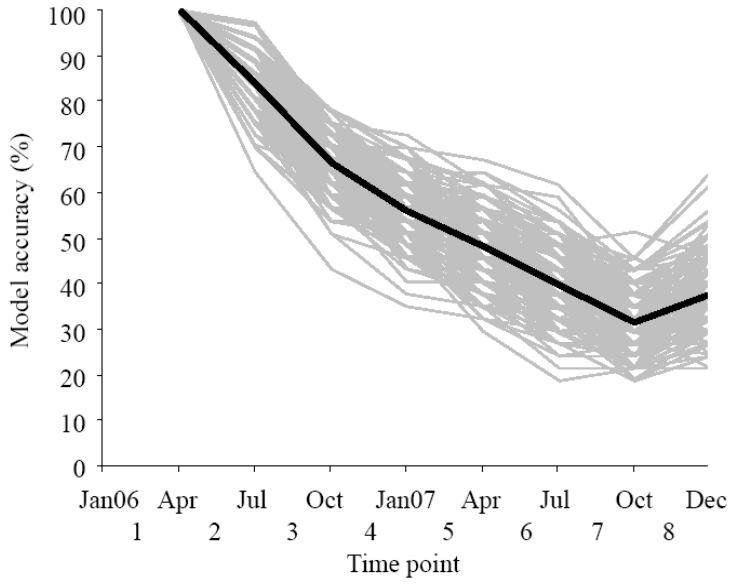

Fig. 3. Accuracy of the best-fitting individual-level dynamic network model. Each grey line signifies a single iteration of the model. The black line represents the mean model accuracy, based on comparison of the model output with the measured infection status of meerkats.

DISCUSSION

This paper illustrates how a suite of epidemiological tools may be applied to the study of a wildlife disease to provide answers to simple questions of disease transmission. Given the

unique challenges that arise when working with wildlife diseases, not all of these techniques will be suitable for every wildlife disease study. Some of the main benefits and limitations of the epidemiological tools used in the present study are summarised in Table 2.

Table 2. Some benefits and limitations of three epidemiological tools for the study of infectious diseases in free-ranging wild animal populations

Tool	Benefits	Limitations
Social network analysis	• Allows comparison of multiple interaction types and their influence on disease transmission. • Can formally compare networks over time. • The effect of intrinsic and external processes on network structure may be determined.	• Requires individual identification of animals (or groups) in order to make repeated observations. • Associations and contacts must be epidemiologically meaningful, and matched in timeframe to the disease being studied. • May be difficult to obtain sufficiently detailed contact data.
Bayesian statistics	• No prior knowledge of diagnostic test performance or disease prevalence is required. • Useful where no gold standard reference test exists. • Simple to perform.	• May require a large amount of empirical data. • Probability intervals may be wide and there may be a high degree of uncertainty in the system if no prior information is available.
Epidemiological modelling	• Provides insights into processes that may be difficult to observe or measure directly in free-living populations. • May indicate the relative effect of different intervention strategies on the transmission of disease. • Does not necessarily require detailed data on all individuals.	• There may be many unknown or immeasurable parameters in wildlife populations. • Requires detailed data on a representative sample of individuals. • Sensitivity analysis may be challenging to perform.

Implications of meerkat social structure for infectious disease transmission

Meerkat social structure was very stable over time, with networks constructed from data collected on one day being significantly correlated with networks containing data from the same groups collected over three months. This pattern held true for all three types of interaction studied, suggesting that data collected over short timeframes will allow valid extrapolation and conclusions to be drawn for intragroup interactions involving the same individuals over longer time periods. Further, predictions about TB transmission could be made from short-term sampling programmes. Stability was more pronounced in patterns of aggression and grooming than in foraging competitions, although all three interaction types were significantly correlated over all timeframes examined. Consistency was found in networks based on both unweighted

data (recording who interacts with whom) and weighted data (recording by how much individuals interact with others). However, stability in network structure depends on consistency in group composition such that networks can only be compared using this correlational method if the number and identity of individuals within a group remains constant. This is fairly straightforward for species that live in stable social groups such as meerkats and badgers. For species with unstable social structures, such as African buffalo (*Syncerus caffer*) which lives in a fission-fusion society (Cross et al., 2005), careful selection of study timeframes is crucial to ensure networks may be meaningfully compared.

For social animals living in tight-knit groups, an obvious question is why *don't* all group members become infected once an infectious disease enters a social group? Networks of interactions between meerkats became less dense as group size increased suggesting that individuals were limited in the number of interactions they could participate in. A concomitant increase in clustering coefficient implied that as group size increased, individuals became more likely to interact locally with a subset of others rather than trying to maintain group-wide interactions. Clustering has been shown to be the dominant effect controlling the growth rate of epidemics (Keeling 1999) with increased clustering reducing R_0, the basic reproductive ratio (Cross et al., 2007). This has important implications for the transmission of infectious diseases such as TB within meerkat networks; such diseases may spread locally within clusters of interacting individuals but be limited from infecting all members of large groups by an apparent threshold in connections between clusters.

Use of diagnostic tests in the absence of a gold standard in wildlife

A Bayesian statistical approach to estimating diagnostic test accuracy seems particularly suited to wildlife, where often no validated tests exist. A prior knowledge of the infection prevalence and likely test performance is not essential as uniform prior distributions (''flat'' probability distributions with equal probability assigned to a large range of parameter values) may be applied (Branscum et al., 2005). The longitudinal nature of the meerkat study with repeated sampling of the same individuals served to improve diagnostic accuracy on an individual level, especially in meerkats repeatedly testing negative (Drewe et al., 2009). Although the tests used in this study were individually of limited diagnostic use, interpreting the results of two tests in parallel produced estimates of sensitivity and specificity that were high enough to usefully inform decision making when determining exposure to TB in wild meerkats and potentially other species in which this infection poses a diagnostic challenge.

Network modelling as a tool to provide insights into TB transmission in wild animals

Network modelling of interactions between humans is well established (Read et al., 2008). Network-based models that incorporate heterogeneity of contact structure between animal groups or individuals are increasingly being used to understand disease transmission (Carrington et al., 2005). To date, animal models have principally focused on the role of farm-to-farm movements in the spread of disease between domestic livestock (e.g., Bigras-Poulin et al., 2006; Vernon & Keeling 2009). Few empirical network-based models of infectious disease transmission in wild animal populations exist. This is despite the huge threat that diseases in wildlife pose to humans, livestock, wildlife and global biodiversity (Daszak et al., 2000).

The results of the model developed in the present study suggest that a grooming encounter between meerkats carries more risk of transmitting *M. bovis* than does an aggressive encounter. Since grooming occurs more commonly than aggression and involves a higher proportion of meerkats (Madden et al., 2009), the importance of *M. bovis* transmission through grooming is

likely to be high in meerkats. Of the four social interactions studied, grooming outdegree (the process of grooming others) was markedly the most important. This supports the findings of Drewe (2010) who found the probability of testing TB positive in the same population of meerkats was positively correlated with grooming outdegree in the previous three months.

The best fitting model was 58% accurate across all time points. When the transmission parameters from the best fitting model were used to generate a new time series and the model fitted to this time series, accuracy rose slightly to 62%, indicating that the majority of the misfitting was due to stochasticity. This suggests that the model developed in the present study is good. The increase in model output accuracy during the last three months of the study period appears to be due to an actual increase in death due to causes other than TB in this period. Since fewer meerkats died in reality during the early part of the model (which assumed a constant risk of death) than would be expected due to chance, the model tended to 'overkill' meerkats during this period. When these meerkats did actually die, the model appeared to become more accurate simply because the classification of 'dead' for these meerkats was now correct. For this reason, the overall mean score was used to judge the accuracy of the model, rather than accuracy at any particular time point.

A limitation of the network model presented here is the assumption that a positive serology result indicates an animal is infected rather than infectious (for which mycobacterial culture was used). Whilst a positive serologic test result for *M. bovis* gives information on the presence of antibody and therefore indicates that an animal has been exposed, a combination of the late production of antibody and the intermittent excretion of *M. bovis* in infected badgers suggests that the main use of serology in this species is actually in the detection of animals likely to be shedding *M. bovis* and therefore infectious to others (Chambers et al., 2002). It is quite possible that a similar pattern occurs in meerkats but it is not possible to say this with confidence in the present study due to the lack of meerkats known to be in early stages of infection at time of sampling. A similar limitation may be present in many wildlife disease studies.

Intermittent excretion of *M. bovis* is a feature of TB in many species (Bengis 1998; Clifton-Hadley et al., 1993). Culture of tracheal washes collected from live animals is therefore likely to result in an underestimation of the infectious proportion of the population (Chambers et al., 2002). In the present study, meerkats were considered infectious for *M. bovis* from their first positive culture result onwards, even if subsequent cultures of samples from the same animals proved negative for *M. bovis*. Whist this is likely to have increased the chances of correctly classifying individuals in later stages of shedding as infectious, it will not have improved detection of animals in early stages of infectiousness. The application of other tests that may detect early stages of *M. bovis* infection, such as the gamma interferon test (Dalley et al., 2008), is one possible solution. Logistical limitations such as the remoteness of the study site precluded the use of this test in the present study.

CONCLUSION

In summary, this paper shows how a suite of epidemiological tools may be applied to the study of a wildlife disease to provide answers to simple questions of disease transmission. Imperfect diagnostic tests and the practical limitations of field research mean that understanding the epidemiology of infectious diseases in wildlife is likely to remain an ongoing challenge. However, a lack of knowledge of both disease prevalence and the true infection status of individuals need not preclude meaningful investigations of infectious diseases in wild animals.

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REFERENCES

Anderson, R.M. and May, R.M. (1991). Infectious Diseases of Humans: Dynamics and Control. Oxford University Press, Oxford, 757p.

Bengis, R.G. (1998). Tuberculosis in free-ranging mammals. In: Zoo & Wild Animal Medicine, 4th edition, Eds: M.E. Fowler and R.E. Miller, W.B. Saunders Company, Philadelphia. pp 101-114.

Bigras-Poulin, M., Thompson, R.A., Chriel, M., Mortensen, S. and Greiner, M. (2006). Network analysis of Danish cattle industry trade patterns as an evaluation of risk potential for disease spread. Prev. Vet. Med. 76, 11-39.

Böhm, M., Palphramand, K.L., Newton-Cross, G., Hutchings, M.R. and White, P.C.L. (2008). Dynamic interactions among badgers: implications for sociality and disease transmission. J. Anim. Ecol 77, 735-745.

Borgatti, S.P., Everett, M.G. and Freeman, L.C. (2002). Ucinet for Windows: Software for Social Network Analysis. Analytic Technologies, Harvard, MA.

Branscum, A.J., Gardner, I.A. and Johnson, W.O. (2005). Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. Prev. Vet. Med. 68, 145-163.

Carrington, P.J., Scott, J. and Wasserman, S. (2005). Models and Methods in Social Network Analysis, Cambridge University Press, Cambridge, 328p.

Chambers, M.A., Pressling, W.A., Cheeseman, C.L., Clifton-Hadley, R.S. and Hewinson, R.G. (2002). Value of existing serological tests for identifying badgers that shed *Mycobacterium bovis*. Vet. Microbiol. 86, 183-189.

Clifton-Hadley, R.S., Wilesmith, J.W. and Stuart, F.A. (1993). *Mycobacterium bovis* in the European badger (*Meles meles*): epidemiological findings in tuberculous badgers from a naturally infected population. Epidemiol. Infect. 111, 9-19.

Clutton-Brock, T.H., Maccoll, A., Chadwick, P., Gaynor, D., Kansky, R. and Skinner, J.D. (1999) Reproduction and survival of suricates (*Suricata suricatta*) in the southern Kalahari. African J. Ecol. 37, 69-80

Corner, L.A., Pfeiffer, D.U. and Morris, R.S. (2003). Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*). Prev. Vet. Med. 59, 147-167

Cross, P.C., Lloyd-Smith, J.O., Bowers, J.A., Hay, C.T., Hofmeyr, M. and Getz, W.M. (2004). Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park. Ann. Zool. Fenn. 41, 879-892.

Cross, P.C., Lloyd-Smith, J.O. and Getz, W.M. (2005). Disentangling association patterns in fission-fusion societies using African buffalo as an example. Anim. Behav. 69, 499-506.

Cross, P.C., Johnson, P.L.F., Lloyd-Smith, J.O. and Getz, W.M. (2007). Utility of R_0 as a predictor of disease invasion in structured populations. J. R. Soc. Interface 4, 315-324.

Dalley, D., Dave, D., Lesellier, S., Palmer, S., Crawshaw, T., Hewinson, G.H. and Chambers, M.A. (2008). Development and evaluation of a gamma interferon assay for tuberculosis in badgers (*Meles meles*). Tuberculosis 88, 235-243.

Daszak, P., Cunningham, A.A. and Hyatt, A.D. (2000). Emerging infectious diseases of wildlife: Threats to biodiversity and human health. Science 287, 443-449.

Defra (2007). Bovine TB: The Scientific Evidence. Final Report of the Independent Scientific Group on Cattle TB. Department for Environment, Food and Rural Affairs, London, 289p.

Drewe, J.A. (2010). Who infects whom? Social networks and tuberculosis transmission in wild meerkats. Proc. R. Soc. B. (Advance online publication, DOI: 10.1098/rspb.2009.1775).

Drewe, J.A., Dean, G.S., Michel, A.L., Lyashchenko, K.P, Greenwald, R. and Pearce, G.P. (2009). Accuracy of three diagnostic tests for determining *Mycobacterium bovis* infection status in live-sampled wild meerkats (*Suricata suricatta*). J. Vet. Diagn. Invest. 21, 31-39.

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Clifton-Hadley, R. and Wint, W. (2005). Cattle movements and bovine tuberculosis in Great Britain. Nature 435, 491-496.

Hamede, R.K., Bashford, J., M^{c}Callum, H. & Jones, M. (2009). Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. Ecol. Lett. 12, 1147–1157.

Keeling, M.J. (1999). The effects of local spatial structure on epidemiological invasions. Proc. Roy. Soc. Lond. B. 266, 859-869.

Keeling, M.J. (2005). The implications of network structure for epidemic dynamics. Theor. Popul. Biol. 67, 1-8.

Krause, J., Croft, D. and James, R. (2007). Social network theory in the behavioural sciences: potential applications. Behav. Ecol. Sociobiol. 62, 15-27.

Kutsukake, N. and Clutton-Brock, T.H. (2006a). Aggression and submission reflect reproductive conflict between females in cooperatively breeding meerkats *Suricata suricatta*. Behav. Ecol. Sociobiol. 59, 541-548.

Kutsukake, N. and Clutton-Brock, T.H. (2006b). Social functions of allogrooming in cooperatively breeding meerkats. Anim. Behav. 72, 1059-1068.

Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E. and Getz, W.M. (2005). Superspreading and the effect of individual variation on disease emergence. Nature 438, 355-359.

Lunn, D.J., Thomas, A., Best, N. and Spiegelhalter, D. (2000) WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. Stat. Comput. 10, 325-337.

Madden, J.R., Drewe, J.A., Pearce, G.P. and Clutton-Brock, T.H. (2009). The social network structure of a wild meerkat population: 2. Intra-group interactions. Behav. Ecol. Sociobiol. 64, 81-95.

Otterstatter, M.C. and Thomson, J.D. (2007). Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies. Oecologia 154, 411–421.

Read, J.M., Eames, K.T. and Edmunds, W.J. (2008). Dynamic social networks and the implications for the spread of infectious disease. J. R. Soc. Interface 5, 1001-1007.

Vernon, M.C. and Keeling, M.J. (2008). Representing the UK's cattle herd as static and dynamic networks. Proc. R. Soc. B. 276, 469-476.

Wasserman, S. and Faust, K. (1994). Social Network Analysis: Methods and Applications, Cambridge University Press, Cambridge, 818p.

USING BIOINFORMATICS TO IDENTIFY RISK FACTORS FOR COMPLEX DISEASES

F. I. LEWIS*, F. BRÜLISAUER AND G. GUNN

SUMMARY

A key issue in identifying risk factors for disease is in separating out those factors which are directly related to infection from those variables which, while correlated with infection, are not risk factors. Standard statistical approaches can identify factors correlated with infection but do not discriminate between correlation and direct dependence. An illustrative case study is presented which explores risk factors for exposure to bovine viral diarrhoea virus (BVDV) in cow-calf herds using Bayesian Network (BN) graphical modelling. Using a generalised linear modelling approach it was found, for example, that the presence of relief stockmen on farms with cow-calf herds is correlated with exposure to BVDV. Using a BN analyses, however, it is possible to objectively discard relief stockmen as a risk factor. The ability of BN modelling to visually summarise complex multivariate dependencies makes it a potentially powerful tool for epidemiological investigations.

INTRODUCTION

The identification of risk factors for disease is an essential part of many epidemiological studies. Typically, such studies seek to identify variables which are statistically significantly correlated with some response variable denoting the presence of, or exposure to, a pathogen. Linear statistical modelling (e.g. generalised linear models, generalised linear mixed models), is the most commonly used method for identifying correlates to disease. Recent examples of these types of studies are Namata et al. (2009); Assie et al. (2009); and Silverlas et al. (2009). In a risk factor analysis what are actually sought, however, are not simply correlates to disease, but rather those factors which directly influence disease.

To discriminate between correlation and direct dependence it is necessary to estimate the joint probability distribution of all variables of interest. A joint probability distribution can be described as a graph, specifically a Directed Acyclic Graph (DAG). A DAG is an intuitive visual representation of the dependency structure between random variables. Figure 1 shows a very simple DAG describing the joint probability distribution between two variables, X and Y. The arc from Y to X denotes that X is conditionally dependent upon Y, that is, the probability that X takes on any particular value is dependent upon the value of Y. An interpretation of Fig.1 is that the probability of disease X being diagnosed is dependent upon on the presence of symptom Y.

*Fraser Lewis, Scottish Agricultural College, King's Buildings, West Mains Road, Edinburgh, EH9 3JG. Email: fraser.lewis@sac.ac.uk

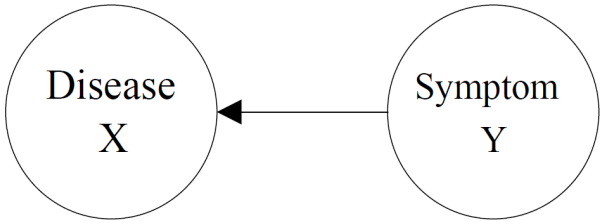

Fig.1 Simple Directed Acyclic Graph (DAG) between two random variables, X and Y

Bayesian Network (BN) modelling (Heckerman et al., 1995) is a machine learning technique designed to elucidate complex probabilistic relationships, and seeks to estimate the joint probability distribution of random variables. BNs are visually represented as DAGs. In recent years, BN modelling has been rapidly adopted across areas of biomedical science, in particular in the analyses of genetic data (e.g. Poon et al., 2007a; Poon et al., 2007b; Jansen et al., 2003). BNs can tease apart direct dependence from correlation, and therefore are a potentially powerful epidemiological tool.

To illustrate the application of BN modelling to veterinary epidemiology, a short overview of the steps required when fitting BN models to data is provided, along with a very brief case study which uses BN modelling to examine potential risk factors for bovine viral diarrhoea virus in cow-calf herds.

MATERIALS AND METHODS

A BN model comprises a DAG, e.g. a network structure, together with a set of parameters specific to that DAG. Fitting BN models to observed data can be split into three inter-related parts: i) parameter learning; ii) network scoring; and iii) structure learning. In i) the individual parameters in a BN are estimated conditional on a fixed network structure, which leads onto ii) where it is estimated how well the given network structure fits available data. In iii) the objective is to search for good fitting (high scoring) network structures amongst the vast number of networks possible.

Parameters in a BN

BN models estimate the joint probability distribution of random variables. As a simple example consider the two binary random variables, X and Y, from Fig.1 where each variable can take the value true (T) or false (F). The DAG says that X is conditionally dependent upon Y. The joint probability of X and Y both equalling T is $P(X=T,Y=T)=P(X=T|Y=T)P(Y=T)$, that is, the conditional probability of $X=T$ given that $Y=T$, multiplied by the probability that $Y=T$. Similarly, the joint probability of $X=T$ and $Y=F$ is $P(X=T,Y=F)=P(X=T|Y=F)P(Y=F)$. This gives a total of three free parameters which require to be estimated: i) $P(Y=T)$; ii) $P(X=T|Y=T)$; and iii) $P(X=T|Y=F)$. If either, or both, variables were instead multinomial rather than binomial then the number of parameters to be estimated increases in an obvious way.

A Bayesian model must have priors defined for each parameter, and the standard assumption in BN modelling is to use independent Beta priors for binomial data at each node, and equivalently independent Dirichlet priors for nodes with multinomial data. Importantly, these parameter priors are conjugate priors for binomial and multinomial data respectively, and hence allow for the posterior distribution to be calculated analytically.

Network Scoring

In most practical circumstances the network structure of a BN is not known a priori, and must be estimated from available observed data. For example, in Fig.1 it was assumed a priori that X and Y were conditionally dependent. Depending on the data available, however, statistical independence where $P(X=T,Y=T)=P(X=T)P(Y=T)$ may be more strongly supported, in which case there would be no arc connecting X and Y in Fig.1. If the network structure is not known then in order to choose between alternative competing networks it is necessary to estimate the goodness of fit of any given BN to available data. The goodness of fit of a BN is summarised by its network score, this is typically the marginal likelihood of the data given the model. Marginal likelihood is the standard Bayesian goodness of fit criterion and is equivalent to the Bayes factor when comparing models with equal priors (Congdon, 2001). Using standard assumptions it is possible to readily calculate the network score for any BN in closed form (see p.212 in Heckerman et al., 1995). In practice, it is usually the log network score which is used to avoid numerical accuracy issues.

There are various network scoring metrics available where these differ in the parameter priors used. The Bayesian Dirichlet equivalence (BDe) metric and the K2 metric are the two most commonly used (see Buntine, 1991, and Heckerman et al., 1995). In the BDe metric the prior for each node (variable) is defined in terms of an equivalent sample size, this is also referred to as the imaginary data base size. The equivalent sample size denotes the number of prior observations which are assumed to have been observed at each node, and the weight of these prior observations is distributed across the individual parameter priors at each node. In contrast to the BDe metric, the K2 metric takes a different approach. Rather than assume a prior equivalent sample size at each node, the K2 metric simply assumes all parameter priors at each node are uninformative, e.g. Beta(1,1) or in the multivariate case Dirichlet(1,…,1).

Structure Learning

Having briefly described the parameters in a BN model, and scoring metrics, a remaining aspect is how to search for optimal BN models given a set of data. Structure learning is computational extremely demanding; for example, for a data set of ten variables there are approximately 10^{18} different possible DAGs (Korb & Nicholson, 2004), far too many to exhaustively search over no matter what computing resources are available. The local search method suggested by Heckerman et al., (1995) is an established, simple and potentially highly effective method of finding optimal BN models. The local search method proceeds as follows: i) choose a random DAG, calculate its network score, and designate this as the current network; ii) iterate over all networks which are a single "perturbation" away from the current network, that is they differ by either the removal, addition or reversal of a single arc; iii) if one of the perturbed networks has a greater network score then replace the current network with this network and go to step ii), if no such network exists then the local search is terminated. This search process is repeated until it is not possible to improve the network score. Other structural learning approaches are possible, in particular the use of model averaging via Markov chain monte carlo methods (e.g. Madigan & York, 1995 and Friedman & Koller, 2003).

The final part of the local search process is summarising the results from many different local searches. While each local search may terminate at a different optimal BN, there is typically a great deal of commonality across these networks. One method of building a single "best" network from all locally optimal networks is to use the idea of a majority consensus network, this is where a single network is constructed comprising of all the arcs which appear in

at least a majority of all locally optimal networks. This is the approach used by Poon et al., 2007a and is borrowed from phylogenetics, where a similar method is used to construct evolutionary trees (e.g. the MrBayes software package, Huelsenbeck & Ronquist, 2001). In the subsequent case study this consensus idea is altered slightly. Instead of building a consensus network from all majority supported arcs, an alternative is to choose the locally optimal network with the highest network score, and then remove all the arcs in this network which have less than majority support across all other locally optimal networks. The latter approach is analogous to the idea of pruning in decision tree analysis (Helmbold & Schapire, 1997).

Software for fitting BNs

There are relatively few software packages available for fitting BNs to data. Two options are the deal library (Boettcher & Dethlefsen, 2003) for use within the R programming environment (R Development Core Team, 2006) and the free Bayes Net toolbox for Matlab written by Kevin Murphy (http://people.cs.ubc.ca/ murphyk/Software/BNT/bnt.html). There is also the commercial business decision support tool called Hugin (http://www.hugin.com) which is based around BN modelling. In the case study analyses later presented the model fitting was done using computer code written by the authors in C using the GNU Scientific Library (Galassi et al., 2006) and is freely available on request.

Case Study – Data

In Brülisauer et al. (2009) a prevalence study for exposure to bovine viral diarrhoea virus (BVDV) in Scottish cow-calf herds was presented. It was found that three distinct prevalence classes exist in the Scottish beef cattle population: herds with exposure-free young stock; herds with very high seroprevalence in young stock (≈96% exposed); and herds with intermediate levels of seroprevalence in young stock (≈32% exposed). Using the method in Brülisauer et al. it was straightforward to allocate each of the 301 study farms into one of the three prevalence classes using maximum likelihood. In the following modelling results the three classes are labelled: DF - disease/exposure free; PI - the high prevalence class is assumed related to the presence of a persistently infected (PI) animal; and Transient - the third class denoting intermediate seroprevalence respectively. The prevalence study also comprised a farm questionnaire detailing management practices and farm characteristics. This data is used to provide a brief, but realistic, illustrative example of how to apply BN models to epidemiological data.

RESULTS

Case Study - BN Results

Figure 2 presents a BN model for analysing potential risk factors for exposure to BVDV in cow-calf herds. In the case study data 33 variables were used. Rather than use a single multinomial variable denoting seroprevalence class, this was coded into three binary variables. Splitting multinomial variables into separate binary variables greatly aids visual interpretation, and adds flexibility since different arcs can now be attached to each different class. This can be seen in Fig.2; the seroprevalence class variables DF, Transient and PI all have different arcs connected to them, if this variable had been kept as a single multinomial variable (with three classes) then the network structure would have to be the same for each class. An important consequence, however, of this approach is that variables DF, Transient and PI are not

independent since if it is known that a farm is not DF or Transient then it is certain that it must be PI. Dependence will therefore exist between these variables and to prevent the structural search process from adding arcs between these variables, which is both computationally wasteful and not real in the sense that this dependence has been introduced as a by-product of allowing more flexibility into the model, the search process is prevented from considering arcs between these variables.

Two "diagnostic" variables were included in the analyses, denoted in Fig.2 by BVD and PI5. These are questionnaire responses from the farmer asking, firstly, if he or she were aware of a BVD problem in their herd, and secondly, whether they had had a PI animal in the herd in the last five years. These questions are effectively direct proxies for prevalence class, and there inclusion in the analyses is mainly as a common sense check that the modelling results are sensible. Both BVD and PI5 variables have three types of response: yes, no and don't know; and were split into separate binary variables with arcs banned between them.

Initially, 10,000 searches were conducted on the case study data. A summary network was produced using the results from all 10,000 searches, and then an additional 90,000 searches were run and the results compared. Using all 100,000 searches the summary network differed slightly from the same network found using 10,000 searches. A further 100,000 searches were run, giving a total of 200,000 search results in all. The summary network produced from 100,000 searches was identical to that from all 200,000 searches, and hence supports that our results from 100,000 searches are statistically robust.

While each search may terminate at a unique, locally optimal network, it is likely that these networks will share a great many structural features. Figure 2 shows a summary network over all 200,000 searches. This network was created by taking the single highest scoring network across all searches and trimming all arcs which did not appear in at least a majority (50%) of all other locally optimal networks. Using the BN in Fig.2 as our single best description of all the relationships within the data, the aspects of most interest are: i) which covariates are related to which other covariates; and ii) how strong are the associations between variables and are these positive or negative relationships.

A simple measure of association in a BN is to use probability ratios, for example, if $X \leftarrow Y$ and these are both binary variables then this can be defined as $P(X=T|Y=T)/P(X=T|Y=F)$. This ratio can be easily calculated from the BN model parameters, and using simulation it is straightforward to estimate 95% confidence intervals as the posterior distributions for each of $P(X=T|Y=T)$ and $P(X=T|Y=F)$ are known. The numerical values in Fig.2 are the mean probability ratios for each individual arc. For example, there is a positive association between farms which attend shows and those which sell animals for breeding, and the mean strength of this association, as measured by the probability ratio is 2.1. Hence, farms which attend shows are twice as likely to sell animals for breeding as farms that do not attend shows. The two diagnostic variables, BVD and PI5, both give intuitively reasonable results; farms with PI level seroprevalence in their young stock are positively associated with answering yes to having had a PI animal in the last five years. Similarly, farms with DF level seroprevalence in their young stock are positively associated with having answered no to a known BVD problem. A similar result is that those farms which said they didn't know whether they had had a BVD problem, also said that they didn't know whether they had had a PI animal in the last five years. Again, an intuitively reasonable result.

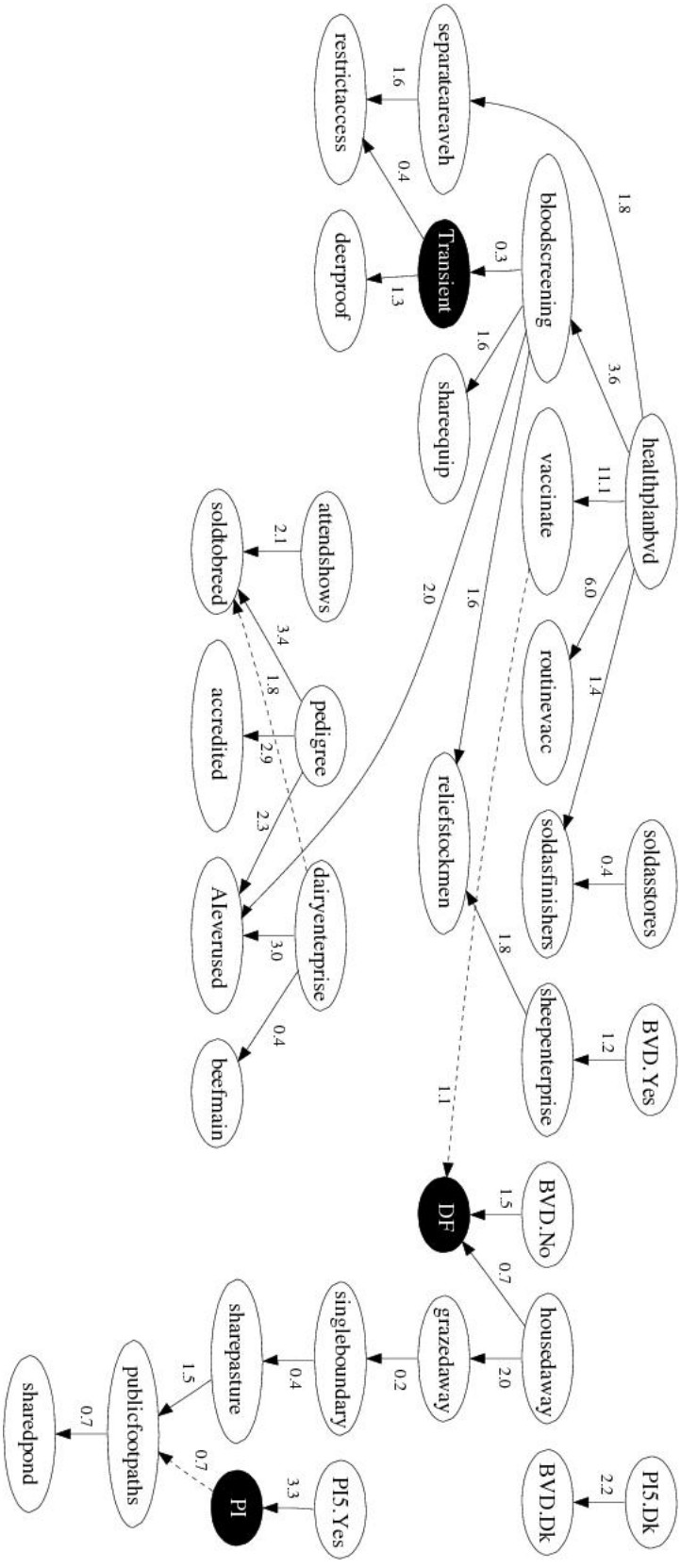

Fig.2 A BN model for analysing potential risk factors for exposure to bovine viral diarrhoea virus in cow-calf herds. Arcs connecting variables denotes conditional dependence between them. Mean probability ratios are shown for each individual arc. Arcs with dotted lines had ratios whose posterior density contained unity (between the 2.5% and 97.5% quantiles).

As a technical footnote, two of the arcs in the summary BN (Fig.2) had probability ratios whose 95% confidence interval included unity. This is not contradictory to using a majority consensus network to ensure that each arc is statistically robust. A 95% confidence interval for the (marginal) strength of association of each arc is simply a different criteria of robustness. From an interpretation point of view, it is obviously desirable if these two criteria strongly overlap, as is indeed the case in Fig.2.

Case Study - GLM Results

As a contrast to the BN approach, a typical generalised linear model (GLM), e.g. binomial response and logit link function, was fitted to the data. This was done separately for each binary "response" variable DF, PI and Transient. In the BN model, the variables directly related to any of the prevalence class variables were housedaway, restrictaccess, deerproof and bloodscreening. Using generalised linear models the same four variables were statistically significant (at at least 5% type one error) against at least one of the prevalence class response variables, however, also strongly statistically significant was reliefstockmen (p-value 0.014). Examining the BN model, this implies that while reliefstockmen may be correlated with Transient it is not directly related. This correlation of reliefstockmen can be explained away by its relationship to bloodscreening, where it is the latter that is directly related to Transient.

DISCUSSION

A brief overview of how to use BN models to explore epidemiological data has been presented. Arguably the single greatest practical strength of using BN modelling is its intuitive graphical nature, which allows ready interpretation of the possibly highly complex relationships with a given data set. This is a unique aspect of this powerful methodology. BNs can also be used for formal statistical inference as each structural feature has posterior parameter estimates available from which various measures of association can be calculated if required.

A drawback of BNs as opposed to less holistic approaches, such as GLMs, is that they require considerable computing resources. For example, the BN in Fig.2 is the culmination of almost 80 hours of computer time on a quad core (Intel Core2 Extreme CPU X9650 @ 3.00GHz) workstation. In contrast, even highly complex generalised linear mixed models can be fitted in seconds. These methods are, however, not competitors, but rather complementary. BNs are excellent at exploring the dependencies in multidimensional data, and the separation of correlation from direct dependence. Results from a BN analyses can then inform further analyses focused more specifically at individual response variables, for example by retaining only directly dependent explanatory variables.

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REFERENCES

Assie, S., Bareille, N., Beaudeau, F. and Seegers, H. (2009). Management- and housing-related risk factors of respiratory disorders in non-weaned french charolais calves. Prev. Vet. Med. 91, 218–225.

Boettcher, S. G. and Dethlefsen, C. (2003). deal: A package for learning bayesian networks. J. Stat. Softw. 8, 1–40.

Brülisauer, F., Lewis, F., Ganser, A., McKendrick, I. and Gunn, G. (2009). The prevalence of bovine viral diarrhoea virus infection in beef suckler herds in scotland. The Veterinary Journal doi:10.1016/j.tvjl.2009.08.011

Buntine, W. (1991). Theory refinement on bayesian networks. In: Proceedings of Seventh Conference on Uncertainty in Artificial Intelligence. Morgan Kaufmann, Los Angeles, CA, USA., pp. 52–60.

Congdon, P. (2001). Bayesian Statistical Modelling. Wiley.

Friedman, N. and Koller, D. (2003). Being bayesian about network structure. a bayesian approach to structure discovery in bayesian networks. Mach. Learn. 50, 95–125.

Galassi, M., Davies, J., Theiler, J., Gough, B., Jungman, G. and Booth, M., F., R. (2006). GNU Scientific Library Reference Manual - Revised Second Edition (v1.8). Network Theory Ltd.

Heckerman, D., Geiger, D., Chickering, D. M. (1995). Learning bayesian networks - the combination of knowledge and statistical-data. Mach. Learn. 20, 197–243.

Helmbold, D. P. and Schapire, R. E. (1997). Predicting nearly as well as the best pruning of a decision tree. Mach. Learn., 51-68.

Huelsenbeck, J. P. and Ronquist, F. (2001). Mrbayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Jansen, R., Yu, H. Y., Greenbaum, D., Kluger, Y., Krogan, N. J., Chung, S. B., Emili, A., Snyder, M., Greenblatt, J. F. and Gerstein, M. (2003). A bayesian networks approach for predicting protein-protein interactions from genomic data. Science 302, 449–453.

Korb, K. B. and Nicholson, A. E. (2004). Bayesian Artificial Intelligence. Chapman and Hall/CRC, Boca Raton.

Madigan, D. and York, J. (1995) Bayesian graphical models for discrete-data. International Stat. Rev. 63, 215–232.

Namata, H., Welby, S., Aerts, M., Faes, C., Abrahantes, J. C., Imberechts, H., Vermeersch, K., Hooyberghs, J., Meroc, E. and Mintiens, K. (2009). Identification of risk factors for the prevalence and persistence of salmonella in belgian broiler chicken flocks. Prev. Vet. Med. 90, 211–222.

Poon, A. F. Y., Lewis, F. I., Pond, S. L. K., Frost and S. D. W. (2007a). Evolutionary interactions between n-linked glycosylation sites in the hiv-1 envelope. Plos Comput. Biol. 3, 110–119.

Poon, A. F. Y., Lewis, F. I., Pond, S. L. K., Frost and S. D. W. (2007b). An evolutionary-network model reveals stratified interactions in the v3 loop of the hiv-1 envelope. Plos Comput. Biol. 3, 2279–2290.

R Development Core Team (2006). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0.

Silverlas, C., Emanuelson, U., de Verdier, K. and Bjorkman, C. (2009). Prevalence and associated management factors of cryptosporidium shedding in 50 Swedish dairy herds. Preventive Veterinary Medicine 90, 242–253.

ASSESSMENT OF THE OVERALL RISK FACTORS FOR A COMPOSITE OUTCOME USING MULTIBLOCK MODELLING. ILLUSTRATION ON LOSSES IN BROILER CHICKEN FLOCKS

S. BOUGEARD*, C. LUPO, S. LE BOUQUIN, E.M. QANNARI AND C. CHAUVIN

SUMMARY

Research in veterinary epidemiology may be concerned with assessing risk factors of complex animal health issues described by several variables. Moreover, veterinary epidemiological data are usually organized in several blocks of variables, consisting in a block of variables to be explained and a large number of explanatory variables organized in some meaningful blocks. We propose an innovative method in the multiblock framework, called multiblock Redundancy Analysis, which is designed to handle most specificities of complex veterinary epidemiological data. The interest and relevance of multiblock Redundancy Analysis is illustrated using a dataset whose aim is to assess the overall risk factors of losses due to mortality and condemnation in broiler chickens, described by four variables. The main aim is to jointly assess the impact of the different production stages on the whole losses and the specific risk factors for each element of losses.

INTRODUCTION

Research in veterinary epidemiology may be concerned with assessing risk factors of complex animal health issues described by several variables. Moreover, veterinary epidemiological surveys usually consist in data gathered from animal characteristics, farm, transport conditions, slaughterhouse features and laboratory results. As a consequence, explanatory variables may be organized into meaningful blocks related to the production stages. In a more formal way, veterinary epidemiological data are organized in (K+1) blocks of variables, consisting in a block of variables to be explained (Y) and a large number of explanatory variables organized in K meaningful blocks (X_1, …, X_K). All these variables are measured on the same epidemiological units, *i.e.* animals or farms. Considering the aim and the specificity of such complex data, the research work focuses on methods related to the multiblock modelling framework. We propose an original method and shall refer to it as multiblock Redundancy Analysis (Bougeard et al., 2008). The underlying principle is that each dataset is summed up by latent variables which are linear combinations of the variables in the dataset

*Stéphanie Bougeard, French Agency for Food Safety (AFSSA), Pig, poultry and fishes Veterinary Research Center, Department of Epidemiology, Zoopole, BP 53, 22440 Ploufragan, France. Email: s.bougeard@afssa.fr

under consideration. It gives valuable tools for the explanation and the investigation of the relationships among variables and datasets. The associated models, based on orthogonalised regressions, explain each variable in Y with explanatory variables. As the method aims at describing datasets with a large numbers of variables, the epidemiologist needs to sum up the complex links between them and between the datasets. Overall indices and graphical displays associated with different interpretation levels are also proposed.

MATERIALS AND METHODS

Multiblock Redundancy Analysis

An original method, called multiblock Redundancy Analysis, is proposed in the multiblock modelling framework, adapted to the setting in which a block Y of several variables is to be explained by K explanatory variable blocks (X_1, …, X_K). The main idea is that each dataset is summed up by a latent variable (or component) which is a linear combination of the variables derived from this dataset (Fig. 1). More precisely, the method derives a global component $t^{(1)}$, related to all the explanatory variables merged in the dataset $X=[X_1|\ldots|X_K]$, closely related to a component $u^{(1)}$, linear combination of the variables in Y. Moreover, the component $t^{(1)}$ sums up the partial components ($t_1^{(1)}$, …, $t_K^{(1)}$) respectively associated with the blocks (X_1, …, X_K).

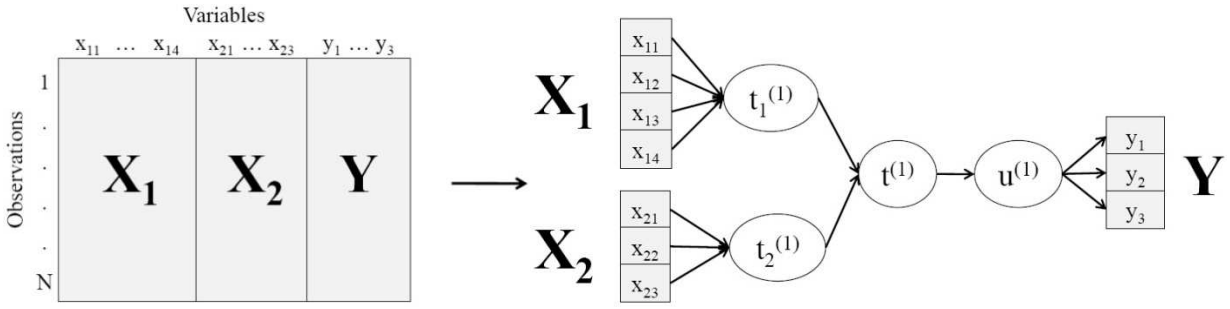

Fig. 1 Example of multiblock data structure and associated conceptual scheme of multiblock Redundancy Analysis, highlighting the relationships between the variable blocks (X_1, X_2, Y) and their associated components ($t_1^{(1)}$, $t_2^{(1)}$, $u^{(1)}$) for the first dimension

These components are computed by maximizing a criterion based on the squared covariance between the global component $t^{(1)}$ and the component $u^{(1)}$, following the maximization problem:

$$cov^2\left(u^{(1)},t^{(1)}\right) \text{ with } t^{(1)} = \sum_{k=1}^{K} a_k^{(1)} t_k^{(1)}, \quad u^{(1)} = Yv^{(1)}, \qquad t_k^{(1)} = X_k w_k^{(1)}, \quad \sum_{k=1}^{K} a_k^{(1)2} = 1 \text{ and } \left\| t_k^{(1)} \right\| = \left\| v^{(1)} \right\| = 1$$

The solutions are derived from the eigenanalysis of a matrix which involves the datasets Y and (X_1, …, X_K), *i.e.* $v^{(1)}$ the eigenvector of $\sum_k Y'X_k(X_k'X_k)^{-1}X_k'Y$ associated with the largest eigenvalue. Thereafter, the partial components $t_1^{(1)}$, …, $t_K^{(1)}$ are therefore given by the normalized projection of $u^{(1)}=Yv^{(1)}$ on each subspace spanned by X_1, …, X_K respectively. The

coefficients $a_k^{(1)}$ are given by $a_k^{(1)} = \frac{cov\left(u^{(1)}, t_k^{(1)}\right)}{\sqrt{\sum_{l=1}^{K} cov^2\left(u^{(1)}, t_l^{(1)}\right)}}$. For further details, refer to (Bougeard et al., 2008).

In order to improve the prediction ability of the model, higher order solutions are obtained by considering the residuals of the orthogonal projections of the datasets (X_1, …, X_K) onto the subspace spanned by the first global component $t^{(1)}$. Subsequent components ($t^{(2)}$, …, $t^{(H)}$) are found by replacing the datasets with their residuals in the same maximization. This allows orthogonalised regression which takes into account all the explanatory variables. The final model may be obtained by selecting the optimal number of components (or model dimension) to be retained, with a validation technique such as cross-validation (Stone, 1974).

For the optimal number of components h, the exponential of the regression coefficients associated with each explanatory variable and each variable to be explained are interpreted as Incidence Rate Ratio (IRR). Bootstrapping simulations are performed to provide standard deviations and tolerance intervals, associated with the regression coefficient matrix. However, if the number of variables in Y is large, the epidemiologist needs to sort the explanatory variables in a global order of priority. The Variable Importance for the Projection (VIP) proposed in the PLS regression framework may be a relevant tool (Wold, 1994; Chong & Jun, 2005). As the VIP indices verify, for a given dimension, the property $\sum_{p=1}^{P} \frac{VIP_p^2}{P} = 1$ where P is the total number of variables in X, they can be expressed as percentages. Associated standard deviations and tolerance intervals, computed using bootstrapped results, may additionally be computed. For the optimal dimension h, each explanatory variable is considered as significantly associated with the Y block if the 95% tolerance interval associated with the VIP does not contain the threshold value 0.8 (Gosselin et al., In Press). Finally, it is also interesting for the epidemiologist to assess the contributions of the explanatory blocks to the modelling task while using the Block Importance in the Prediction, BIP (Vivien et al., 2005), based on the weighted average values of the coefficients ($a_1^{(h)2}$, …, $a_K^{(h)2}$). As the BIP indices verify, for a given dimension, the property $\sum_{k=1}^{K} \frac{BIP_k^2}{K} = 1$ where K is the number of explanatory blocks, they can be expressed as percentages. Associated standard deviations and tolerance intervals, computed using bootstrapped results, may be given. It follows that each block X_k is considered to be significantly associated with the overall block Y or with each variable in Y, if the 95% tolerance interval associated with the BIP does not contain the threshold value 0.8.

Illustration dataset

The study population consists in a cohort of broiler chicken flocks slaughtered during 2005 in the main French regions of production (for details on data collection, refer to (Lupo et al., 2009)). A large number (404) of broiler chicken flocks are randomly selected by a two-stage sampling, stratified per slaughterhouse and based on random selection of the day of slaughter and of the flock sequence number in the slaughtering schedule of that day. From the 404 broiler chicken flocks, 351 are retained for this study considering the occurrence of missing data. The aim is to assess the overall risk factors for a composite outcome: the losses in broiler chickens (Y), described by four variables, *i.e.* the first-week mortality rate, the mortality rate during the rest of the grow out (2nd week to slaughter), the mortality rate during the transport to the

slaughterhouse and the condemnation rate at slaughterhouse. The explanatory variables are firstly selected on the basis of the main factors reported in the literature and on an earlier univariate screening step applied to each variable to be explained. These 68 selected variables are organized in four thematic blocks related to farm structure and systematic husbandry management practices (X_1, 16 variables), flock characteristics and on-farm history of the chicks at placement (X_2, 14 variables), flock characteristics during the rearing period (X_3, 17 variables) and catching, transport - lairage conditions, slaughterhouse and inspection features (X_4, 21 variables). Indicator (dummy) variables are considered for the categorical variables. All the 68 putative explanatory variables are included in the multiblock analysis, fitted with a manual backward-selection procedure.

As all the variables are expressed in different units, they are column centered and scaled to unit variance. It is worth noting that as the explanatory variables have been standardized to unit variance, the total variance in each block is equal to the number of variables in this block. This motivates the block weighting in order to put the blocks to the same footing (Wold et al., 1996; Westerhuis & Coenegracht, 1997). Each of the (K=4) explanatory block is accommodated by an isotropic scaling factor to set them to the same total variance (chosen equal to 1/K). Therefore, the merged explanatory dataset $X=[X_1|X_2|X_3|X_4]$ has a total variance equal to one. In the same way, the total variance of the Y block is also set to one. Moreover, within the Y dataset, the four variables to be explained do not have the same importance for the epidemiologist. As a matter of fact, the loss is more important for the condemnation at slaughterhouse than for the early mortality. While keeping the Y total variance set to one, the variance of each variable in Y is weighted according to its relative impact, estimated considering the amount of live weight (kilograms) lost at each stage. It turns out that the variance is set equal to 0.03 for the early-mortality rate, 0.4 for the mortality rate during the rest of the grow out, 0.11 for the mortality rate during the transport to the slaughterhouse and 0.46 for the condemnation rate at slaughterhouse. These weightings affect all the results given in the section.

RESULTS

Preliminary analysis

First of all, the 68 explanatory variables are included in a multiblock analysis where each variable in Y has the same weight, this model being more stable. Among the 68 explanatory variables, 20 are significant risk factors for at least one element of the losses and are retained for the final model (see variable description in Table 1). Among these 20 variables, five pertain to the farm structure and the systematic husbandry management practices (X_1), four are selected from the flock characteristics and the on-farm history of the chicks at placement (X_2), six relate to the flock characteristics during the rearing period (X_3) and five relate to the catching, transport - lairage conditions, slaughterhouse and inspection features (X_4). The detailed results concerning this model are given in (Bougeard et al., Submitted). Therefore, these 20 selected variables are included in a weighted multiblock analysis, where each variable in Y is weighted according to its relative loss. The cross-validation results led us to a model with (h=3) components (m_{cv}=500 cross-validated resamples). This model explains 92% of the variation in Y.

Overall risk factors for losses

A predictive model is set up by regressing each variable to be explained on the basis of the first three global components. The regression coefficients, transformed into Incidence Rate Ratio (IRR), the associated standard deviations and the tolerance intervals (m_{bt}=500 bootstrapped resamples) of the 20 explanatory variables are computed for each variable to be explained. Table 1 gives the explanatory variables which are significantly associated with the four variables related to the losses. Each variable in Y is significantly related to a specific set of explanatory variables, except the first-week mortality rate whose weight in the analysis is too weak. Furthermore, in order to sort these explanatory variables in a global order of priority which reflects their overall contribution to the explanation of the Y block, the Variable Importance for the Projection (VIP) are given. Figure 2 gives the VIP^2, expressed as percentages, of the main explanatory variables, *i.e.* $VIP^2 \geq 3\%$, with their 95% tolerance interval. It turns out that three explanatory variables have a significant impact on the overall losses: the stress occurrence during rearing (VIP^2_{Stress}=15.4% [6.3;24.5]), the carcass withdrawal at the evisceration line (VIP^2_{Evisc}=11.6% [6.0;17.3]), the type of loading system ($VIP^2_{LoadType}$=9.0% [3.1;14.9]).

Table 1. Contribution of the explanatory variables to the explanation of the four variables related to the losses, through significant Incidence Rate Ratio (IRR) associated with their 95% tolerance interval (351 chicken broilers)

	First-week mortality rate	Mortality rate during the rest of the grow out	Mortality rate during the transport to slaughterhouse	Condemnation rate at slaughterhouse
Weight in the analysis	**3%**	**40%**	**11%**	**46%**
X_1 block: Farm structure and systematic husbandry management practices				
Total area for chicken on the farm	NS	NS	0.21 [0.08;0.34]	0.34 [0.07;0.60]
Cleaning step in decontamination of chicken house: yes (*vs* no)	NS	-0.27 [-0.42;-0.12]	-0.17 [-0.30;-0.04]	-0.32 [-0.56;-0.08]
Heating system in the chicken house: gas heater (*vs* radiant)	NS	-0.24 [-0.42;-0.04]	NS	NS
Sorting practice: yes (*vs* no)	NS	NS	NS	0.24 [0.01;0.47]
Age of the facilities: >12 years (*vs* recent or renovated)	NS	NS	NS	0.30 [0.05;0.54]

X_2 block: Flock characteristics and on-farm history of the chicks at placement				
Vitamins and minerals during the starting period: yes (*vs* no)	NS	-0.15 [-0.29;-0.02]	NS	-0.16 [-0.30;-0.03]
Frequency of farmer visits during the starting period	NS	NS	NS	-0.31 [-0.48;-0.14]
Homogeneity of chicks at placement: yes (*vs* no)	NS	NS	NS	NS
Number of chicks at placement	NS	NS	0.21 [0.10;0.32]	NS
X_3 block: Flock characteristics during the rearing period				
Production type: standard (*vs* other[b])	NS	0.39 [0.15;0.62]	NS	-0.32 [-0.58;-0.07]
Homogeneity of chickens at the end of rearing: yes (*vs* no)	NS	NS	0.14 [0.03;0.26]	-0.44 [-0.71;-0.17]
Genetic strain: X (vs other)	NS	-0.64 [-1.00;-0.27]	-0.13 [-0.25;-0.01]	-0.37 [-0.60;-0.14]
Locomotor disorder observed: yes (*vs* no)	NS	0.56 [0.1;0.97]	NS	0.37 [0.04;0.69]
Stress occurrence[c] during rearing: yes (*vs* no)	NS	1.00 [0.64;1.35]	0.17 [0.01;0.33]	0.38 [0.11;0.65]
Frequency of farmer visits during rearing	NS	NS	NS	-0.32 [-0.49;-0.16]
X block: Catching, transport - lairage conditions, slaughterhouse and inspection features				
Type of loading system: mechanical (*vs* manual)	NS	NS	0.35 [0.19;0.51]	NS
Meteorological conditions during lairage: rain and/or wind (*vs* neither rain nor wind)	NS	NS	0.16 [0.03;0.29]	NS
Stocking density in transport crates	NS	0.23 [0.04;0.42]	0.20 [0.02;0.39]	NS
Average duration of waiting time on lairage	NS	NS	0.16 [0.01;0.30]	-0.24 [-0.43;-0.06]
Withdrawal of carcasses at the evisceration line: yes (*vs* no)	NS	NS	NS	0.66 [0.44;0.89]

[a] NS means "Non Significant".
[b] "Others" includes light or heavy production types.
[c] Stress occurrence gathers feeding system defection, electrical defect, etc.

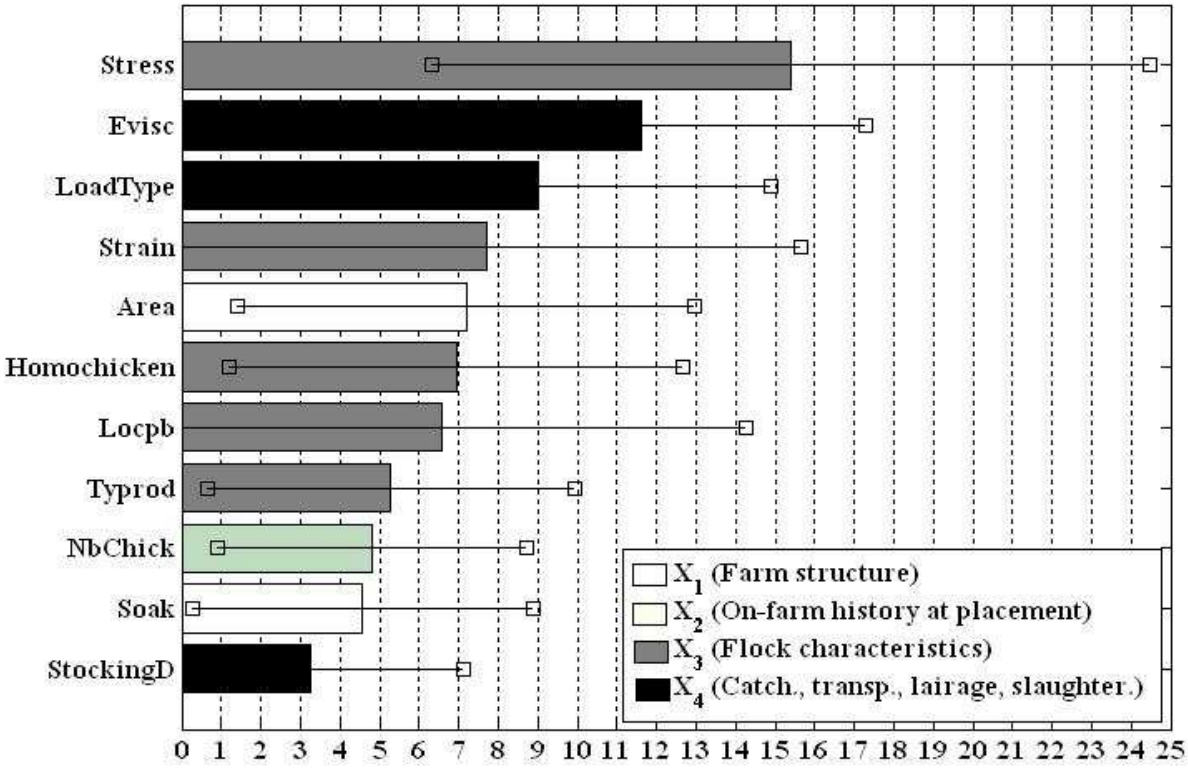

Fig. 2 Contribution of the main explanatory variables ($VIP^2 \geq 3\%$) to the explanation of the overall losses, through Variable Importance for the Projection (VIP^2 expressed as percentages) associated with their 95% tolerance interval

Block importance in the explanation of losses

Figure 3 depicts the relative importance of the four production stages, *i.e.* the four explanatory blocks, in the overall losses explanation, highlighting the significant importance of two blocks (in our example, the BIP^2 significant limit is 16%). More precisely, 45.0% [34.6;55.4] of the overall losses variation are explained by the flock characteristics during the rearing period (X_3) and 28.5% [20.1;36.9] by the catching, transport - lairage conditions, slaughterhouse and inspection features (X_4). In addition, the relative importance of the four production stages in the explanation of each element of the losses are given in Table 2. Neither the farm structure (X_1) nor the on-farm history at placement (X_2) have a significant impact on any variable of the losses. The most important findings are that the mortality rate during the rest of the grow out and the condemnation rate at slaughterhouse are mainly explained by the flock characteristics during rearing (X_3), and that the mortality rate during the transport to slaughterhouse is mainly related to the catching, transport - lairage conditions, slaughterhouse and inspection features (X_4).

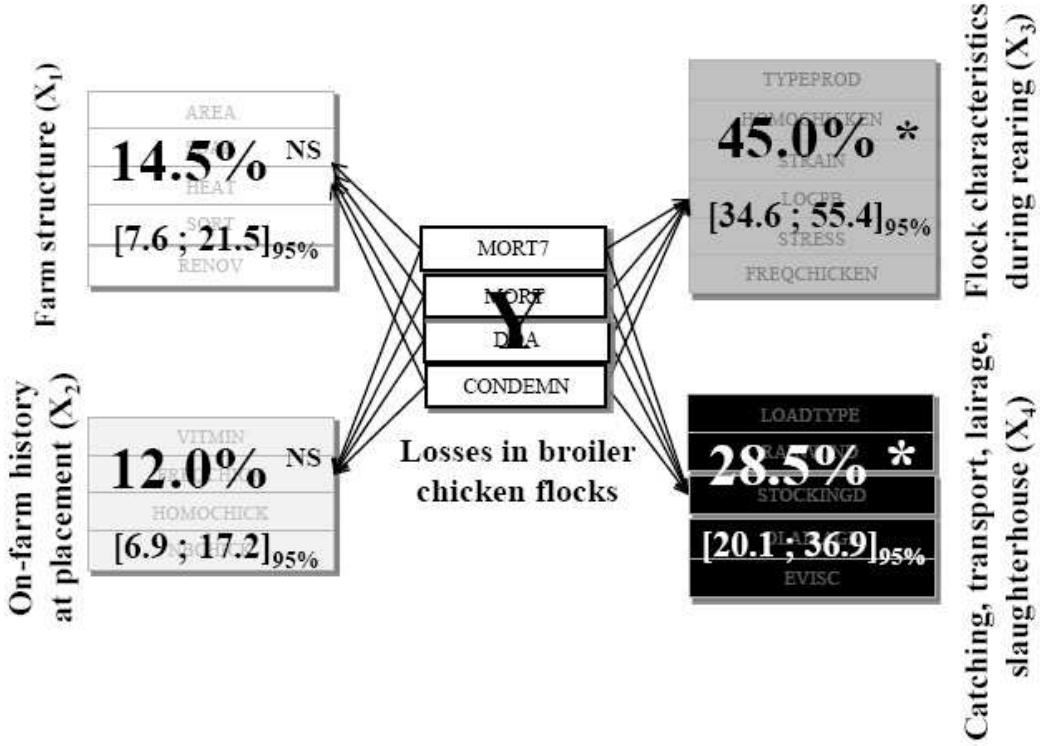

Fig. 3 Contribution of the four explanatory blocks (X_1, …, X_4) to the explanation of the overall losses, through Block Importance in the Prediction (BIP^2 expressed as percentages) associated with their 95% tolerance interval.

Table 2. Contribution of the explanatory blocks (X_1, …, X_4) to the explanation of each variable related to the losses, through Block Importance in the Prediction (BIP^2 expressed as percentages) associated with their 95% tolerance interval.

	First-week mortality rate	Mortality rate during the rest of the grow out	Mortality rate during the transport to slaughterhouse	Condemnation rate at slaughterhouse
Weight in the analysis	**3%**	**40%**	**11%**	**46%**
Farm structure and systematic husbandry management practices (X_1)	15.6 [6.5;24.6] NS	11.2 [3.8;18.5] NS	15.6 [6.4;28.8] NS	16.9 [5.6;28.1] NS
Flock characteristics and on-farm history at placement (X_2)	12.4 [5.2;19.7] NS	9.7 [3.8;15.6] NS	15.1 [5.4;24.8] NS	11.9 [4.9;18.9] NS
Flock characteristics during rearing (X_3)	44 [18.4;69.6] *****	**59.0** [46.7;71.3] *****	14.3 [0.0;32.1] NS	**54.9** [39.3;70.5] *****
Catching, transport, lairage, slaughterhouse (X_4)	28.0 [5.7;50.4] NS	20.1 [11.2;29.1] NS	**55.0** [37.1;72.8] *****	16.3 [6.5;26.2] NS

DISCUSSION

Multiblock Redundancy Analysis is a relevant and useful tool to handle the specificity of complex veterinary epidemiological data, as it enhances their interpretation and unveils new information for the epidemiologist. It makes possible to assess, with a single analysis, the overall risk factors for a composite outcome: the losses in broiler chickens, described by four variables, *i.e.* the first-week mortality rate, the mortality rate during the rest of the grow out (2^{nd} week to slaughter), the mortality rate during the transport to the slaughterhouse and the condemnation rate at slaughterhouse. Moreover, the method allows to weight each variable in Y, according to its relative impact, estimated considering the amount of live weight lost at each stage. The obtained risk factors are consistent with those from specific studies reported in the literature, but most of these studies focused on one particular processing step and are moreover related to each specific element of losses. In addition, this method makes it possible to shed light on the significant explanatory variables affecting a composite dependent variable and to detect the overall location of the problems in the various explanatory blocks.

Multiblock Redundancy Analysis allows the use of a large number of variables and individuals in a single model, and restricts the tricky and unsatisfactory selection of variables in comparison with usual statistical processes. Moreover, the method uses additional information available for grouping the variables into meaningful blocks and allows to be explained several variables at the same time. This avoids to set up separate models or to combine several dependent variables which might result in a loss of information.

However, multiblock methods present some limitations in comparison with Generalized Linear Models and further investigations will be undertaken to handle more epidemiological data specificities. For instance, multiblock modelling does not efficiently handle the information from relevant external variables, such in Hierarchical Generalized Linear Models. Furthermore, further developments in multiblock analysis should allow new breakthrough in the statistical processing of veterinary epidemiological data. Multiblock Redundancy Analysis can be directly adapted to more complex data, *e.g.* explain several blocks of health events, each being described by several variables, or the evolution of a complex health event at different times. These kinds of methods can also be extended to the case where the explanatory blocks of variables are linked with each others. These developments together with the increase of the volume and the complexity of data in biology will certainly contribute to make the use of multiblock modelling more and more popular.

REFERENCES

Bougeard, S., Hanafi, M., Lupo, C. and Qannari, E.M. (2008). From multiblock Partial Least Squares to multiblock redundancy analysis. A continuum approach. In: International Conference on Computational Statistics, Porto (Portugal). 607-616

Bougeard, S., Lupo, C., Le Bouquin, S., Qannari, E.M. and Chauvin, C. (Submitted). Use of multiblock modelling to assess the overall risk factors of a composite outcome. Illustration on losses in broiler chickens. Prev. Vet. Med.

Chong, I.G. and Jun, C.H. (2005). Performance of some variable selection methods when multicollinearity is present. Chemom. Intell. Lab. 78, 103-112

Gosselin, R., Rodrigue, D. and Duchesne, C. (In Press). A Bootstrap-VIP approach for selecting wavelength intervals in spectral imaging applications. Chemom. Intell. Lab.

Lupo, C., Le Bouquin, S., Balaine, L., Michel, V., Peraste, J., Petetin, I., Colin, P. and Chauvin, C. (2009). Feasibility of screening broiler chicken flocks for risk markers as an aid for meat inspection. Epidemiol. Infect. 137, 1086-1098

Stone, M. (1974). Cross-validatory choice and assessment of statistical predictions. J. Roy. Stat. Soc. 36, 111-147

Vivien, M., Verron, T. and Sabatier, R. (2005). Comparing and predicting sensory profiles by NIRS: Use of the GOMCIA and GOMCIA-PLS multi-block methods. J. Chemometr. 19, 162-170

Westerhuis, J.A. and Coenegracht, P.M.J. (1997). Multivariate modelling of the pharmaceutical two-step process of wet granulation and tableting with multiblock partial least squares. J. Chemometr. 11, 379-392

Wold, S. (1994). PLS for multivariate linear modeling. In: Waterbeemd, H.v.d. (Ed.) QSAR: Chemometric methods in molecular design: Methods and principles in medicinal chemistry. Verlag-Chemie, Weinheim, Germany

Wold, S., Kettaneh, N. and Tjessem, K. (1996). Hierarchical multiblock PLS and PC models for easier model interpretation and as an alternative to variable selection. J. Chemometr. 10, 463-482

EQUINE EPIDEMIOLOGY

A CLUSTER-RANDOMISED CONTROLLED TRIAL TO EVALUATE KNOWLEDGE-TRANSFER INTERVENTIONS FOR RURAL WORKING EQUID USERS IN ETHIOPIA

A.P. STRINGER*, C.E. BELL, R.M. CHRISTLEY, F. GEBREAB, G. TEFERA, K. REED, A. TRAWFORD AND G.L. PINCHBECK

SUMMARY

A cluster-randomised controlled trial (c-RCT) was used to evaluate the effectiveness of 3 knowledge-transfer interventions on knowledge change about equid health, in rural Ethiopian working equid users. Groups were exposed to either an audio programme, a village meeting or a diagrammatic handout, and were compared to a control group who received no intervention. A total of 516 randomly selected participants undertook the pre-intervention questionnaire and 504 of these undertook the post-dissemination questionnaire, a follow-up response rate of 98%. All interventions significantly improved the overall 'change in knowledge' score on the questionnaire compared to the control, with the diagrammatic handout (coefficient (coef) 9.2, *s.e.* = 0.6) and the village meeting (coef 9.7, *s.e.* = 0.5) having a greater impact than the audio programme (coef 5.2, *s.e.* = 0.6). This study should aid others in the design of education materials for adult learning amongst working equid users in a developing country.

INTRODUCTION

There are estimated to be 1.8 million horses, 377,000 mules and 4.3 million donkeys working in Ethiopia, the largest population of donkeys in Africa and the second largest donkey population in the world after China (Anon, 2007). Their role in the socio-economics of the country is substantial, with the majority of the Ethiopian population dependent on traditional subsistence agricultural production (DFID, 2006). Livelihoods are predominantly based on agriculture, which accounts for 85% of employment, 45% of national income and over 90% of export earnings, but Ethiopian agriculture still remains low-input, low-value and subsistence-oriented (DFID, 2006). Equids are used for the transportation of goods, people and in some areas for agricultural purposes (Gebreab, 1993; Garuma et al., 2007). They have a direct effect on the lives of rural people by reducing the transport burdens of water, fuel wood and goods (Garuma et al., 2007). Although often considered a secondary animal in relation to oxen, donkeys provide an effective entry point for assisting women in both domestic responsibilities and income generating activities (Marshall & Ali, 1997).

Working equids in Ethiopia suffer from low productivity as a result of prevalent parasitic and infectious disease, low nutritional standards and poor management practices (Yilma et al.,

*Andrew Stringer, School of Veterinary Science, Faculty of Health and Life Sciences, University of Liverpool, Leahurst Campus, Chester High Road, South Wirral, CH64 7TE, UK. E-mail: stringer@liv.ac.uk

1990). These causes contribute to the short life span of Ethiopian donkeys (9-13 years) compared to a possible 37-40 years in the UK (Svendsen, 1994). Due to their perceived low social status, and their single purpose role within farming systems, few farmers keep more donkeys than are needed to fulfil their immediate work requirements (Tesfaye & Smith, 2000). Farmers who do not own a donkey have a relatively weak economic base compared to those who own donkeys, and also own fewer other livestock (Alemayehu et al., 2000).

Wounds are one of commonest health concerns to afflict working donkeys in many countries (Curran et al., 2005; Pritchard et al., 2005; Biffa & Woldemeskel, 2006; Burn et al., 2007; Sells et al., 2009). In addition, studies of donkeys in Ethiopia have demonstrated that back sores and wounds are the most commonly observed health problem (Tesfaye & Curran, 2005). The majority of these wounds are iatrogenically caused, which is in contrast to the majority of wounds on equids in developed countries which are predominantly due to accidental injury. Wounds in working donkeys are seen on the legs, girth, tail, saddle and wither regions (Pritchard et al., 2005; Sells et al., 2009). These wounds are often caused by a combination of poorly fitting and designed tack or harnesses, beating with sticks and improper management practices (Pearson et al., 2003). Differences in wound severity and location can often be attributed to the different uses of the donkeys: for example, they may be ridden, carry packs or be used for draught, and also to differences in saddle and harness design (Sells et al., 2009).

One approach to decrease the prevalence of wounds is through education of donkey users. Ethiopian farmers have themselves identified a need for greater knowledge through training (Tesfaye et al., 2005). However, there is limited published work available that evaluates the impact of different knowledge-transfer methods on adult learning in developing countries, and to the authors' knowledge no published work is available that evaluates the effect of knowledge-transfer interventions on the education of working equid users. A variety of knowledge-transfer methods have been widely used for educating people in the developing world including: handouts and leaflets (Tu & Giang, 2002); rural radio (Valente et al., 1994; de Silva & Garforth, 1997; Chapman et al., 2003); drama (Harvey et al., 2000; Soldan, 2004); video (Rimm, 2003); information and communication technology (ICT) (Dawson & Joof, 2005) and community educators (Klepp et al., 1997). However, few studies have utilised randomised controlled trials to assess the impact of knowledge-transfer interventions on their target audience (Eaden et al., 2002; Kelly et al., 2003; Thomas et al., 2003; Bell et al., 2005). The aims of this study were to develop a number of different knowledge-transfer interventions for rural working equid users in Ethiopia and, subsequently, to assess the efficacy of these on knowledge change using a c-RCT.

MATERIALS AND METHODS

Content and design of knowledge-transfer interventions

Both the focus of the education programme, and the design of the knowledge-transfer interventions were informed by a Participatory Situation Analysis (PSA) (Stringer et al., 2009). The PSA gathered information on the perceptions of working equid owners on the health and disease concerns about their animals, and their existing contact and social networks. The PSA identified wounds as an important owner perceived concern about their donkeys. This information was then triangulated with both clinical records from a veterinary non-governmental organisation (NGO) involved in providing free veterinary care for working equids in Ethiopia, and with available published literature. Results of the triangulation process then informed the development of ten learning objectives, designed to address key issues associated with wounds

and wound management in donkeys, including their causes, sites, treatment, prevention and relevance (Table 1).

Table 1. Learning Objectives on the topic of wounds and wound management in donkeys in Ethiopia used to develop knowledge-transfer interventions.

	LEARNING OBJECTIVES
1	Be able to list 4 causes of manmade wounds.
2	Identify 4 common sites/areas affected by manmade wounds.
3	Be aware of good and bad topical treatments for wounds.
4	Describe how to prepare an appropriate salt solution for cleaning wounds.
5	Be able to list 3 steps involved in cleaning wounds appropriately.
6	Recognise 2 signs of an early harness wound.
7	Select appropriate material as a base layer for the harness.
8	Describe 3 important features of the padding on the harness.
9	Describe an important feature of harness base layer care.
10	Recognise 3 disadvantages of your donkey having wounds.

These ten learning objectives were then incorporated into the design and development of three different knowledge-transfer interventions; an audio programme (A), a village meeting facilitated by an animal health worker (VM) and a diagrammatic handout (HO). The interventions chosen for inclusion in the c-RCT were informed by results of other relevant published studies, and future sustainability, economics and logistical considerations. All interventions were designed with content that was both culturally and socially acceptable. Each intervention underwent extensive phases of pretesting, piloting and reverse translation prior to release to study participants.

The diagrammatic handout was designed to be predominately image-based with as little text as necessary, having taken into consideration the low levels of literacy and visual literacy identified amongst study participants during the PSA and pretesting phase. The handout consisted of four laminated colour pages of A4 paper (available on request), containing high quality photos with background detail removed using Adobe Photoshop CS2. Text on the handout was limited to single words or short sentences in Oromo (regional language of the Oromia region). The handout was distributed to participants on an individual basis at the end of the pre-intervention questionnaire, and was not accompanied by any discussion or clarification of the content.

The audio programme was developed in the format of a short (12 minute) radio drama which comprised of a discussion in Oromo between a wise old livestock owner and a young, inexperienced owner, and was recorded by recognised local radio actors. This programme was recorded using digital software (Audacity 1.2.6, http://audacity.sourceforge.net) and was broadcast via an MP3 player and loudspeakers to all participants in a village on a group basis following administration of the pre-intervention questionnaire.

The village meeting consisted of a standardised talk accompanied by both visual ‘poster’ displays and demonstrations given by a local, qualified animal health worker in Oromo. It was delivered to all participants in a village as a group after the pre-intervention questionnaire and also involved a short question and answer session for clarification at the end of the meeting which could be in either Oromo or Amharic.

Design of c-RCT

A c-RCT design was used to compare the effects of three knowledge-transfer interventions with a control group (that received no knowledge-transfer intervention) on change in knowledge of equid users. Villages (Kebeles) and rural donkey users were randomly selected and the same intervention was randomly assigned to all participants within each village. Cluster randomisation was necessary to prevent "contamination" between owners belonging to one village via sharing of information. Identical questionnaires were administered both pre- and post-dissemination to assess changes in knowledge levels. Follow up questionnaires were administered 11-18 days post intervention (median 14, mode 14).

Sample size calculation

Sample size calculations were performed for a clustered design using a cluster sample size calculator (Campbell et al., 2004). An estimate of the variance at village level from previous studies in developing countries (Bell et al., 2005) was used, giving a design effect of 2.3 and an intra-cluster correlation coefficient of 0.14. A total of 8 villages each with 15 owners (total 480 participants) per type of intervention were required to detect a 30% change in knowledge (e.g. an increase from a baseline of 20% to 50%) with 95% confidence and 80% power. Therefore 32 villages with at least 25 owners per village were selected to allow for potential loss to follow up. A blocked design was used such that, within each set of 8 randomly selected villages, each knowledge-transfer intervention and control was assigned randomly to two villages. This was to avoid runs of one type of intervention being selected by chance, as we hypothesized that season and seasonal job activities of farmers may affect the response rates.

Study sites and participants

The c-RCT was carried out between November 2008 and July 2009 in one of the seven regional zones of Ethiopia (Oromia). Within this region, one zone (Arsi) was selected due to: a lack of previous exposure to an equine veterinary NGO; a known high density of donkey users, and logistical considerations. Within this area four woredas (administrative departments) (Sire, Hitosa, Tiyo, Degeluna Tijo) were selected and a complete list of villages within the woredas was obtained from each woreda agricultural office. Thirty two villages were randomly selected using random numbers generated in a spreadsheet programme Microsoft Excel 2007 (Microsoft Cooperation, USA). Villages were excluded if there was no road access; the Development Agent (DA) was deemed too inexperienced or new to that village; if there were inadequate villager records or if selected villages shared a major market at which contamination may occur. Development agents were recruited to liaise with each selected village and to aid in the participant recruitment process. Lists of village inhabitants were obtained from village agricultural offices or municipality offices, and participants in villages were randomly selected using random numbers generated in Microsoft Excel 2007 (Microsoft Cooperation, USA). Participants were eligible for inclusion in the c-RCT if they were male, owned or used a donkey, over 18 years of age, and able to attend the study visits. All participants were recruited on a volunteer basis and were free to refuse participation or leave the trial at any point. Once recruited, participants were assigned a unique ID number and ID card, and were also paid a nominal monetary incentive for participation in each study visit. The visit dates for both the pre-intervention visit and the follow up visit were predetermined and DA's were responsible for ensuring that participants were informed of the correct date and time.

Data collection and analysis

Baseline data were collected at the pre-intervention visit which included age, formal education level, radio use, literacy ability (Oromo and Amharic), number of donkeys owned, length of donkey user-ship, other animals owned, housing of donkey, exposure to equine veterinary NGO's, responsibility in household and responsibility for decisions regarding donkey management. Participants' knowledge-change was measured using 12 closed questions about donkey wounds and wound management. These questions corresponded to the ten defined learning objectives, and were identical in both pre-intervention and follow up questionnaires. The 12 questions required participants to volunteer between one and four correct responses per question, to achieve a total maximum score of 28. Questionnaires were extensively piloted and reversed translated. All questionnaires were administered on an individual basis by one trained animal health worker (AHW) in either of two regional languages (Oromo and Amharic) in a consistent and controlled manner with no additional clarification. To avoid contamination of later participants by individuals who had already completed the questionnaire, all participants were kept in two separated groups until the time that either a group intervention could be administered (in villages allocated as either audio or village meeting intervention), or participants who had completed the questionnaire could leave the village (in villages allocated as either control groups or handout interventions). The administrators of the questionnaires and those assessing the outcomes of the trial were not blinded due to nature of this intervention trial.

Data were entered into a spreadsheet (Microsoft Excel 2007, Microsoft Cooperation, USA). Data were analysed using SPSS v17 (SPSS Inc, Chicago, Illinois, USA) and MLwiN v2.02 (Centre for Multilevel Modelling, Bristol, UK). Data analysis included comparison of baseline data between intervention groups to check for adequate randomisation using Chi-square tests for categorical data and Kruskal-Wallis and Mann-Whitney tests for continuous data. The primary outcome measure used was a continuous variable reflecting the change in score between pre- and post-intervention questionnaires. The change in score of individual respondents for the different knowledge-transfer interventions was assessed using multilevel linear regression models to allow for clustering of individuals within villages. Analysis was carried out per-protocol due to no data being available on the outcome of those participants lost at short term follow up. However due to the small number of participants lost (n=12) it is unlikely to have a large effect. The effects of all covariates that varied at baseline were also considered. Continuous variables (age and pre-intervention score) were centred by subtraction of the sample mean from all observations. A backward-step process was used, with covariates remaining in the model if they were statistically significant, or if they altered the effect of other covariates.

RESULTS

Descriptive Results

Eighty villages were assessed for eligibility and 24 were excluded due to lack of road access (n=9), the Development Agent (DA) was either too inexperienced or new to village (n=2), inadequate villager records (n=3) or due to the sharing of a major market (n=10). From the remaining 56 villages 32 were randomly selected and interventions randomly assigned (Fig. 1). A total of 516 participants from 32 villages undertook the pre-intervention questionnaire; 504 of these participants undertook the post-intervention questionnaire, a response rate of 98%. Reasons for seven of the 12 participants who were lost at follow-up were obtained; these included personal and family illness, funeral attendance and late arrival at the follow-up visit

(Fig. 1). There was no significant difference in the proportion of participants lost across intervention groups.

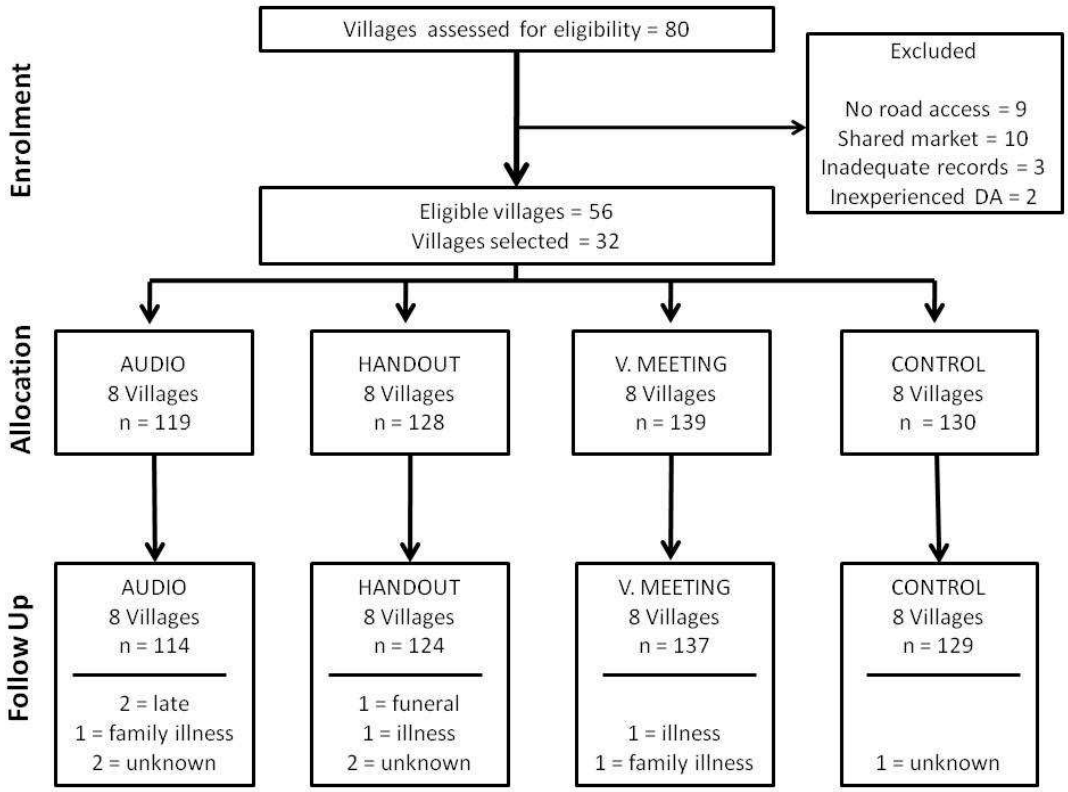

Fig. 1 c-RCT flow diagram indicating number of participants and villages at each stage of the trial.

Table 2. Age and pre-intervention score, and comparison across intervention groups for 516 participants in a c-RCT in Oromia region, Ethiopia.

Variable		Overall	INTERVENTION				Kruskal-Wallis P value
			Control	Audio	Handout	Village Meeting	
Age	Mean	45.66	44.45	48.85	42.27	47.01	0.01
	Median	45.00	43.00	48.00	40.00	48.00	
	Percentiles (25)	33.00	33.00	36.00	32.00	32.00	
	Percentiles (75)	57.00	54.25	60.00	50.75	60.00	
Pre-intervention Score	Mean	6.28	6.40	6.69	5.95	6.10	0.08
	Median	6.00	6.75	7.00	6.00	6.00	
	Percentiles (25)	5.00	5.00	5.00	5.00	4.00	
	Percentiles (75)	8.00	8.00	8.00	7.00	8.00	

Baseline information on participants (Tables 2 and 3) revealed low formal levels of education, with the majority of participants having only attended formal schooling until primary level. The majority owned one donkey, with a large majority owning cattle and oxen as well. Baseline information revealed low levels of literacy, with a greater proportion of participants unable to read Oromo than Amharic. The majority of the participants listened to the radio daily.

Table 3. Baseline information and comparison across intervention groups for categorical data 516 participants in a c-RCT in Oromia region, Ethiopia.

Variable		Overall	INTERVENTION				Chi-square P value
			Control	Audio	Handout	Village Meeting	
		(%)	n (%)	n (%)	n (%)	n (%)	
Education level	No Education	24.6	26 (20)	30 (25)	34 (27)	37 (27)	0.6
	Adult Education only	14.5	14 (11)	21 (18)	19 (15)	21 (15)	
	Primary	33.3	49 (38)	33 (28)	40 (31)	50 (36)	
	Junior	13.8	19 (15)	18 (15)	15 (12)	19 (14)	
	Higher	13.6	22 (17)	17 (14)	19 (15)	12 (9)	
	Other (Advanced)	0.2	0 (0)	0 (0)	1 (1)	0 (0)	
Literacy (Oromo)	No	78.5	101 (78)	99 (83)	102 (80)	103 (74)	0.4
	Yes	21.5	29 (22)	20 (17)	26 (20)	36 (26)	
Literacy (Amharic)	No	44.6	50 (39)	53 (45)	56 (44)	71 (51)	0.2
	Yes	55.4	80 (62)	66 (56)	72 (56)	68 (49)	
Listen to radio daily	No	20.0	25 (19.2)	23 (19.3)	19 (14.8)	36 (25.9)	0.2
	Yes	80.0	105 (80.8)	96 (80.7)	109 (85.2)	104 (74.1)	
Number of donkeys	0	6.0	6 (4.6)	8 (6.7)	7 (5.5)	10 (7.2)	0.04
	1	52.1	60 (46.2)	79 (66.4)	60 (46.9)	70 (50.4)	
	2	27.9	43 (33.1)	22 (18.5)	41 (32.0)	38 (27.3)	
	3	10.3	19 (14.6)	6 (5.0)	13 (10.2)	15 (10.8)	
	>3	3.7	2 (1.5)	4 (3.4)	7 (5.5)	6 (4.3)	
Own horse	No	71.1	71 (54.6)	76 (63.9)	109 (85.2)	111 (79.9)	<0.001
	Yes	28.9	59 (45.4)	43 (36.1)	19 (14.8)	28 (20.1)	
Own mule	No	97.5	124 (95.4)	116 (97.5)	128 (100)	135 (97.1)	0.1
	Yes	2.5	6 (4.6)	3 (2.5)	0 (0)	4 (2.9)	
Own cattle/ox	No	6.4	5 (3.8)	6 (5.0)	11 (8.6)	11 (7.9)	0.3
	Yes	93.6	125 (96.2)	113 (95.0)	117 (91.4)	128 (92.1)	
Own sheep	No	37.0	33 (25.4)	37 (31.1)	69 (53.9)	52 (37.4)	<0.001
	Yes	63.0	97 (74.6)	82 (68.9)	59 (46.1)	87 (62.2)	
Own goat	No	74.2	107 (83.3)	96 (80.7)	82 (64.1)	98 (70.5)	<0.001
	Yes	25.8	23 (17.7)	23 (19.3)	46 (35.9)	41 (29.5)	
Own dog	No	27.9	18 (13.8)	35 (29.4)	36 (28.1)	55 (39.6)	<0.001
	Yes	72.1	112 (86.2)	84 (70.6)	92 (71.9)	84 (60.4)	
Own Poultry	No	21.9	30 (23.1)	25 (21.0)	22 (17.2)	36 (25.9)	0.4
	Yes	78.1	100 (76.9)	94 (79.0)	106 (82.8)	103 (74.1)	

Baseline comparison of randomisation

Analysis of baseline data to check the randomisation process showed that a number of variables (age, number of donkeys owned and ownership of horses, sheep, goats and dogs) were significantly different between intervention groups (Tables 2 and 3) and pre-intervention scores approached significance. Further analysis revealed that the pre- intervention scores of the audio group were significantly higher than those of both the handout and village meeting groups. Baseline comparison of age amongst the intervention groups revealed significant differences, with the audio group being older than the control and handout intervention groups. The village meeting intervention group was significantly older than the handout intervention group. The effects of these differences were explored in the multivariable analysis.

Multilevel linear regression analysis

All interventions significantly improved the overall change in score between pre- and post-intervention questionnaires compared to the control (Table 4), with the handout and village meeting having a significantly greater impact than the audio programme. The initial model only considered the interventions (Model 1). The final model (Model 2) considered all intervention types and those covariates which were significant at baseline comparison. The only covariates shown to have a significant effect on the outcome were age and pre-intervention score. The higher the pre-intervention score and the older the age of participant, the less the change in score at follow-up. However, although both had a significant effect on the outcome (change in score) they had a minimal effect on the intervention coefficients (Table 4).

Table 4. Multilevel linear regression models showing the impact of different interventions on a change in score between questionnaires in 504 participants in a c-RCT in Oromia region, Ethiopia.

	Model 1		Model 2	
Intervention	Coefficient (S.E)	P value	Coefficient (S.E)	P value
Control (intercept)	0.6		0.6	
Audio	4.8 (0.6)	<0.001	5.2 (0.6)	<0.001
Handout	9.5 (0.6)	<0.001	9.2 (0.5)	<0.001
Village meeting	9.7 (0.6)	<0.001	9.7 (0.5)	<0.001
Age (years) [a]			-0.07 (0.009)	<0.001
Pre-intervention score [a]			-0.4 (0.06)	<0.001
Village variance	0.5 (0.3)		0.6 (0.3)	
Individual variance	10.7 (0.7)		9.3 (0.6)	

[a] Indicates variables were centred. Therefore the intercept represents the change in score for controls of average age and average pre-intervention score.

DISCUSSION

To the authors' knowledge this is the first study to evaluate the effectiveness of different knowledge-transfer interventions for adult learning amongst working equid users in a developing country. All interventions improved post-intervention knowledge of the target audience, however, the handout and village meeting improved these scores by approximately twice as much as the audio programme. The final model showed that the age of the participant

and pre-intervention score (baseline knowledge) had an effect on the outcome variable (change in score) but these had a minimal effect on the efficacy of the intervention. This is consistent with another study, suggesting that older age groups have a lower literacy proficiency than younger adults (Desjardins, 2003), which could lead to a reduced ability for knowledge acquisition. There are few comparable studies in the literature which utilise randomised controlled trials to show the efficacy of different knowledge-transfer interventions, however, our results are supported by one study that shows an increase in knowledge with a variety of interventions amongst Tanzanian smallholder dairy farmers (Bell et al., 2005).

The use of defined learning objectives to guide the design and development of all three interventions was essential to ensure that the core content of each was consistent, and that their effect on knowledge change could then be objectively evaluated (Ramhani, 2006). Learning objectives can provide an explicit overview of both what 'the learner has achieved' and 'what should be assessed' at the end of an educational programme, and it is recommended that they are formulated at the start of an educational programme to aid robust curriculum design and delivery (Harden, 2002).

Both the formal education levels and literacy levels of the target audience were expected to be low, and this was considered during the design and development phase of the interventions prior to commencement of the c-RCT. There were also concerns over the visual literacy ability of our target audience. Research carried out during both the PSA and the piloting of interventions identified low levels of literacy ability amongst the target audience in both regional languages (Oromo or Amharic), suggesting that a more diagrammatic/pictorial intervention was required. The success of the handout in this study suggests that the considerable efforts made during the piloting and development phase resulted in the production of a handout that was understandable and appropriate to the visual literacy of the study participants.

The village meeting provided the largest change in knowledge of the three study interventions. The combination of an oral presentation with demonstrations and visual images were likely to have accommodated all levels of literacy, visual literacy and language issues. The participants in our study revealed low levels of literacy, with a greater percentage of participants unable to read Oromo than Amharic. Despite this, our interventions were designed in Oromo as this is the official language of the region, the language currently being taught at school and the language that the majority of our target audience communicate in (even if illiterate in this language). However, the opportunity for a question and answer session within this format allowed participants to clarify any areas of confusion or any missed messages in either language.

The audio programme was designed to simulate a possible future radio broadcast. Previous studies have shown high radio ownership amongst households within Ethiopia with regular radio listeners (Farr et al., 2005) and this was consistent with our findings which showed that 80% of participants listened to a radio on a daily basis. Formats using a drama performed by local actors were shown to be most popular amongst farmers listening to agricultural extension programmes (Chapman et al., 2003) and the effect of this format shown to be greatest among uneducated (no formal education) individuals (Valente et al., 1994). This concurs with the findings in this study that showed a greater improvement in knowledge change amongst participants with a lower pre-intervention score, with those with the lowest pre-intervention score being those with the lowest levels of education. Although the audio programme in this study had the least impact on change in knowledge when compared to the two other interventions it still significantly improved knowledge. The potential benefits of a successful audio intervention may be the ability to 'reach' thousands of listeners with relative ease of administration and low cost, which may

outweigh or complement the greater knowledge impacts of the more labour intensive interventions.

All data in this study were gathered via questionnaire interviews with participants in either of two regional languages (Oromo or Amharic). Although the questionnaires utilised closed questions, the accuracy of all the data must be considered carefully, especially as the information required by the authors was translated. Reliability of participant information was not validated and may be imperfect or biased by participant reporting of perceived correct answers. However due to the study design (c-RCT), we would expect this bias or measurement error to be randomised across all participants, in all intervention groups, and therefore to have minimal effect on the estimates of the effects of interventions. Randomisation is designed to equally distribute potentially confounding factors across intervention groups; whether or not to present adjusted or unadjusted results is a subject of active debate (Dohoo et al., 2003). Results presented in this study have been adjusted for potential confounders and are presented in Model 2 which shows that they had a minimal effect on the interventions. Use of a control group allowed us to monitor participants for the 'Hawthorne Effect' (that a participant in the control group receiving a pre-intervention questionnaire would subsequently have a greater level of knowledge at the follow up visit) (Campbell et al., 1995). Little evidence of the 'Hawthorne Effect' was seen in this study as the average improvement in the control group was only 0.6 marks and was not significant.

In future, knowledge-transfer interventions developed for rural equid users in this region of Ethiopia should consider the formal education level, literacy/visual literacy ability and age of audience as key issues, and should be thoroughly piloted and refined before final release. Although this study showed that direct contact with a specifically trained animal health worker, in combination with a mixture of demonstration, presentation and question and answer session was the most effective knowledge transfer method, interventions based on visual images, designed and piloted with the intended audience, have also been shown to be successful. Ethiopia, with its large population of equids, is ideally placed to benefit from appropriate education or extension programmes for the owners and users of equids. The results from this study may be beneficial to other populations of livestock owners, particularly in sub-Saharan Africa, however, it is likely that different issues associated with learning across different communities may exist, and these must be carefully considered when designing education programmes.

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REFERENCES

Alemayehu, M., Pearson, R.A., Bekure, T. and Kebede, G. (2000). Survey on Donkey uses in urban and rural areas of the central parts of Ethiopia. In: Smith, D., Tesfaye, A., More, L.

(Eds.), Alleviating poverty in peri-urban Ethiopia by improving the health, welfare and management of donkeys., Debre Zeit, Ethiopia, pp. 11-15.

Anon. (2007). FAO Statistical database website. Food and Agricultural Organisation of the United Nations. http://faostat.fao.org/. Accessed 22 October 2009.

Bell, C.E., French, N.P., Karimuribo, E.D., Ogden, N.H., Bryant, M.J., Swai, E.M., Kambarage, D.M. and Fitzpatrick, J.L. (2005). The effects of different knowledge-dissemination interventions on the mastitis knowledge of Tanzanian smallholder dairy farmers. Prev. Vet. Med. 72, 237-251.

Biffa, D. and Woldemeskel, M. (2006). Causes and factors associated with occurence of external injuries in working equines in Ethiopia. Int. J. Appl. Res. Vet. Med. 4, 1-7.

Burn, C.C., Pritchard, J.C., Farajat, M., Twaissi, A.A.M. and Whay, H.R. (2007). Risk factors for strap-related lesions in working donkeys at the World Heritage Site of Petra in Jordan. Vet. J. 178, 263-271.

Campbell, J.P., Maxey, V.A. and Watson, W.A. (1995). Hawthorne Effect - implications for prehospital research. Ann. Emerg. Med. 26, 590-594.

Campbell, M.K., Thomson, S., Ramsay, C.R., MacLennan, G.S. and Grimshaw, J.M. (2004). Sample size calculator for cluster randomized trials. Comput. Biol. Med. 34, 113-125.

Chapman, R., Blench, R., Kranjac-Berisavljevic, G. and Zakariah, A.B.T. (2003). Rural radio in agricultural extension: The example of vernacular radio programs on soil and water conservation in N.Ghana., Agren: Agricultural Research and Extension Network Paper, 127. ODI, London.

Curran, M.M., Feseha, G. and Smith, D.G. (2005). The impact of access to animal health services on donkey health and livelihoods in Ethiopia. Trop. Anim. Health Prod. 37, 47-65.

Dawson, A. J. and Joof, B.M. (2005). Seeing, thinking and acting against malaria: a new approach to health worker training for community empowerment in rural Gambia. Rural Remote Health (online). 5 (353), 1-6.

Desjardins, R. (2003). Determinants of literacy proficiency: a lifelong-lifewide learning perspective. Int. J. Edu. Res. 39, 203-245.

DFID (Department for International Developement) (2006). DFID Ethiopia Country Assistance Plan, 2006-10, Consultation draft. www.dfid.gov.uk.

Dohoo, I., Martin, W. and Stryn, H. (2003). Veterinary Epidemiological Research. AVC Inc., Charlottetown, Prince Edward Island.

Eaden, J., Abrams, K., Shears, J. and Mayberry, J. (2002). Randomized controlled trial comparing the efficacy of a video and information leaflet versus information leaflet alone on patient knowledge about surveillance and cancer risk in ulcerative colitis. Inflamm. Bowel Dis. 8 (6), 407-412.

Farr, A.C., Witte, K., Jarato, K. and Menard, T. (2005). The effectiveness of media use in health education: Evaluation of an HIV/AIDS radio campaign in Ethiopia. J. Health Comm. 10 (3), 225-235.

Garuma, S., Lemecha, F., Sisay, A. and Jemal, E. (2007). Study on gender distribution of ownership of animal-drawn carts and its effect on women's life in Adami Tulu and Dugda Bora districts. Draught Animal News. CVTM, Edinburgh, pp. 29-34.

Gebreab, F. (1993). Use of Equines in Ethiopia. In: Proceedings of the Fourth National Livestock Improvement Conference. Addis Ababa, Ethiopia, pp. 51-58.

Harden, R.M. (2002). Learning Outcomes and Instructional Objectives: is there a difference? Med. Teach. 24, 151-155.

Harvey, B., Stuart, J. and Swan, T. (2000). Evaluation of a drama-in-education programme to increase AIDS awareness in South African high schools: a randomized community intervention trial. Int J STD AIDS 11, 105-111.

Kelly, N.R., Huffman, L.C., Mendoza, F.S. and Robinson, T.N. (2003). Effects of a videotape to increase use of poison control centers by low-income and Spanish-speaking families: a randomized, controlled trial. Pediatrics 111, 21-26.

Klepp, K.I., Ndeki, S.S., Leshabari, M.T., Hannan, P.J. and Lyimo, B.A. (1997). AIDS education in Tanzania: promoting risk reduction among primary school children. Am J Public Health 87, 1931-1936.

Marshall, K. and Ali, Z. (1997). Gender issues in donkey use in rural Ethiopia. In: Starkey, P., Fielding, D., (Eds.), Donkeys, People and development, a resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA). pp. 62-68.

Pearson, R.A., Simalenga, T.E. and Krecek, R.C. (2003). Harnessing and Hitching Donkeys, Mules and Horses for Work. CVTM, University of Edinburgh.

Pritchard, J.C., Lindberg, A.C., Main, D.C.J. and Whay, H.R. (2005). Assessment of the welfare of working horses, mules and donkeys, using health and behaviour parameters. Prev. Vet. Med. 69, 265-283.

Ramhani, S. (2006). Twelve Tips to promote excellence in medical teaching. Med. Teach. 28, 19-23.

Rimm, M. (2003). Extension materials for meat-borne parasitic diseases in developing countries. Acta Trop. 87, 171-175.

Sells, P.D., Pinchbeck, G.L., Mezzane, H., Ibourki, J. and Crane, M. (2009). Pack wounds of donkeys and mules in the Northern High Atlas and lowlands of Morocco. Equine Vet. J. *In press.*

de Silva, D.N. and Garforth, C.J. (1997). The effectiveness of different radio programme formats for the dissemination of information on the safe use of insecticides in paddy cultivation in Mahaweli system-C of Sri Lanka. Trop. Agric. Res. 9, 302-316.

Soldan, V.A.P. (2004). How family planning ideas are spread within social groups in rural Malawi. Stud. Family Plann. 35, 275-290.

Stringer, A.P., Christley, R.M., Bell, C.E., Gebreab, F., Tefera, G., Reed, K., Trawford, A. and Pinchbeck, G.L. (2009). Owner perceptions of working equid health and disease in Ethiopia - a Participatory Situation Analysis (PSA). Poster: Society for Veterinary Epidemiology and Preventive Medicine, London, available at: www.svepm.org.uk.

Svendsen, E.D. (1994). The main types of parasites affecting donkey's health in various parts of the world. In: Bakkoury, M., Prentis, R.A. (Eds.), Second International Colloquim on Wokring Equines, Rabat, Morocco.

Tesfaye, A. and Curran, M.M. (2005). A longitudinal survey of market donkeys in Ethiopia. Trop. Anim. Health Prod. 37, 87-100.

Tesfaye, A., Jemal, I., Ferede, S. and Curran, M.M. (2005). Technology transfer pathways and livelihood impact indicators in central Ethiopia. Trop. Anim. Health Prod. 37, 101-122.

Tesfaye, A. and Smith, D.G. (2000). Comparison of donkey owners and non-owners in the household economy. In: Smith, D.G., Tesfaye, A., More, L. (Eds.), Alleviating poverty in peri-urban Ethiopia by improving the health, welfare and mananagement of donkeys, Debre Zeit, Ethiopia. pp 76-92.

Thomas, D.M., Ray, S.M., Morton, F.J., Drew, J.S., Offutt, G., Whitney, C.G. and Jacobson, T.A. (2003). Patient education strategies to improve pneumococcal vaccination rates: randomized trial. J. Investig. Med. 51, 141-148.

Tu, N.V. and Giang, T.T. (2002). Improving the efficiency of aquaculture extension activity in the southeastern provinces of Southern Vietnam. In: Edwards, P., Little, D.C., Demaine, H. (Eds.), Rural aquaculture. CABI Publishing, Wallingford, pp. 285-300.

Valente, T.W., Kim, Y.M., Lettenmaier, C., Glass, W. and Dibba, Y. (1994). Radio promotion of family planning in The Gambia. International Family Planning Perspectives 20, 96-100.

Yilma, J.M., Gebreab, F.A., Svendsen, E.D. and Mohammed, A. (1991). Health Problems of Working Donkeys in Debre Zeit and Menagesha Regions of Ethiopia. In: Fielding, D., Pearson, R.A. (Eds.), Donkeys, Mules and Horses in Tropical Agricultural Development, CVTM, Edinburgh. pp 151-155.

LONGITUDINAL STUDY OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* IN THE FAECES OF HORSES ADMITTED TO AN EQUINE HOSPITAL

T. MADDOX*, N.J. WILLIAMS, P. CLEGG, A. O'DONNELL, S. DAWSON AND G. PINCHBECK

SUMMARY

The increasing prevalence of antimicrobial resistant bacteria represents a significant problem for human and veterinary medicine, with resistance in *Escherichia coli* from horses documented in commensal and pathogenic bacterial strains. A longitudinal study of 103 horses in an equine hospital was conducted, with faecal samples cultured for antimicrobial resistant *E. coli*. High sample prevalence for resistant *E. coli* was identified for several antimicrobials. The prevalence of resistance was lower at admission, rising on days 2 and 4 post admission. Risk factors were identified using multilevel, multivariable modelling and included the day the sample was obtained, antimicrobial treatment in the previous seven days, location within the hospital and the total daily dosages of cotrimoxazole prescribed in the hospital. High levels of multidrug resistant (49.2% of samples) and extended spectrum β-lactamase producing *E. coli* (28.7% of samples) were recovered; such bacteria could complicate treatment if they were the cause of infection.

INTRODUCTION

Escherichia coli is a Gram-negative bacteria of the *Enterobacteriaceae* family and part of the normal commensal gastrointestinal flora of most animals and humans, including horses (van Duijkeren et al., 2000). As a commensal, *E. coli* is frequently exposed to antimicrobial agents used in the treatment of infections caused by other organisms, potentially allowing it to acquire resistance determinants and act as a reservoir for resistance genes (Hart et al., 2006; Karami et al., 2007).

Antimicrobial resistance amongst bacteria is recognised as an important and increasing problem in human and veterinary medicine, with significant economic implications, as well as increased patient morbidity and mortality (Paladino et al., 2002). Resistance in *E. coli* isolated from animals was identified as an emerging problem over forty years ago (McKay et al., 1965). Shortly after this, multidrug antimicrobial resistance (resistance to three or more antimicrobial drug classes) was identified in equine *E. coli* isolates, in addition to extensive resistance to tetracycline (Harihara & Barnum, 1973). Multidrug resistant (MDR) bacteria, including *E. coli*, have been the cause of disease outbreaks in equine hospitals on several occasions (Ward et al., 2005; van Duijkeren et al., 2009).

*Thomas W. Maddox, National Centre for Zoonosis Research, School of Veterinary Sciences, University of Liverpool, UK, CH64 7TE. E-mail: Thomas.Maddox@liverpool.ac.uk

Bacterial resistance to antimicrobials can be intrinsic or acquired. Intrinsic resistance is the consequence of a structural or functional trait allowing tolerance of an antimicrobial drug by all members of a bacterial group; it is usually expressed by chromosomal genes and vertically inherited. Acquired resistance is a trait associated with only some strains of a bacterial species or genus and is due to genetic change in the bacterial genome. This may be via chromosomal mutation or more usually by horizontal acquisition of foreign genetic material, including resistance genes on conjugative plasmids or other mobile genetic elements (Hall & Collis, 1995).

Extended spectrum β-lactamase (ESBL) enzymes produced by *E. coli* represent a resistance mechanism of particular significance, providing resistance to β-lactam antimicrobials, including the third-generation cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime). The majority of ESBL enzymes are mutations derived from the classical TEM or SHV β-lactamases (Payne et al., 1989) and, being plasmid-borne, have become widespread within many bacteria species. However, cefotaximase enzymes (CTX-M) represent a distinct class of ESBLs, which have become prevalent in *E. coli* from humans and other animals (Liebana et al., 2006).

Several factors have been found to be associated with antimicrobial resistant *E. coli* in animals. The use of antimicrobial drugs is generally accepted to increase the prevalence of resistance; some environmental factors and increased age (for very young animals) have also been associated (Berge et al., 2005). The use of some antimicrobial drugs and hospitalisation were found to be associated with antimicrobial resistance in 216 horses in a North American study (Dunowska et al., 2006).

The aim of this study was to determine the prevalence of antimicrobial resistant *E. coli* in horses on entry to an equine hospital and during hospitalisation and to identify risk factors associated with its presence.

MATERIALS AND METHODS

Study overview

Repeated faecal samples were collected from horses in an equine hospital over an 18-month period (from December 2007 through May 2009). Antimicrobial resistant *E. coli* were isolated from samples and investigated to determine their susceptibility to a panel of seven antimicrobial agents. The presence of resistance in samples was analysed using multilevel multivariable modelling.

Study population

The study population consisted of horses admitted to the University of Liverpool's Philip Leverhulme Equine Hospital (PLEH). Horses admitted for less than 48 hours were excluded, as were patients undergoing radioactive gamma scintigraphic examination, un-weaned foals and mares with un-weaned foals. Recruitment days were selected on a convenience basis, but on these days all eligible horses were enrolled. Informed ethical consent was obtained from all owners.

The primary study hypothesis was that exposure to antimicrobial treatment in the hospital would increase the odds of a horse having antimicrobial resistant *E. coli* in its subsequent faecal

samples. Sample size estimates for a study investigating horses exposed and non-exposed to antimicrobials were conducted using the EpiInfo software package (EpiInfo version 6 EpiInfo 6, CDC & WHO, Geneva). Assuming a ratio of 1:2 for untreated:treated with antimicrobials and an expected prevalence of antimicrobial resistance of 50% in the unexposed group (as pilot studies indicated prevalence would be high), a total of 144 horses would be required to detect odds ratios of three or more, with 95% confidence and 80% power.

Sample collection

A fresh faecal sample was obtained from each horse within 12 hours of admission and then subsequently every two days until discharge from the hospital. Signalment data, admission reason, previous veterinary history, details of the horse's home yard and management were collected by a questionnaire administered to the owner.

Information from hospital database records

Whilst hospitalised, details of drug administration, veterinary procedures and location within the hospital were recorded for each horse. From the PLEH clinical records system the total number of horses hospitalised and the total number of defined daily doses (DDD) prescribed for all antimicrobials were determined for each day of the study period. DDD per day was calculated as the daily total amount of an antimicrobial prescribed divided by the total daily dose required for an average (500kg) horse; adapted from the standard World Health Organisation calculation used for determining defined daily dosages for human drugs (WHO, 2007).

Sample processing

From the samples, 2.5g of faeces was added to a 10ml volume of brain-heart infusion broth containing 5% glycerol and mixed using a stomacher (Stomacher 80, Seward). The surface of one eosin methylene blue agar (EMBA) plate and one MacConkey agar plate were inoculated for confluent bacterial growth and seven antimicrobial impregnated discs (MAST Ltd, UK) were applied to the surface, in accordance with the direct plating method of Bartoloni et al. (2006). The antimicrobials used and their potencies were: ampicillin (10µg), co-amoxiclav (30µg) ciprofloxacin (1µg), gentamicin (10µg), nalidixic acid (30µg), tetracycline (30µg) and trimethoprim (2.5µg). The faecal suspension was streaked onto three further EMBA plates; one plate containing the cephalosporin cefotaxime (1µg/ml), one containing ceftazidime (1µg/ml), and one with no antimicrobials incorporated. After incubation, bacterial growth morphologically consistent with *E. coli* was subcultured for further testing. From the MacConkey and EMBA plates with antimicrobial discs applied, any colonies growing inside the general inhibition zone of each antimicrobial disc were selected. At least one colony was taken from each of the EMBA plates with cefotaxime and ceftazidime (if bacterial growth had occurred), and three colonies were randomly selected from the EMBA plate with no antimicrobial.

Antimicrobial sensitivity testing

Isolates were prepared for antimicrobial sensitivity testing in accordance with British Society for Antimicrobial Chemotherapy (BSAC) guidelines. The surface of one Iso-Sensitest agar plate was inoculated for semi-confluent bacterial growth and the same seven antimicrobial discs detailed previously were applied. After incubation, the diameter in millimetres of the zones of inhibition around each of the antimicrobial discs was recorded.

Suspected ESBL-producing isolates taken from the EMBA plates containing cefotaxime or ceftazidime were subjected to the paired disc diffusion test (MAST Ltd, UK) in accordance with the method of M'Zali et al. (2000). An Iso-Sensitest agar plate was inoculated for confluent bacterial growth and three pairs of antimicrobial discs were applied to its surface. The antimicrobial pairs used and their potencies were: cefpodoxime (30μg) and cefpodoxime/clavulanic acid (30/10μg), ceftazidime (30μg) and ceftazidime/clavulanic acid (30/10μg), cefotaxime (30μg) and cefotaxime/clavulanic acid (30/10μg). An increase in inhibition zone size of 5mm or more in the presence of clavulanic acid for any or all of the antimicrobial pairs indicates confirmation of ESBL production.

Identification of *E. coli*

Isolates with biochemical profiles consistent with *E. coli* were confirmed by polymerase chain reaction (PCR) assay for the *E. coli* specific *uidA* gene (McDaniels et al., 1996).

Statistical analysis

Independent (risk factor) variables were derived from information obtained from owner questionnaires, clinical and hospital records, and are detailed in Tables 1-3.

Faecal samples were considered the level one unit of interest, the binary outcome for each sample was the presence or absence of an *E. coli* isolate with resistance to one of seven antimicrobials. Resistance to each of the seven antimicrobials was considered as a separate outcome. Additionally, the presence in a sample of an *E. coli* with multidrug resistance (to three or more antimicrobial classes) or with ESBL-mediated resistance were considered as two further outcomes. Due to repeated measures, data were clustered within horses (level two units); therefore, factors affecting the occurrence of antimicrobial resistant *E. coli* were examined using separate multivariable, multilevel models with a binomial distribution and logit link function. Within-horse clustering was accounted for as a random intercept in all models. In order to make allowance for the autocorrelation of repeated measures data, an additional variable was created (resistance in previous sample), defined as the presence or absence of the resistance outcome in the preceding faecal sample.

For continuous variables with *P*-value <0.25, the functional form of the variable with respect to each outcome was assessed using generalised additive models (GAM). The GAM models were fitted using cubic spline smoothers in the S-Plus software package (S-plus 2000, Mathsoft Inc). The functional forms of the relationships were then used to inform the polynomial fits (of centred data) in the multivariable logistic regression models, which were then tested for significance.

All variables that showed some association with the presence of resistant *E. coli* in univariable analysis (a *P*-value <0.25) were considered for incorporation into a final multivariable model for that outcome. For any pair of variables with a correlation coefficient of $\geq$0.70, only the variable with the smallest *P*-value was considered for further analysis. The final models were constructed by a manual backwards stepwise procedure where variables with a Wald *P*-value <0.05 were retained in the model. The resistance in previous sample variable was retained for all final models. First order interaction terms were tested for biologically plausible variables remaining in the final models.

Data were analysed using the MLwiN statistical software package (MLwiN Version 2.1 Centre for Multilevel Modelling, University of Bristol). Initial univariable and multivariable calculations were performed using penalised quasi-likelihood estimates (2^{nd} order PQL). The final models were fitted using Monte Carlo Markov Chain utilising Metropolis Hasting sampling with diffuse priors, a burn in period of 10,000 iterations and a run of 500,000 iterations. The number of iterations required was determined by examining the MCMC diagnostics, including the Brooks-Draper and Rafferty-Lewis Nhat statistics (Browne, 2009).

Table 1. Dichotomous variables considered for inclusion in the final multilevel multivariable models, with the number and percentage of samples in each category.

Variable	Number of samples (%)	
	No	Yes
Any antibiotic on sampling day	332 (72.6)	125 (27.4)
Penicillin on sampling day	376 (82.3)	81 (17.7)
Gentamicin on sampling day	449 (98.2)	8 (1.8)
Trimethoprim on sampling day	416 (91.0)	41 (9.0)
Ceftiofur on sampling day	453 (99.1)	4 (0.9)
Enrofloxacin on sampling day	449 (98.2)	8 (1.8)
Any antibiotic in previous 2 days	313 (68.5)	144 (31.5)
Penicillin in previous 2 days	351 (76.8)	106 (23.2)
Gentamicin in previous 2 days	448 (98.0)	9 (2.0)
Trimethoprim in previous 2 days	411 (89.9)	46 (10.1)
Ceftiofur in previous 2 days	453 (99.1)	4 (0.9)
Enrofloxacin in previous 2 days	450 (98.5)	7 (1.5)
Any antibiotic in previous 7 days	284 (62.1)	173 (37.9)
Penicillin in previous 7 days	308 (67.4)	149 (32.6)
Gentamicin in previous 7 days	443 (96.9)	14 (3.1)
Trimethoprim in previous 7 days	401 (87.7)	56 (12.3)
Ceftiofur in previous 7 days	450 (98.5)	7 (1.5)
Enrofloxacin in previous 7 days	451 (98.7)	6 (1.3)
Surgical procedure in 24hr prior to sample	435 (95.2)	22 (4.8)
Surgical procedure in 48hr prior to sample	401 (87.7)	56 (12.3)
Prior hospitalisation in previous 3 months	405 (88.6)	52 (11.4)

Table 2. Categorical variables considered for inclusion in the final multilevel multivariable models, with the number and percentage of samples in each category.

Variable	Categories	Number of samples (%)
Day of hospitalisation	Day 0	103 (22.5)
	Day 2	103 (22.5)
	Day 4	99 (21.7)
	Day 6	71 (15.5)
	Day 7 or later	81 (17.7)
Sex	Female entire	150 (32.8)
	Male entire	25 (5.5)
	Male neutered	282 (61.7)
Admission condition	Musculoskeletal	140 (30.6)
	Medical	120 (26.3)
	Gastrointestinal (medical)	162 (35.4)
	Gastrointestinal (surgical)	16 (3.5)
	Soft tissue surgery	19 (4.2)
Hospital yard on sampling day	Medical yard	163 (35.7)
	Orthopaedic yard	22 (4.8)
	Overflow yard	44 (9.6)
	Colic/intensive care	39 (8.5)
	Pony yard	46 (10.1)
	Mixed yard	132 (28.9)
	Temporary yard	11 (2.4)

Table 3. Continuous variables considered for inclusion in the final multilevel multivariable models, with the median values and interquartile range for each variable.

Variable		Median Value (IQR)
Antimicrobial doses prescribed in hospital:	0-24hr prior to sample	6.6 DDD (0, 29.6)
	24-48hr prior to sample	8.7 DDD (0, 35.8)
Penicillin doses prescribed in hospital:	0-24hr prior to sample	0.8 DDD (0, 8.2)
	24-48hr prior to sample	1.5 DDD (0, 8.2)
Cotrimoxazole doses prescribed in hospital:	0-24hr prior to sample	0 DDD (0, 11)
	24-48hr prior to sample	0 DDD (0, 13)
Gentamicin doses prescribed in hospital:	0-24hr prior to sample	0 DDD (0, 1.9)
	24-48hr prior to sample	0 DDD(0, 2.1)
Total number of horses hospitalised:	0-24hr prior to sample	23 horses (8, 30)
	24-48hr prior to sample	22 horses (8, 30)
Age of horse		6 years (9,12)
Number of horses on home yard		15 horses (8, 35)

IQR = interquatile range; DDD = defined daily doses (see Materials and Methods)

RESULTS

Descriptive statistics

In total 110 horses were enrolled in the study; seven were subsequently excluded as they remained hospitalised for only one night. A total of 457 faecal samples were collected from the remaining 103 horses; the median duration of hospitalisation was 6 days (IQR 2-4 days).

At least one antimicrobial resistant *E. coli* isolate was recovered from 309 of the faecal samples (67.6%; 95% confidence interval (CI) 63.3, 71.9). The total number of non-duplicate resistant *E. coli* isolated from all samples was 694, with a total of 42 distinct antimicrobial resistance phenotypes identified. The overall prevalence of resistant *E. coli* isolates in faecal samples was over 25% for all the antimicrobials examined except co-amoxiclav (8.1%). Multidrug resistant *E. coli* (resistant to three or more antimicrobial classes) were identified in 49.2% of samples. ESBL-producing *E. coli* were recovered from 131 samples (28.7%; 95% CI 24.5, 32.8). The sample and isolate prevalence of resistance to each antimicrobial, multidrug resistance and presence of an ESBL producing *E. coli* are detailed in Table 4.

Table 4. Number and percentage of isolates and samples with resistance to all antimicrobials tested, multidrug resistance and ESBL-mediated resistance.

	Isolates (n= 694)		Samples (n= 457)	
	No. resistant	% (95% CI)	No. resistant	% (95% CI)
Ampicillin	466	67.1 (63.7, 70.6)	232	50.8 (46.2, 55.3)
Co-amoxiclav	56	8.1 (6.0, 10.1)	36	7.9 (5.4, 10.3)
Ciprofloxacin	169	24.4 (21.2, 27.5)	121	26.5 (22.4, 30.5)
Gentamicin	298	42.9 (39.3, 46.6)	164	35.9 (31.5, 40.3)
Nalidixic acid	228	32.9 (29.4, 36.3)	147	32.2 (27.9, 36.4)
Tetracycline	418	60.2 (6.6, 63.9)	226	49.5 (44.9, 54.0)
Trimethoprim	644	92.8 (90.9, 94.7)	297	65.0 (60.6, 69.4)
Multidrug	422	60.8 (57.2, 64.4)	225	49.2 (44.7, 53.8)
ESBL mediated	142	20.5 (17.5, 23.5)	131	28.7 (24.5, 32.8)

95% CI = 95% confidence intervals

Prevalence figures for each day of admission are shown in Fig. 1. A distinct pattern of antimicrobial resistance during hospitalisation was shown for all drugs. On admission there was consistently low prevalence of resistance, with a sharp increase observed on days 2 and 4, and then a slight decrease for samples collected on day 6 or later.

Generalised Additive Models results

The GAMs constructed showed that total number of antimicrobials doses prescribed (DDD) in the previous 0-24 and 24-48 hours, and the total number of horses hospitalised in the previous 0-24 and 24-48 hours demonstrated a significant non-linear relationship (p <0.05) with some of the outcomes examined. Appropriate polynomial terms were considered during the multivariable analysis for the relevant outcomes, however these variables did not remain in the final models.

Univariable analysis

Univariable analysis found many of the variables described in Tables 1-3 to be significantly associated with the outcomes considered (data not shown). Individual horse antimicrobial treatment within the previous 24 hours, 48 hours and seven days were all significantly correlated with each other. For all outcomes considered, overall antimicrobial treatment in the previous seven days was determined as the best fit for the models. Similarly, antimicrobial defined daily dosages prescribed in the hospital in the previous 0-24 and 24-48 hours were significantly correlated, as were undergoing a surgical procedure in the previous 24 and 48 hours. The variable pertaining to exposure in the previous 48 hours proved to be the best fit for the model for all of these variables.

Variables including number of horses in the hospital in the 0-24 and 24-48 hours before sampling, and undergoing surgery in the 24 and 48 hours before sampling, were highly significant on univariable analysis (P <0.0001) but failed to remain in the final models. In addition age, sex and previous hospitalisation were only found to be significantly associated with outcomes considered on univariable analysis.

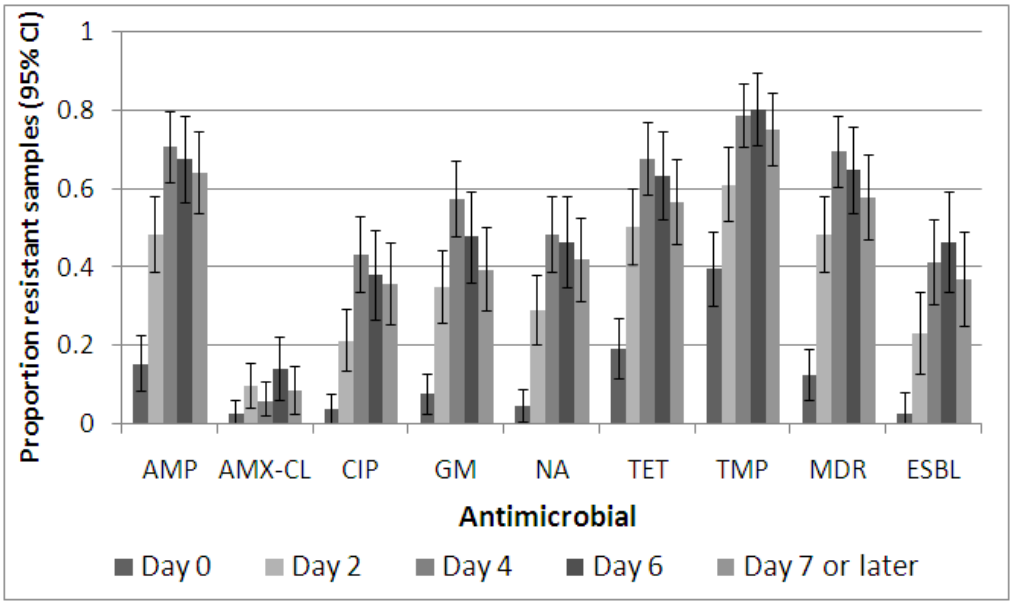

Fig. 1 Sample prevalence of antimicrobial, multidrug and ESBL-mediated resistances for each day of sampling in 457 faecal samples from 110 hospitalised horses. (AMP = ampicillin; AMX-CL = co-amoxiclav; CIP = ciprofloxacin; GM = gentamicin; NA = nalidixic acid; TET = tetracycline; TMP = trimethoprim; MDR = multidrug resistant, ESBL = ESBL mediated).

Multivariable analysis

The final multivariable, multilevel logistic regression models are shown in Tables 5 and 6. For all outcomes except trimethoprim resistance, the day the sample was obtained was significant, with increased risk of resistance for samples taken on day 2 or later. For all outcomes except ESBL-mediated resistance, having had antimicrobial treatment in the seven days prior to a sample also significantly increased the risk of resistance. The amount prescribed (DDD) of cotrimoxazole in the hospital in the 24-48 hours prior to a sample was a significant risk factor for all outcomes except ciprofloxacin, tetracycline, and ESBL-mediated resistance. Patients admitted for gastrointestinal surgery were at increased risk of nalidixic acid and particularly ciprofloxacin resistance. Horses in the orthopaedic yard were at increased risk of trimethoprim and ciprofloxacin resistant *E. coli* and those on the mixed yard were at increased risk of ESBL-mediated resistant *E. coli*.

As expected, there was significant clustering of resistance outcomes within horses; however, random slope effects were not significant, suggesting that there was no major difference in covariate effects in different horses. No significant interactions (Wald P-value <0.05) were found for the variables remaining in the final models. The MCMC diagnostics performed (Browne, 2009) indicated that the fits were smooth and regular, with adequate mixing of chains for all fixed effect variables. Sufficient iterations were performed to give certainty about the estimates for the model parameters.

Table 5. Results of multivariable multilevel analysis for the outcomes of tetracycline, ampicillin, multidrug and gentamicin resistance in 457 faecal samples from 110 horses admitted to the Philip Leverhulme Equine Hospital, University of Liverpool.

Variable		Tetracycline resistance			Ampicillin resistance			Multidrug resistance			Gentamicin resistance		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Day of sample	Day 0	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-
	Day 2	4.01	1.7, 9.45	<0.001	3.15	1.21, 8.21	0.02	5.18	1.81, 14.9	0.002	6.57	2.15, 20.1	<0.001
	Day 4	9.33	3.22, 27.0	<0.001	9.66	2.90, 32.3	<0.001	14.1	3.55, 55.9	<0.001	16.5	4.22, 64.8	<0.001
	Day 6	4.45	1.72, 17.2	0.004	6.21	1.69, 22.8	0.005	8.34	1.99, 34.9	0.003	9.69	2.31, 40.7	0.002
	Day 7+	3.06	1.04, 9.03	0.04	3.90	1.21, 12.6	0.02	4.27	1.16, 15.7	0.03	5.2	1.36, 19.9	0.02
Resistant at previous sample		1.96	0.86, 4.44	0.1	2.40	0.98, 5.86	0.05	2.49	0.96, 6.64	0.06	2.59	1.1, 6.08	0.03
Antimicrobial previous 7 days		4.17	1.97, 8.82	<0.001	3.64	1.77, 7.49	<0.001	3.54	1.67, 7.48	<0.001	3.63	1.73, 7.9	<0.001
Cotrimoxazole doses prescribed (DDD) in hospital previous 48hrs		-	-	-	1.02	1.01, 1.04	0.006	1.02	1.0, 1.03	0.04	1.02	1.0, 1.03	0.03
Variance (standard error)			2.0 (0.9)			2.2 (1.0)			2.6 (1.3)			1.9 (1.1)	

95% CI = 95% credible intervals; *P* values are from the Wald chi-squared test

Table 6. Results of multivariable multilevel analysis for the outcomes of nalidixic acid, ESBL-mediated, trimethoprim and ciprofloxacin resistance in 457 faecal samples from 110 horses admitted to the Philip Leverhulme Equine Hospital, University of Liverpool.

Variable		Nalidixic acid resistance			ESBL-mediated resistance			Trimethoprim resistance			Ciprofloxacin resistance		
		OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Day of sample	Day 0	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-
	Day 2	4.35	1.37, 13.8	0.01	27.1	5.63, 131	<0.001	0.89	0.39, 2.03	0.7	6.25	1.6, 24.3	0.008
	Day 4	9.0	2.8, 28.9	<0.001	97.8	17.2, 555	<0.001	2.65	1.0, 6.98	0.05	30.6	7.0, 134	<0.001
	Day 6	6.47	1.86, 22.5	<0.001	94.6	15.0, 595	<0.001	2.51	0.85, 7.4	0.1	17.9	3.63, 88.8	<0.001
	Day 7+	4.6	1.35, 15.7	0.01	36.2	6.42, 205	<0.001	1.42	0.52, 3.85	0.08	13.1	2.82, 61.2	0.001
Resistant at previous sample		3.69	1.84, 7.41	<0.001	1.25	0.48, 3.25	0.6	2.83	1.25, 6.39	0.01	1.11	0.42, 2.92	0.8
Antimicrobial previous 7 days		4.57	2.28, 9.18	<0.001	-	-	-	4.36	2.04, 9.33	<0.001	12.8	3.84, 42.5	<0.001
Cotrimoxazole doses prescribed (DDD) in hospital previous 48hrs		1.01	1.0, 1.03	0.04	-	-	-	1.02	1.01, 1.04	0.01	-	-	-
Admission condition	Soft tissue surgery	(Ref)	-	-	-	-	-	-	-	-	(Ref)	-	-
	Musculoskeletal	1.83	0.82, 4.07	0.1	-	-	-	-	-	-	3.69	0.94, 14.4	0.06
	Medical	0.69	0.34, 1.42	0.3	-	-	-	-	-	-	1.18	0.34, 4.18	0.8
	Gastrointestinal (surg)	7.06	1.29, 38.7	0.02	-	-	-	-	-	-	114	4.9, 2590	0.003
	Gastrointestinal (med)	1.72	0.39, 7.58	0.5	-	-	-	-	-	-	10.0	0.59, 169	0.11
Hospital yard	Medical yard	-	-	-	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-
	Orthopaedic yard	-	-	-	7.71	0.78, 76.0	0.08	4.94	1.13, 21.5	0.03	9.71	1.41, 66.9	0.02
	Overflow yard	-	-	-	2.59	0.39, 17.0	0.3	0.75	0.25, 2.21	0.6	0.57	0.10, 3.17	0.5
	Colic/intensive care	-	-	-	1.88	0.26, 13.3	0.6	2.45	0.67, 9.01	0.2	0.21	0.03, 1.74	0.2
	Pony yard	-	-	-	4.73	0.79, 28.3	0.09	1.96	0.64, 5.98	0.2	2.73	0.58, 12.9	0.2
	Mixed yard	-	-	-	11.6	2.75, 48.8	<0.001	1.22	0.56, 2.67	0.6	1.11	0.35, 3.55	0.9
	Temporary yard	-	-	-	4.23	0.39, 46.5	0.2	4.45	0.49, 39.8	0.2	4.06	0.49, 33.2	0.2
Variance (standard error)			0.3(0.4)			4.1 (1.9)			4.2 (1.7)			2.7 (1.46)	

95% CI = 95% credible intervals; *P* values are from the Wald chi-squared test

DISCUSSION

The overall prevalence of antimicrobial resistant *E. coli* in horse faecal samples identified in this study was high (67.7%), but similar studies have shown comparable prevalence levels. A study from Ireland showed a prevalence for multidrug resistant *E. coli* of 65% in hospitalised horses (Bryan et al., 2008), whilst a North American equine hospital reported resistance in 62% of *E. coli* isolated from horses (Dunowska et al., 2006). The prevalence of ESBL-producing bacteria identified in this study was higher than expected, with 53.4% of horses having at least one ESBL-positive *E. coli* isolated during hospitalisation. ESBL-producing *E. coli* have been identified in diagnostic submissions of pathogenic isolates from horses in the Netherlands; all were of the CTX-M type (Vo et al., 2007). A further study looking at commensal *E. coli* in a Czech equine hospital reported a prevalence of 19% in 27 horses sampled on a single occasion; again they were of the CTX-M type (Dolejska et al., 2008).

The identified increase in the prevalence of antimicrobial resistant *E. coli* after admission could occur via a number of means. Possibilities include: acquisition of resistant *E. coli* from other hospitalised horses or the hospital environment; acquisition of genetic resistance determinants from similar sources; an increase in resistant *E. coli* already present (but previously undetectable) in the gastrointestinal flora or transfer of resistance determinants from other members of the gut flora. The two most important identified risk factors were day of hospitalisation and treatment with antimicrobials in the seven days prior to a sample. An association with antimicrobial treatment agrees with a study that included a group of hospitalised horses (Dunowska et al., 2006), which identified aminoglycoside, cephalosporin and cotrimoxazole administration as risk factors for antimicrobial resistance in *E. coli*. Resistance was not restricted to samples for which there was antimicrobial treatment in the previous seven days; the sample prevalence of resistance for the majority of outcomes was higher than the proportion of samples with previous antimicrobial treatment (37.9%). Dunowska et al. (2006) identified hospitalisation as an independent risk factor (in addition to antimicrobial treatment) on comparison of resistant *E. coli* prevalence in groups of hospitalised and non-hospitalised horses.

Few animal studies have attempted to document the influence of hospital-level factors such as number of hospitalised animals or total antimicrobial usage on the presence of resistance, although this is more common for human hospital studies (de With et al., 2006; Pakyz et al., 2008). The number of horses in the hospital was not a significant risk factor for any outcome. However, for most outcomes the number of doses prescribed in the hospital of cotrimoxazole was a significant risk factor. As previously stated, the present study suggests that at an individual horse level, antimicrobial treatment results in an increased prevalence of resistant *E. coli* in its faecal samples. This could translate to higher numbers of such bacteria present in the hospital environment, increasing other horses' exposure to these bacteria (or their resistance determinants) and increasing the likelihood for transmission.

The decrease in resistant *E. coli* prevalence noted from day six onward is interesting. Regardless of exactly how it occurs, the increased prevalence of resistant *E. coli* associated with hospitalisation may signify some form of disturbance of the horses' normal gastrointestinal flora. The subsequent decrease identified could represent the re-establishment of a more normal bacterial population, particularly if the cause of the disturbance occurs early during hospitalisation or the selection pressure is not maintained.

Reason for admission was a significant risk factor only for nalidixic acid and ciprofloxacin resistance; for both outcomes admission for a surgical gastrointestinal condition represented increased risk. It is unclear whether the condition itself or some aspect of subsequent treatment is the important factor. Non-surgical gastrointestinal disease did not represent increased risk and undergoing surgery was not a significant risk factor for any outcome. It is uncertain whether hospital yard represents a direct environmental factor or is merely a reflection of the reason for admission, type of treatment or severity of disease. Although the descriptions given to the yards represent their primary intended use, they are not strictly adhered to and the yard is more likely to represent an environmental factor. One yard (orthopaedic) was shown to be significantly associated with increased risk for ciprofloxacin and trimethoprim resistance, and the mixed-purpose yard (which also houses the largest number of horses) was also significantly associated with ESBL-mediated resistance. Environment sampling in a Czech equine hospital study isolated multidrug resistant *E. coli* from most water troughs sampled (Dolejska et al., 2008).

The emergence of multidrug resistant bacteria in animals is a concern for public health as well as veterinary medicine (Barza, 2002). Transfer of *E. coli* from animals to in-contact people is difficult to confirm but household members, including pets, have been shown to share *E. coli* strains (Murray et al., 2004; Damborg et al., 2009) and a longitudinal study documented repeated transfer of pathogenic *E. coli* strains between a pet and members of its owner family (Johnson et al., 2008). Similar opportunities for bacterial transfer may exist for horses. The antimicrobial resistant *E. coli* recovered in this study were not causing disease and are likely to represent commensal strains of the organism. However, under certain circumstances such strains may be able to cause disease, either through the acquisition of virulence determinants or via inoculation of a non-gastrointestinal site. Isolates such as the multidrug resistant *E. coli* identified in this study could prove refractory to treatment by most of the antimicrobials available for use in equine medicine. Additionally, humans involved with the care of hospitalised horses are frequently in close contact with the animals and their faeces, representing considerable opportunity for potential transfer of resistant *E. coli* strains to humans.

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REFERENCES

Bartoloni, A., Benedetti, M., Pallecchi, L., Larsson, M., Mantella, A., Strohmeyer, M. and Kronvall, G. (2006). Evaluation of a rapid screening method for detection of antimicrobial resistance in the commensal microbiota of the gut. Trans. R. Soc. Trop. Med. Hyg. 100, 119-125.

Barza, M. (2002). Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals. Clin. Infect. Dis. 34, S123-S125.

Berge, A.C.B., Atwill, E.R. and Sischo, W.M. (2005). Animal and farm influences on the dynamics of antibiotic resistance in faecal Escherichia coli in young dairy calves. Prev. Vet. Med. 69, 25-38.

Browne, W.J. (2009). MCMC Estimation in MLwiN, v2.13. Centre for Multilevel Modelling, University of Bristol.

Bryan, J., Leonard, F.C., Katz, L.M., Duggan, V. and Fanning, S. (2008). The development of multiple-antimicrobial resistance in commensal faecal *Escherichia coli* of hospitalised horses. In, 48th British Equine Veterinary Association Congress, Birmingham, 244p.

Damborg, P., Nielsen, S.S. and Guardabassi, L. (2009). Escherichia coli shedding patterns in humans and dogs: insights into within-household transmission of phylotypes associated with urinary tract infections. Epidemiol. Infect. 137, 1457-1464.

de With, K., Maier, L., Steib-Bauert, M., Kern, P. and Kern, W.V. (2006). Trends in antibiotic use at a university hospital: Defined or prescribed daily doses? Patient days or admissions as denominator? Infection 34, 91-94.

Dolejska, M., Duskova, E., Machkova, G., Kohoutova, L. and Cizek, A. (2008). Detection of ESBL-producing Escherichia coli in horses, personel and the environment within a veterinary teaching hospital. In, Conference proceedings; Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, Copenhagen, Denmark, pp. 57-58.

Dunowska, M., Morley, P.S., Traub-Dargatz, J.L., Hyatt, D.R. and Dargatz, D.A. (2006). Impact of hospitalization and antimicrobial drug administration on antimicrobial susceptibility patterns of commensal Escherichia coli isolated from the feces of horses. J. Am. Vet. Med. Assoc. 228, 1909-1917.

Hall, R.M. and Collis, C.M. (1995). Mobile Gene Cassettes and Integrons - Capture and Spread of Genes by Site-Specific Recombination. Mol. Microbiol. 15, 593-600.

Harihara, H. and Barnum, D.A. (1973). Drug Resistance among Pathogenic Enterobacteriaceae from Animals in Ontario. Can. J. Public Health 64, 69-69.

Hart, W.S., Heuzenroeder, M.W. and Barton, M.D. (2006). A study of the transfer of tetracycline resistance genes between Escherichia coli in the intestinal tract of a mouse and a chicken model. J. Vet. Med. Series B-Infectious Diseases and Veterinary Public Health 53, 333-340.

Johnson, J.R., Clabots, C. and Kuskowski M.A. (2008). Multiple-Host Sharing, Long-Term Persistence, and Virulence of Escherichia coli Clones from Human and Animal Household Members. J. Clin. Microbiol. 46, 4078-4082.

Karami, N., Martner, A., Enne, V.I., Swerkersson, S., Adlerberth, I. and Wold, A.E. (2007). Transfer of an ampicillin resistance gene between two Escherichia coli strains in the bowel microbiota of an infant treated with antibiotics. J. Antimicrob. Chemother. 60, 1142-1145.

Liebana, E., Batchelor, M., Hopkins, K.L., Clifton-Hadley, F.A., C. J. Teale, C.J., A. Foster, A., Barker, L., Threlfall, E.J. and Davies, R.H. (2006). Longitudinal Farm Study of Extended-Spectrum beta -Lactamase-Mediated Resistance. J. Clin. Microbiol. 44, 1630–1634.

M'Zali, F.H., Chanawong, A., Kerr, K.G., Birkenhead, D. and Hawkey, P.M. (2000). Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. J. Antimicrob. Chemother. 45, 881-885.

McDaniels, A.E., Rice, E.W., Reyes, A.L., Johnson, C.H., Haugland, R.A. and Stelma, G.N. (1996). Confirmational identification of Escherichia coli, a comparison of genotypic and phenotypic assays for glutamate decarboxylase and beta-D-glucuronidase. Appl. Environ. Microbiol. 62, 3350-3354.

McKay, K.A., Ruhnke, H.L. and Barnum, D.A. (1965). The Results of Sensitivity Tests on Animal Pathogens Conducted over the Period 1956-1963. Can. Vet. J. 6, 103-111.

Murray, A.C., Kuskowski, M.A. and Johnson, J.R. (2004). Virulence factors predict Escherichia coli colonization patterns among human and animal household members. Ann. Intern. Med. 140, 848-849.

Pakyz, A., Powell, J.P., Harpe, S.E., Johnson, C., Edmond, M. and Polk, R.E. (2008). Diversity of antimicrobial use and resistance in 42 hospitals in the United States. Pharmacotherapy 28, 906-912.

Paladino, J.A., Sunderlin, J.L., Price, C.S. and Schentag, J.J. (2002). Economic consequences of antimicrobial resistance. Surg Infect (Larchmt) 3, 259-267.

Payne, D.J., Marriott, M.S. and Amyes, S.G.B. (1989). Mutants of the Tem-1 Beta-Lactamase Conferring Resistance to Ceftazidime. J. Antimicrob. Chemother. 24, 103-110.

van Duijkeren, E., Moleman, M., Sloet van Oldruitenborgh-Oosterbaan, M., Multem, J., Troelstra, A., Fluit, A.C., van Wamel, W.J.B., Houwers, D.J., de Neeling, A.J. and Wagenaar, J.A. (2009). Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: An investigation of several outbreaks. Vet. Microbiol. *In press.*

van Duijkeren, E., van Asten, A. and Gaastra, W. (2000). Characterization of Escherichia coli isolated from adult horses with and without enteritis. Vet. Q. 22, 162-166.

Vo, A.T.T., van Duijkeren, E., Fluit, A.C. and Gaastra, W. (2007). Characteristics of extended-spectrum cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae isolates from horses. Vet. Microbiol. 124, 248-255.

Ward, M.P., Brady, T.H., Couetil, L.L., Liljebjelke, K., Maurer, J.J. and Wu, C.C. (2005). Investigation and control of an outbreak of salmonellosis caused by multidrug-resistant Salmonella typhimurium in a population of hospitalized horses. Vet. Microbiol. 107, 233-240.

WHO (2007). Guidelines for ATC classification and DDD assignment 2007. WHO Collaborating Centre for Drugs Statistics Methodology, available at: www.whocc.no/atcdd. Accessed 10 October 2009.

USE OF SOCIAL NETWORK ANALYSIS TO CHARACTERISE EQUINE MOVEMENTS AND IDENTIFY PREMISES AT HIGH RISK FOR DISEASE INTRODUCTION AND SPREAD IN SPAIN

B. MARTÍNEZ-LÓPEZ*, A.M. PEREZ AND J.M. SÁNCHEZ-VIZCAÍNO

SUMMARY

Social network analysis (SNA) and graph theory have recently been used to describe movement patterns and to identify premises that may play an important role in the introduction or spread of animal diseases. Because of the relatively recent introduction of SNA in the field of veterinary medicine, SNA has never been used to characterize the network of contacts among equine premises. SNA was used here to explore the equine network of movements, to identify premises highly connected with other premises and to illustrate the potential risk of introduction and spread of equine diseases in Castile and Leon (CyL) region of Spain. Characteristics of the equine network were also compared with pigs and cattle networks in CyL. Results may be used to update contingency plans for management of foreign diseases such as African horse sickness or West Nile fever and, ultimately, to establish risk-based surveillance and control programs in Spain or other EU countries.

INTRODUCTION

The equine industry of developed countries has recently undergone a remarkable transformation. Horses were replaced by motorised vehicles in practices traditionally associated with equines and, conversely, there was an increment in the number of horses used in alternative activities such as sport, entertainment and meat production. Changes in the scope and objectives of the equine industry resulted in a reduction of the world horse population by almost 14 million animals during the first part of the last century. However, the world horse population has remained stable at around 58 million horses since 1980 (FAOSTAT, 2009). Approximately 7% of the world horse population is located in the European Union (EU) and Spain, with 424,000 animals, holds the fourth largest population of horses in the EU (WAHID, 2009).

The horse industry is threatened by a large number of diseases and syndromes. There are 21 infectious diseases to which equines are susceptible and that are notifiable to the World Organisation for Animal Health (OIE). Eleven of these (African horse sickness (AHS), contagious equine metritis, dourine, Western equine encephalomyelitis, equine infectious anaemia, equine influenza, equine piroplasmosis, equine rhinopneumonitis, equine viral arteritis, glanders, and Venezuelan equine encephalomyelitis) affect equines exclusively. The ten

* Beatriz Martínez-López, Centro de Vigilancia Sanitaria Veterinaria (VISAVET) and Animal Health Department, Complutense University of Madrid, Av. Puerta de Hierro s/n. 28040, Madrid. Spain. E-mail: beatriz@sanidadanimal.info

remaining horse diseases for which reporting is mandatory to the OIE and that affect multiple species are Eastern equine encephalomyelitis, Japanese encephalitis, new world screwworm (*Cochliomyia hominivorax*), old world screwworm (*Chrysomya bezziana*), Q fever, rabies, surra, tularemia, vesicular stomatitis, and West Nile fever (OIE, 2009). The complexity of the equine industry, relatively high frequency of international movements, limitations of current horse disease surveillance systems and shortage of resources allocated to the prevention and control of equine diseases make Spain and other EU countries particularly vulnerable to the introduction and spread of horse diseases.

The objective of this study was to apply social network analysis (SNA) to explore the patterns of the equine network of movements and identify important premises which may be targeted for control and surveillance systems. Here, a region of Spain for which complete and updated data were available was used to illustrate the methodology and type of results that could be obtained. The analysis and methods described here could be easily expanded to other regions and countries and may be useful to develop risk-based contingency and surveillance programs for management of equine diseases.

MATERIALS AND METHODS

The region of Spain selected for the study was the autonomous community of Castile and Leon (CyL) (Fig. 1). CyL was selected because of the willingness of regional authorities to make all data required for the analysis available. Horse shipment records included information on the day of shipment, number of equines shipped and location (latitude and longitude) and production type of the premises of origin and destination of the shipment. Location (latitude and longitude), production type and the number of horses (i.e. premises size) were also available for every premises in CyL.

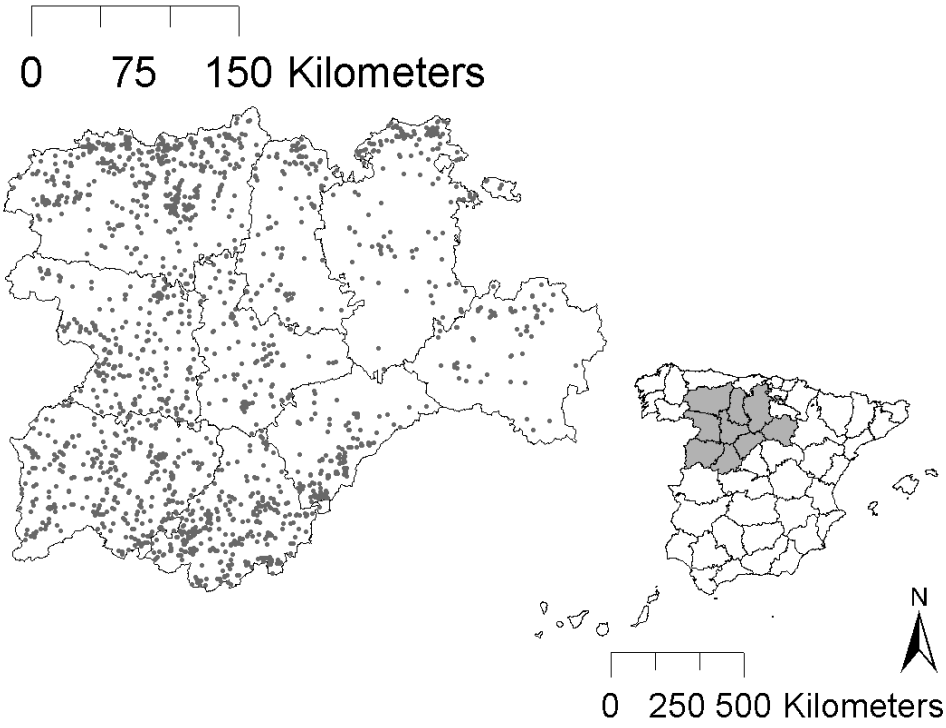

Fig. 1 Location of equine premises in CyL region of Spain

The horse population of CyL included 49,036 horses distributed over 2,135 premises (Fig. 1). Horse premises were classified, according to their objectives, as production farms (78.2%), entertainment, game or sport facilities (15.2%), free-grazing farms (2.2%), markets (1.5%), dealers (0.9%), slaughterhouses (0.8%), bullrings (0.2%) and non-specified purpose (1%). The mean (S.D.) herd size was 57 (70) for dealers, 25 (48) for production farms, 14 (30) for entertainment, game or sport facilities, 10 (21) for free-grazing farms and 10 (20) for farms with non-specified purpose, whereas there was no permanent population of horses in markets, slaughterhouses and bullrings.

SNA and graph theory were used to estimate premises-specific indicators of the frequency of live horses shipments into CyL. A review of SNA and graph theory and their application in preventive veterinary medicine is available elsewhere (Martínez-López et al., 2009a). Briefly, SNA may be defined as the analytical approach that intends to describe the nature and extent of the interactions among the elements of a group in order to understand the collective behavior of the network (Laumann & Pappi, 1976; Anderson & Jay, 1985). Graph theory provides the theoretical framework to study the characteristics, properties and important components of the network. SNA and graph theory have recently been used in preventive veterinary medicine to assess the patterns of animal movements and introductions into a region in studies that could have important applications, for example, to formulate risk-based surveillance programs (Martínez-López et al., 2009a,b).

In this study, two directed networks were created using premises as nodes or vertices and equine shipments as links or connections (Martínez-Lopez et al., 2009a). Shipments with origin in a premises located outside CyL and with destination in a premises located within CyL were used to construct a network referred to as the external network (EN). Shipments with origin and destination within CyL were included into a so-called internal network (IN). Thus, the EN and IN were proxies for the risk of disease introduction and spread in CyL, respectively. Relative importance for introduction and/or spread of disease of every horse premises *i* was estimated by computing the *degree* (Dc_i) and *closeness* (Cc_i) *centrality measures*. *Degree centrality* refers to the number of contacts that a premises has. Directed networks have two measures of degree centrality, one for the incoming contacts (*in-degree*, IDc_i) and other for the outgoing contacts (*out-degree*, ODc_i). Generally, the relative or normalized in- and out-degree centrality measures ($RIDc_i$, $RODc_i$) were used, which consider the total number of vertices in the network (N) and were computed as $IDc_i/N-1$ and $ODc_i/N-1$, respectively. *Closeness centrality* (Cc_i) is an estimate of how closely connected a vertex is to all other vertices of the network. The formula to compute the closeness centrality of vertex *i* is

$$Cc_i = \frac{1}{\sum_{j=1}^{N} d(n_i n_j)}$$

where $\sum_{j=1}^{N} d(n_i n_j)$ is the sum of *shortest path lengths* from vertex *i* to all other vertices in the network. Similar to the degree centrality, directed networks have two closeness centrality measures referred to as in- and out- closeness, which were computed similarly to the above equation but using the sum of *shortest path lengths* of the incoming and outgoing contacts, respectively (Martínez-López et al., 2009a). All computations were performed using Pajek v.1.23 (Batagelj V. and Mrvar A.,University of Ljublijana, Slovenia).

RESULTS

The EN included 47 (2%) premises and 103 shipments, with a total of 655 horses introduced into the CyL region (Fig. 2). The mean (S.D.) number of horses moved per shipment was 6 (10). The mean (S.D.) number of equine shipments per week was 2 (2) and 28% of the shipments took place on Fridays. The distance covered by 50%, 75% and 95% of the shipments was 66.4 km, 122.6 km, and 393.8 km, respectively. Entertainment, game or sport facilities, production farms, free-grazing farms, slaughterhouses, markets, dealers and bullrings received 33%, 28%, 17%, 16%, 3%, 2% and 1% of the shipments, respectively. The largest in-degree (IDc_i = 12) was estimated for an entertainment, game or sport facility. The average (median) value of IDc_i was 2 (1).

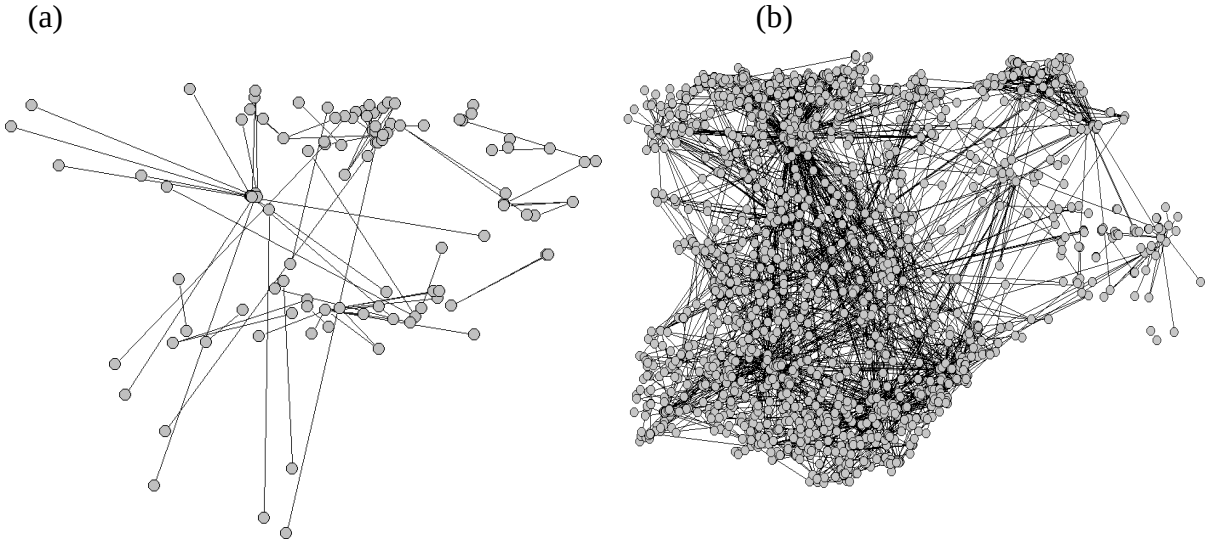

Fig. 2 Network of equine shipments with origin in other regions of Spain and destination within CyL, referred to as external network (EN) (a) and with origin and destination within CyL, referred to as internal network (IN) (b)

The IN included 2,118 (99%) premises and 3,645 shipments (Fig. 2). The mean (S.D.) number of equine shipments per week was 69 (34) and the 28% of the shipments occured on Friday. The mean (S.D.) number of equine moved per shipment was 4 (6). The distance covered by 50%, 75% and 95% of the shipments was 37.9 km, 74.6 km and 173.9 km, respectively. Specifically, production farms, entertainment, game or sport facilities and markets accounted for 73%, 11% and 8%, respectively, of the shipments in the IN. The destination of 48%, 22%, 12% and 10% of the shipments corresponded to production farms, markets, entertainment, game or sport facilities and slaughterhouses, respectively. The highest probability of contact was among production farms (0.34) and from production farms to markets (0.18) (Table 1).

The largest in-degree (IDc_i = 270) and out-degree (ODc_i = 80) for the IN were estimated for markets. Premises with high in- and out- degree centralities were also found to have high in- and out-closeness centrality values, respectively (Fig. 3). Conversely, most premises with high in-degree and in-closeness values did not correspond with premises with high out-degree and out-closeness values, respectively (Fig. 3).

Premises with high values of in- and out- degree and closeness centrality measures were broadly distributed throughout CyL (Fig. 4).

Table 1. Probability of contact among different types of equine premises into Castile and Leon (CyL) region of Spain during 2008.

		DESTINATION					
		Markets	Entertainment, game or sport	Slaughterhouse	Production	Other	TOTAL
ORIGIN	Market	0.000	0.009	0.001	0.067	0.004	0.081
	Entertainment, game or sport	0.030	0.024	0.002	0.044	0.008	0.109
	Production	0.184	0.084	0.066	0.335	0.062	0.730
	Other	0.009	0.006	0.028	0.033	0.005	0.080
	TOTAL	0.222	0.122	0.097	0.479	0.079	1.000

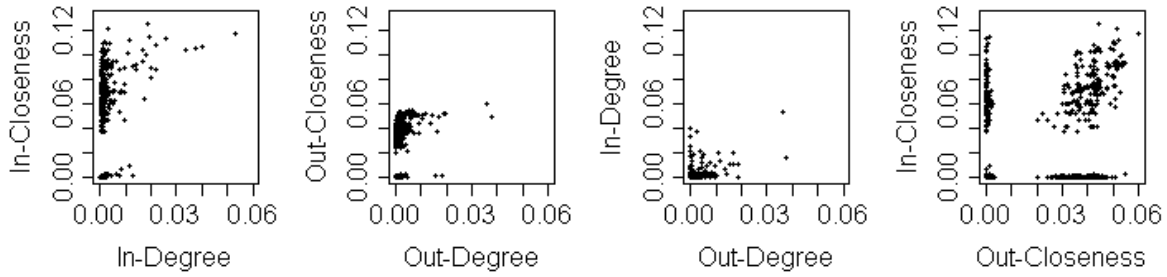

Fig. 3 Relationship between in- and out- relative degree and closeness centrality measures for the internal network (IN) in CyL region of Spain

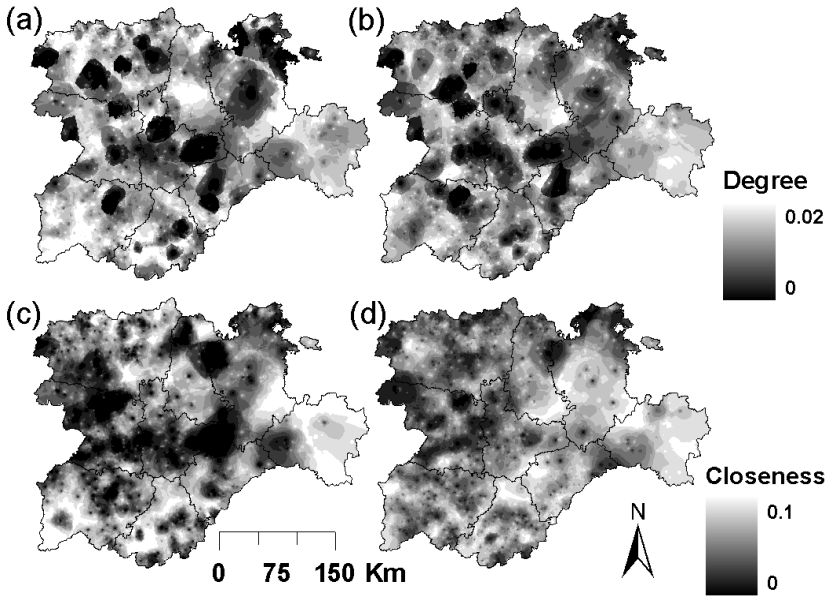

Fig. 4 Spatial distribution of premises with high values of in-degree (a), out-degree (b), in-closeness (c) and out-closeness (d) into CyL region. The isopleth map was generated using inverse distance weight (IDW)

DISCUSSION

Implementation of effective surveillance programs aimed at early detection of incursions of foreign animal diseases into equine populations of Spain and Europe has been impaired by factors inherent in the complex dynamics of the equine industry and by the shortage of resources available to official veterinary services for design of equine disease control and prevention programs. Here, we present a relatively simple analytical approach aimed at identifying premises at highest risk of introduction or spread of equine foreign diseases in a region. Such areas and periods of time may be selectively targeted as part of an active disease prevention program in order to increase the sensitivity and effectiveness of the surveillance system. Although the approach could be applied to any region and easily adapted to any equine disease, here it was used to identify high risk premises for introduction and/or spread of equine diseases in a limited region of Spain.

One of the most important drawbacks for the implementation of active surveillance programs for equine diseases is the relative shortage of financial and human resources available to veterinary services for horse disease control and prevention programs, compared with the poultry and livestock sectors. The characterization of the contact patterns among equine premises in this study may be useful for the development of policies to prevent and control equine diseases.

Compared to the networks of swine and cattle contacts in CyL, previously described by Martínez-López et al. (2009c), the equine network of CyL included a lower number of premises and shipments (2,118 premises and 3,645 shipments in the equine network compared with 24,442 and 220,337 in cattle and 8,457 and 78,888 in pigs, respectively). However, an interesting observation is that most equine premises (99.2%) had at least one shipment per year.

This is not the case in the cattle and swine industries in which the percentage of premises with at least one shipment per year was 80% and 30%, respectively. Equine shipments covered longer distances than cattle shipments but similar distances to swine shipments (50%, 75% and 95% of the shipments covered 37.9 km, 74.6 km and 173.9 km in the equine network; 25.7 km, 55.1 km and 140 km in the cattle network and 40.5 km, 75.1 km and 172.8 km in the swine network) and were more homogeneously distributed throughout the year than cattle and swine shipments. Equine premises were more heterogeneous, including more diverse production types than cattle and swine premises and being more frequently connected to markets than cattle and pig premises, which may result in higher risk for disease spread compared to cattle and pigs. Slaughterhouses were also less important in the reception of shipments from equine premises than for swine and cattle networks. Specifically, 73%, 11% and 8% of equine shipments originated from production farms, entertainment, game or sport facilities and markets, respectively, whereas in cattle 98.5% originated from production farms and 1.5% from markets, and in swine 100% originated from production farms. Destination of shipments in the equine network was distributed as 48%, 22%, 12% and 10% to production farms, markets, entertainment, game or sport facilities and slaughterhouses, respectively, whereas in the cattle network the distribution was 50.4%, 39.2% and 10.3% to slaughterhouses, production farms and markets, respectively, and in the swine network 73.1% and 26.9% of shipments were to slaughterhouses and production farms, respectively. Those differences in the nature and structure of the equine network of contact patterns compared with other livestock animals should be considered in the development and implementation of surveillance and control programs.

The analytical approach developed here may be improved by the addition of certain epidemiological data that were not available at the time of the analysis. For example, one assumption of the prototype presented here was that the risk of disease introduction or spread was the same for every movement, regardless of the origin of the shipment. Alternatively, risk assessment may be used to quantify the risk that shipped horses were infected at the time of introduction into the premises in order to weight the shipments by their risk of infection of certain equine diseases. However, there is a notable lack of information on the prevalence of the diseases in endemic regions, which prevented the inclusion of those factors into this analysis. The analysis here was restricted only to horse movements. However, the risk associated with introduction of other susceptible species may also be considered. Inclusion of other equine species may be particularly important for diseases such as AHS, where the disease mortality rate is quite high in horses (House, 1993; Meiswinkel, 1998) but certain susceptible species, such as donkeys or zebras, show negligible clinical signs, which may increment the risk of introduction associated with these species. Thus, introduction of equine species other than horses, which was assumed to be nil in CyL but may be important in other regions of Spain and Europe, may be considered in order to enhance the sensitivity of the approach. Another potential expansion of the work here is the combination of SNA with techniques for identification of clusters in time and space to develop compartmentalisation and regionalisation strategies with application in prevention and control of disease spread. Such an objective is beyond the scope of this manuscript, but see, for example, Martínez-López et al. (2009b), for an example of how SNA and cluster analysis techniques allow identification of not only relations, but also areas and time periods at higher risk of introduction or spread of disease.

In conclusion, the study here is the first to use SNA to identify premises at high risk of equine diseases introduction and/or spread into a region of Spain. Methods and results presented here may be used to update contingency plans for early detection of incursions of foreign diseases such as AHS or West Nile fever in CyL, and ultimately, to establish cost-effective strategies to enhance the sensitivity of surveillance programs in Spain and other EU countries.

ACKNOWLEDGEMENTS

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REFERENCES

Anderson, J.G. and Jay, S.J. (1985). Computers and clinical judgment: the role of physician networks. Soc. Networks 14, 137-161.

FAOSTAT (2009). Available at: http://faostat.fao.org. Accessed 16 December 2009.

House, J.A. (1993). African horse sickness. Vet. Clin. North. Am. Equine Pract. 9, 355-364.

Laumann, E.O. and Pappi, F. (1976). Networks of Collective Action: A Perspective on Community Influence Systems. Academic Press, New York.

Martínez-López, B., Perez, A.M. and Sánchez-Vizcaíno, J.M. (2009a). Social Network Analysis. Review of General Concepts and Use in Preventive Veterinary Medicine. Transbound. Emerg. Dis. 56, 109-120.

Martínez-López, B., Perez, A.M. and Sánchez-Vizcaíno, J.M. (2009b). Combined application of social network and cluster detection analyses for temporal-spatial characterization of animal movements in Salamanca, Spain. Prev. Vet. Med. 91, 29-38.

Martínez-López, B., Perez, A.M. and Sánchez-Vizcaíno, J.M. (2009c). Network analysis of the cattle and swine movements in the Spanish region of Castile and Leon: implications for disease spread. 12th International Symposium on Veterinary Epidemiology and Economics, 10-14 August, 2009, Durban, South Africa.

Meiswinkel, R. (1998). The 1996 outbreak of African horse sickness in South Africa - the entomological perspective. Arch. Virol. Suppl. 14, 69-83.

OIE (2009). Available at: http://www.oie.int/eng/maladies/en_classification.htm#ListeOIE. Accessed 10 December 2009.

WAHID (2009). Available at: http://www.oie.int/wahis/public.php?page=home. Accessed 23 October 2009.

DYNAMIC MODELLING

MODELLING PCV-2 WITHIN-HERD DYNAMICS:

A FOCUS ON PIG-MANAGEMENT PRACTICES

M. ANDRAUD*, N. ROSE, B. GRASLAND, A. JESTIN AND F. MADEC

SUMMARY

Porcine Circovirus type 2 (PCV-2) is commonly recognised as the aetiological agent of Post-weaning Multisystemic Wasting Syndrome (PMWS) in pigs. Epidemiological studies suggest that within-herd PCV-2 dynamics could be a pivotal factor for PMWS development. The aim of this modelling study was to assess the impact of different management practices and batch-rearing procedures on PCV-2 dynamics within a farrow-to-finish pig herd. A stochastic individual-based model was built to simulate the population dynamics within a typical farrow-to-finish pig farm and coupled with an epidemiological model describing the PCV-2 infectious process. Specific experimental trials were carried out to estimate the PCV-2 transmission parameters (direct and indirect transmission, time-dependent transmission rate). A clear effect of the batch-farrowing system on within-herd course of PCV-2 infection was found, independently of husbandry practices, the risk being increased by 20% for the weekly-batch farrowing procedure compared to the three-week system. Management practices were also found to play a major role in infection spread by modifying the contact structure between individuals with different infectious statuses.

INTRODUCTION

Porcine Circovirus type 2 (PCV-2) is a small single-stranded DNA virus commonly recognised as the aetiological agent of Post-weaning Multisystemic Wasting Syndrome (PMWS) (Allan & Ellis, 2000; Segalés & Domingo, 2002; Madec et al., 2008). However, retrospective studies have evidenced that PCV-2 has been present in the field for decades without any reported clinical consequence (Magar et al., 2000; Rodríguez-Arrioja et al., 2003). There is no clear relationship between PCV-2 infection and PMWS occurrence and additional factors seem to be necessary for clinical signs to develop. Different PCV-2 genotypes, with possibly different pathogenicities, have been distinguished recently (Grierson et al., 2004; Allan et al., 2007) but a clear demonstration of strain-related virulence is lacking.

Deviations in management procedures in the early life of growing pigs were rapidly identified as possible triggering factors of the PMWS outbreak in France in the late 90's (Madec et al., 2000; MacKenzie & Bishop, 2001). Several epidemiological studies of risk factors for PMWS have been carried out during the last decade (Rose et al., 2003; Lopez-Soria et al., 2005; Rose et al., 2009). These studies showed that the younger the pigs were infected, the higher the risk of developing clinical PMWS, suggesting that within-herd PCV-2 dynamics could be a

*Mathieu Andraud, Epidemiology Unit, Afssa-LERAPP, 22440F Ploufragan, France.
E-mail : m.andraud@afssa.fr

pivotal factor for PMWS development. Risk factor analysis confirmed that husbandry practices, such as intensive cross-fostering or mingling practices and pen-size in the nursery facilities, could enhance within-herd PMWS spread.

In France, farrow-to-finish farms are usually organised according to a batch-segregated rearing procedure. This system makes it possible to separate contemporary piglets into different batches that are then grown in separate rooms. Hence, the farrowing, nursery and fattening rooms are organised according to an all-in / all-out policy that facilitates cleaning and disinfection and allows a down period of the facilities before a new batch is brought in. The most common batch-rearing systems are based on a 3-week interval between batches (7 batches of sows) or on a 1 week-interval (21 batches of sows) for larger farms. This latter system results in shorter down-periods in the different sectors due to the short interval between batches. The influence of the batch-rearing system has never been explored but previous epidemiological studies have shown that larger farms are usually more likely to be PMWS affected (Rose et al., 2003).

The aim of this work was therefore to determine the impact of the batch-rearing system, together with husbandry practices, on within-herd PCV-2 dynamics using a modelling approach. For this purpose, a stochastic individual-based model was developed to represent the population dynamics within a farrow-to-finish pig farm and was coupled with an epidemiological model describing the PCV-2 infectious process. This epidemiological model was built in accordance with data from the literature. Moreover, two transmission experiments were carried out to accurately characterise PCV-2 transmission and to parameterise the epidemiological model. The first one (Andraud et al., 2008) was designed to investigate the difference in transmission by direct and indirect contact (within- and between-pen transmission), and in the second (Andraud et al., 2009a) serial transmission experiments were conducted to define the transmission rate in terms of time elapsed since infection . In this study, 2 batch-farrowing systems (based on 1-week and 3-week intervals respectively), with different levels of mixing in the farrowing and nursery facilities that might influence the within-herd PCV-2 course of infection, were compared. The impact of the different management strategies on the age at which individuals became infected, known to be a major risk factor for PMWS, was studied.

MATERIALS AND METHODS

Population dynamics model

A stochastic individual-based model was developed to describe the population dynamics within a farrow-to-finish pig farm and has been described in detail in Andraud et al. (2009b; 2009c). However, in these studies the herd was managed according to a 3-week batch-farrowing system and recent modelling improvements now permit representation of a weekly batch-farrowing system. Major modifications of the model concern the herd size, which has been increased to a mean number of 567 productive sows distributed between 21 batches, and the number of rooms within the pig-farm (5 farrowing, 6 nursery and 18 fattening facilities compared with 2, 3 and 5 rooms respectively for the 3-week batch-farrowing procedure). Briefly, all animals are individually represented using a set of variables describing their physiological status and their location (room and pen) at each time-step (day) of the simulation. The contact structure (within pens, between pens) is known and used at each time-step to assess the transmission occurrence. Events directly involving the animals, such as deaths, abortions or insemination failures, are modelled stochastically using Monte-Carlo procedures.

Epidemiological model

The epidemiological model was developed in accordance with data from the literature concerning the PCV-2 infectious process and has been fully described in Andraud et al (2009c). Briefly, the model is based on a classical SEIR model (Susceptible, Exposed, Infectious, Recovered), to which 5 infectious states were added to account for both pseudo-vertical infections (through infected semen) and passive immunity in new born piglets. The model parameters were mainly derived from specific experimental trials designed to analyse: (i) transmission in pseudo-vertically infected piglets and the effect of passive immunity (Rose et al., 2007); (ii) transmission according to the contact structure between individuals (within- and between-pen transmissions (Andraud et al., 2008)) and (iii) the evolution of transmission with time since infection (Andraud et al., 2009a). Six different transmission rates, depending on the type of infection (horizontal or pseudo-vertical) and the location of each infected individual, were identified. These transmission rates were expressed in terms of time elapsed since infection. The within-pen force of infection λ was then computed daily taking into account the within and between-pen interactions (Andraud et al., 2009c), the infectious process being governed by Monte-Carlo processes.

Pig management practices

As the population dynamics model was developed on an individual basis, it was possible to focus on the impact of different management strategies involving individual movements (i.e., cross-fostering and post-weaning mixing in nursery pens) on the course of infection, which have been identified as major risk factors for clinical PMWS occurrence.

Batch-farrowing systems: Two batch-farrowing systems, based on one- and three-week intervals between two successive batches, were considered in this study. For both systems, the sow cycle was fixed at 147 days including 114 days of gestation, 28 days of lactation and a weaning-to-oestrus interval of 5 days. The herd characteristics according to these two batch-farrowing systems are given in Table 1.

Table 1. Herd characteristics according to batch-farrowing systems

	THREE-WEEK	ONE-WEEK
Sow cycle (days)	147	147
Mean number of productive sows	166	567
Number of batches	7	21
Number of farrowing rooms	2	5
Number of farrowing crates per facility	25	30
Number of nursery rooms	3	6
Number of fattening rooms	5	18

The following husbandry strategies were tested for the two batch-farrowing systems to analyse the effect of management modifications on the age-related probability of PCV-2 infection.

Cross-fostering: Three levels of cross-fostering were represented:

(i) No cross-fostering allowed

(ii) 15% cross-fostered piglets, corresponding to cross-fostering of piglets from large litters

(iii) Random-mixing of piglets to obtain homogeneous litter size (percentage of foster-piglets >15%)

Nursery management: Once weaned, the piglets were transferred to the nursery room where they were grouped into pens. As in the previous study (Andraud et al., 2009c), two pen sizes were tested:

(i) Small pens housing 20 to 40 pigs

(ii) Large pens housing 40 to 70 pigs.

In each case, the piglets could either be grouped by litter or randomly mixed.

Statistical analysis

Experimental design: A complete factorial experimental design was used to test the influence of the batch-farrowing systems combined with management strategies on the within-herd course of PCV-2 infection, resulting in 24 combinations of husbandry practices. One hundred simulations representing a 1-year period were carried out for each combination of management practices.

Survival analysis of age at infection: The age at infection was studied by recording from simulations the ages at which piglets were infected. The age-to-infection events were subjected to survival analysis (Proc Lifetest, SAS 9.1. SAS Institute Inc., 2000). Batch-farrowing systems and management strategies were tested by modelling the time-to-event using a Cox proportional hazard model with a sandwich estimate of the variance–covariance matrix to take into account the non-independence of pigs within batches (Proc PHREG, SAS 9.1.SAS Institute Inc., 2000). Modifications of management practices were entered as main effects in the Cox proportional hazard model. All interactions between main effects were tested and were removed from the model using a backward procedure until a final model was obtained with all factors significant ($p<0.05$).

RESULTS

All the main effects (batch-farrowing system, cross-fostering, grouping practices and pen size in nursery) were found to be significant in the final model. The effect of the batch-farrowing system was not modified by any of the other practices as all interaction terms involving this variable were insignificant. Thus, the risk of early infection, whatever the other practices, was increased by 20% (HR=1.20 [1.03; 1.38]) in a herd managed according to a weekly-batch-farrowing system as compared to a herd managed on a 3-week basis with equivalent management practices. However, significant interactions between cross-fostering, grouping practices and pen size in nursery were found (Table 2). Thus the effects of these variables on time-to PCV-2 were influenced by the other practices. To facilitate interpretation, hazard ratios were calculated for different combinations of these main effects (Table 3).

Rearing piglets in large pens (50 pigs/pen) in the nursery was found to drastically increase the risk of early infections mainly when the other practices were at a low risk level. When there was no cross-fostering and the piglets were grouped by litters in the nursery, the risk of early infections was increased by 35% (HR=1.35 [1.31; 1.39]) whereas when the piglets had already been mixed intensively (>15% cross-fostering and random grouping of piglets), the risk of early infections was only increased by 9%. Cross-fostering was found to play a major role in the course of PCV-2 infection, especially if the proportion of cross-fostered piglets exceeded 15% (HR=1.20 [1.13; 1.27]). Moreover, the influence of grouping practices was moderate for low levels of cross-fostering whereas it dramatically increased the risk of early infections when more than 15% of the piglets were cross-fostered. In that case, the effects of high-level cross-fostering and random-grouping practices were not only added but the interaction between these two practices led to a hazard ratio of 1.58 [1.50; 1.66].

Table 2. Final Cox proportional hazard model of time-to PCV-2 infection with significant variables and estimated coefficients

VARIABLE	ESTIMATE (β)	SE(β)	P-VALUE
Batch-farrowing system			
3-week	-	-	
1-week	0.18	0.07	0.02
Cross-Fostering (CF)			
No	-	-	
15%	0.04	0.02	0.07
>15%	0.18	0.03	<0.0001
Group			
Grouped by litters	-	-	
Random-mixing	0.05	0.01	0.001
Pens			
Small	-	-	
Large	0.30	0.02	<0.0001
CF*Group			
No and litter-mixing	-		
15% and random-mixing	0.05	0.02	0.006
>15% and random-mixing	0.23	0.03	<0.0001
CF*Pens			
No and Small	-	-	
15% and Large	0.00	0.02	0.86
>15% and Large	-0.07	0.02	0.0006
CF*Pens*Group			
No, Small and Litter-mixing	-	-	
15%, Large and Random-mixing	-0.05	0.01	<0.0001
>15%, Large and Random-mixing	-0.15	0.01	<0.0001

Table 3. Hazard ratio estimates according to husbandry practices

VARIABLES			HR	CI (95%)
CROSS-FOSTERING	GROUPING PRACTICES	PEN SIZE		
No	By litter	Small	1.00	-
		Large	1.35	(1.31; 1.39)
	Random	Small	1.05	(1.02; 1.08)
		Large	1.42	(1.33; 1.50)
15%	By litter	Small	1.04	(1.00; 1.09)
		Large	1.40	(1.34; 1.47)
	Random	Small	1.15	(1.10; 1.20)
		Large	1.47	(1.40; 1.54)
>15%	By litter	Small	1.20	(1.13; 1.27)
		Large	1.51	(1.44; 1.59)
	Random	Small	1.58	(1.50; 1.66)
		Large	1.73	(1.64; 1.82)

DISCUSSION

PCV-2, commonly recognised as the aetiological agent of Post-weaning Multisystemic Wasting Syndrome (PMWS), is mainly related to disease expression by the age at infection: the earlier the infection, the higher the risk (Lopez-Soria et al., 2005; Rose et al., 2009). Husbandry practices were also shown to be related to the likelihood of PMWS occurrence (Rose et al., 2003). The aim of this study was therefore to analyse the effect of husbandry practices and batch-farrowing procedures on the course of PCV-2 infection, by means of a modelling approach, to see whether the relationship between PMWS and management could be explained by the observed modifications in the course of PCV-2 infection. A stochastic individual-based model representing the population dynamics within a farrow-to-finish pig herd was developed and coupled with an epidemiological model representing the PCV-2 infectious process. The main parameters of the latter were based on literature data and specific transmission experiments, to limit the degree of uncertainty (Andraud et al., 2008; Andraud et al., 2009a). Moreover, the good consistency of the model structure and parameters was confirmed by comparing the outcome of the simulation model with field serological data (Andraud et al., 2009c).

The two batch-farrowing systems most commonly found in France, based on three week- or one week- intervals, were tested in this study. The latter batch-rearing procedure is adapted to large herds and even if the batch-size is consistent with the 3-week system, three times more batches are produced in the same amount of time. As a consequence of this increased productivity, the intervals between batches are short and the down periods are drastically reduced which leads to increased infection pressure mainly in the farrowing sector.

The effects of twelve combinations of management practices on the within-herd course of infection were investigated taking into account different levels of mixing in the farrowing (cross-fostering) and nursery facilities (grouping, pen size). A clear relationship between cross-

fostering and the nursery-grouping policies was established: the higher the level of cross-fostering, the greater the impact of random mingling of piglets in the nursery pens. This could be explained by a direct modification of the contact structure between individuals with different infectious statuses (highly influenced by cross-fostering) when entering the nursery room. The in-nursery contact structure could also be modified by increasing the pen-size in the nursery rooms, leading to a higher number of pigs per pen and increasing the probability of PCV-2 transmission compared with small groups of segregated pigs. This sole structural modification, with no cross-fostering and the pigs gathered by litter, increased the risk of early infection by 35%. Hence, all the efforts to keep piglets with their littermates in the early days of life could be greatly jeopardised by the subsequent constitution of large groups in the nursery. Conversely, the effect of pen size decreased (to 15%) when the pigs had already been intensively mixed in the farrowing and nursery rooms. Mixing policies (cross-fostering and random-grouping in the nursery) are thus of primary importance with regards to the course of PCV-2 infection.

REFERENCES

Allan, G. and Ellis, J. (2000). Porcine circoviruses : a review. J. Vet. Diagn. Invest. 12, 3-14.

Allan, G.M., McNeilly, F., McMenamy, M., McNair, I., Krakowka, S.G., Timmusk, S., Walls, D., Donnelly, M., Minahin, D., Ellis, J., Wallgren, P. and Fossum, C. (2007). Temporal distribution of porcine circovirus 2 genogroups recovered from postweaning multisystemic wasting syndrome affected and nonaffected farms in Ireland and Northern Ireland. J. Vet. Diagn. Invest. 19, 668-673.

Andraud, M., Grasland, B., Durand, B., Cariolet, R., Jestin, A., Madec, F. and Rose, N. (2008). Quantification of porcine circovirus type 2 (PCV2) within- and between-pen transmission in pigs. Vet. Res. 39, 43-55.

Andraud, M., Grasland, B., Durand, B., Cariolet, R., Jestin, A., Madec, F., Pierre, J.S. and Rose, N. (2009a). Modelling the time-dependent transmission rate for porcine circovirus type 2 (PCV2) in pigs using data from serial transmission experiments. J. R. Soc. Interface 6, 39-50.

Andraud, M., Leon, E.A., Pierre, J.S., Madec, F. and Rose, N. (2009b). Modelling PCV2 infection within a farrow-to-finish pig farm. Pig J. 62.

Andraud, M., Rose, N., Grasland, B., Pierre, J.-S., Jestin, A. and Madec, F. (2009c). Influence of husbandry and control measures on Porcine Circovirus type 2 (PCV2) dynamics within a farrow-to-finish pig farm: a modelling approach. Prev. Vet. Med. 92, 38-51.

Grierson, S.S., King, D.P., Sandvik, T., Hicks, D., Spencer, Y., Drew, T.W. and Banks, M. (2004). Detection and genetic typing of type 2 porcine circoviruses in archived pig tissues from the UK. Arch. Virol. 149, 1171-1183.

Lopez-Soria, S., Segalès, J., Rose, N., Viñas, M.J., Blanchard, P., Madec, F., Jestin, A., Casal, J. and Domingo, M. (2005). An exploratory study on risk factors for postweaning multisystemic wasting syndrome (PMWS) in Spain. Prev. Vet. Med. 69, 97-107.

MacKenzie, K. and Bishop, S.C. (2001). Developing stochastic epidemiological models to quantify the dynamics of infectious diseases in domestic livestock. J. Anim. Sci. 79, 2047-2056.

Madec, F., Eveno, E., Morvan, P., Hamon, L., Blanchard, P., Cariolet, R., Amenna, N., Marvan, H., Truong, C., Mahé, D., Albina, E. and Jestin, A. (2000). Post-weaning multisystemic wasting syndrome (PMWS) in pigs in France: clinical observations from follow-up studies on affected farms. Livest. Prod. Sci. 63, 223-233.

Madec, F., Rose, N., Grasland, B., Cariolet, R. and Jestin, A. (2008). Post-weaning Multisystemic Wasting Syndrome (PMWS) and other PCV2-related problems in pigs: a twelve-year experience. Transbound. Emerg. Dis. 55, 273-283.

Magar, R., Larochelle, R. and Müller, P. (2000). Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. Can. J. Vet. Res. 64, 184-186.

Rodríguez-Arrioja, G.M., Segalés, J., Rosell, C., Rovira, A., Pujols, J., Plana-Durán, J. and Domingo, M. (2003). Retrospective study on porcine circovirus type 2 infection in pigs from 1985 to 1997 in Spain. J. Vet. Med. B. 50, 99-101.

Rose, N., Larour, G., Le Diguerher, G., Eveno, E., Jolly, J.-P., Blanchard, P., Oger, A., Le Dimna, M., Jestin, A. and Madec, F. (2003). Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. Prev. Vet. Med. 61, 209-225.

Rose, N., Blanchard, P., Cariolet, R., Grasland, B., Amenna, N., Oger, A., Durand, B., Balasch, M., Jestin, A. and Madec, F. (2007). Vaccination of Porcine Circovirus type 2 (PCV2)-infected sows against Porcine Parvovirus (PPV) and Erysipelas: Effect on Post-weaning Multisystemic Wasting Syndrome (PMWS) and on PCV2 Genome Load in the Offspring. J. Comp. Pathol. 136, 133-144.

Rose, N., Eveno, E., Grasland, B., Nignol, A.C., Oger, A., Jestin, A. and Madec, F. (2009). Individual risk factors for Post-weaning Multisystemic Wasting Syndrome (PMWS) in pigs: A hierarchical Bayesian survival analysis. Prev. Vet. Med. 90, 168-179.

Segalés, J. and Domingo, M. (2002). Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. Vet. Q. 24, 109-124.

THE INFLUENCE OF CONTACT STRUCTURE ON DISEASE TRANSMISSION IN A DAIRY HERD USING PARATUBERCULOSIS AS AN EXAMPLE

C. MARCÉ*, P. EZANNO, H. SEEGERS, D. U. PFEIFFER AND C. FOURICHON

SUMMARY

Pathogen transmission occurs by direct or indirect contact between susceptible and infected animals. The population structure is known to influence disease spread and persistence. The objective of this study was to evaluate how herd management influences contact structure and *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) transmission within a dairy herd. A stochastic compartmental model of *Map* transmission in a closed herd was developed. Indirect transmission via the environment was explicitly modelled. Calves were housed in individual or group pens and different levels of exposure of calves to a contaminated environment were studied. It was found that, calf contact structure during the first weeks of life influenced *Map* prevalence. In addition, it was found that reducing the exposure of calves to adults had a larger impact on *Map* prevalence than hygiene measures and could lead to the fadeout of infection. The systematic analysis of contact structure used here can be applied to other diseases when developing realistic models to support decision making.

INTRODUCTION

Pathogens can spread within a herd either through direct contact between susceptible and infected animals or via contaminated materials, which can either be mobile (e.g. farmer boots, vehicles, vectors) or not (e.g. environment, drinking trough). When disease spread is studied, the term contact structure usually includes direct contacts such as nose-to-nose contacts and indirect contacts through ingestion of faeces or milk, for example. In a typical dairy farm, contacts are important because dairy herds are structured in multiple groups, depending on age and production characteristics. Therefore, all animals do not contact each other to the same extent. The influence of housing and management on contacts between young and adult animals and contacts between animals of the same age is important to consider, as well as hygiene measures if indirect transmission is possible. Depending on the pathogen being studied, the key features of the contact structure within a herd which are linked to pathogen transmission may vary.

Modelling has been used to study the influence of herd structure on disease transmission (Turner et al., 2003; Ezanno et al., 2008). Due to the fact that paratuberculosis infection occurs mainly in young stock and is characterised by a long latency period (1 to ≥ 15 years) before any

*Clara Marcé, INRA, Veterinary School of Nantes, UMR1300 Bio-aggression, Epidemiology and Risk analysis, BP 40706, F-44307 Nantes, France / Veterinary Epidemiology & Public Health Division, Royal Veterinary College, University of London, Hatfield AL9 7TA, Hertfordshire, United Kingdom.
Email: marce@vet-nantes.fr

clinical signs may arise, field studies investigating the transmission of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) are difficult and expensive. Field studies are further complicated by the low and varying sensitivity of diagnostic tests. Epidemiological models are suitable for studying *Map* transmission within a herd, taking account of the main factors influencing disease transmission such as contact structure. Several *Map* transmission models already exist and have been reviewed in Marcé et al. (2010b). However, the effect of dairy herds being managed as multiple sub-groups has never been studied. In this study, the impact of totally or partially decreasing exposure of calves to faeces was considered. In particular, the focus was on reducing exposure to other calf faeces via hygiene methods and to adult faeces through the use of different housing facilities.

Mycobacterium avium paratuberculosis subspecies is indirectly transmitted through the ingestion of contaminated faeces (faecal-oral transmission), or contaminated milk or colostrum (Taylor et al., 1981; Chiodini et al., 1984; Streeter et al., 1995). However, there is limited information on the effect of environmental *Map* contamination on transmission. It has long been thought that only adults could shed the bacteria in their faeces (Chiodini et al., 1984). However, *Map* faecal shedding has now also been described in young stock (Bolton et al., 2005; Antognoli et al., 2007), as has calf-to-calf transmission (Van Roermund et al., 2007). The importance of this transmission route on within-herd infection dynamics has not yet been evaluated. Calf-to-calf transmission occurs locally and indirectly through contamination of the shared pen environment, whereas adult-to-calf transmission is influenced by a contaminated environment of the whole farm. Adult-to-adult transmission is assumed not to exist for paratuberculosis because calves are the main susceptible group.

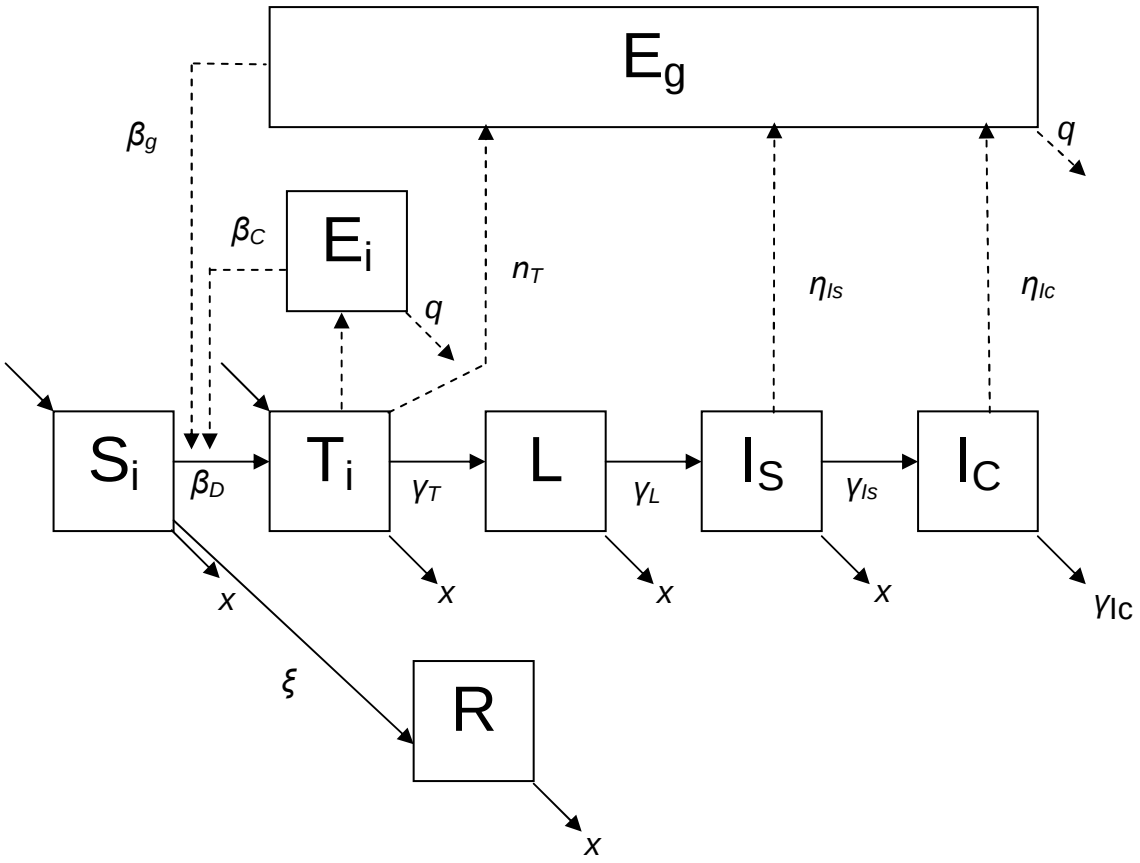
In this study, a new model that incorporates calf-to-calf transmission and explicitly represents indirect transmission via local and global contaminated environments was developed. The objective was to assess the effect of contact structure on *Map* transmission in persistently infected dairy herds and to specifically assess the effect of calf isolation from other calves and from adults during the first weeks of life.

MATERIALS AND METHODS

To represent both the population dynamics and the spread of *Map* within a dairy herd of 110 cattle, a stochastic compartmental model was developed, with a time step of one week (Fig. 1). It was assumed that farmers raise their own replacement cattle for economic and bio-security reasons, thus closed herds were modelled; this is fairly common in France. Initially one infected heifer was introduced into these primarily susceptible closed dairy herds. This study then focused on endemic infected herds; endemic infected herds being defined as herds still infected 25 years after pathogen introduction. Each simulation was run 400 times, from which 122 were persistently infected in the reference scenario.

In contrast, to other models published on dairy herds that considered faecal-oral indirect transmission as being directly linked to the presence of infectious animals (Marcé et al., 2010b), the persistence of *Map* in the environment was considered here by modelling environmental contamination explicitly. Two types of environment were differentiated: the local environment of a pen, and the global environment of the farm. Calves are separated in groups (i), each having its specific local environment E_i. E_g represents the global environment of the farm.

The parameters for herd management as well as infection were derived from published data and expert's knowledge in order to obtain a realistic representation of a dairy herd infected with *Map*. A detailed description of the full model is provided in Marcé et al. (2010a). The model was implemented in Scilab 5.1.



Legend: β_g = 'general' indirect transmission parameter; β_C = group specific indirect parameter; β_D = Direct transmission parameter (milk/colostrum ingestion); q = exit rate of *Map*; η = shedding rate in faeces; x = exit rate of cattle; 1/ γ_T = mean time spent in T compartment; 1/ γ_L = mean time spent in L compartment; 1/ γ_{Is} = mean time spent in I_S compartment; 1/ γ_{Ic} = mean time spent in I_C compartment; $1/\xi$ = mean time spent in S compartment

Fig. 1 Flow diagram representing the *Mycobacterium avium paratuberculosis* (*Map*) transmission model, incorporating the infectious states, S: susceptible; T: transiently infected; L: latently infected; Is: subclinically infected; Ic: clinically infected; R: resistant and transitions between states

Within-herd spread of *Map*

In the model, only animals under 1 year old were assumed to be susceptible to infection; the younger, the more susceptible. An exponential decrease in susceptibility with age was modelled. It was assumed that both infected adults and calves could be infectious. However, the quantity of *Map* shed was assumed to vary depending on the age of the animal and its infection status. *Map* can be shed in colostrum, milk and calf and adult faeces and survive for several weeks in the

environment; these factors were incorporated. Transmission was assumed to occur *in utero* or horizontally by ingestion of *Map*. Indirect faecal-oral transmission occurred when cattle ingested bacteria from a contaminated local or global environment.

Within the model, calves can be born transiently infected or susceptible. If susceptible, a calf can become transiently infected by ingestion of *Map*. Contamination of colostrum and milk can occur directly (direct shedding by an infected cow) or indirectly (presence of faeces in the liquid). A transiently infected calf can shed *Map* for 25 weeks before becoming latently infected. A latently infected animal will not shed *Map* until it becomes subclinically infected. The last infection state for an animal, if not culled, is the clinically infected state. If not infected at one year old, an animal was considered to be resistant to *Map* infection. It was assumed that recovery from infection was not possible.

Within-herd contact structure

Contact structure was defined by the possible indirect contacts between animals either because they shared the same environment at the same time, or because they shared the same environment at different periods of time, or because of the presence of fomites. Infectious animals shed *Map* in their faeces and contaminate associated environments because *Map* can persist for a long period of time in the environment, outside the host. The model included 2 types of environment: a local environment, specific to each group housing facility and a global environment to which all shedding animals contribute. Calf-to-calf transmission occurred through the shared local environment; while adult-to-calf transmission occurred through the global contaminated environment of the farm.

After the separation from the dam, in the reference scenario, calves were housed 2 weeks in individual pens before moving to group pens. In the other scenarios considered, time spent in individual housing could last from 0 to 8 weeks as stated by the European regulation (Council Directive 91/629/EEC and Council Directive 97/2/EC). After a maximum of 8 weeks spent in individual housing, calves are moved to a group pen. Two different types of individual housing could be used: hutches or single pens. A hutch is a separate cubicle in which a calf does not have direct contact with other calves and does not have contact with any environment contaminated by adults (for example fomites, or water). It was assumed that perfect cleaning was undertaken between each calf. A single pen is a pen in which it is possible that a calf can have social contacts with calves in contiguous pens through the hemstitch inner walls. They could also be in contact with the faeces of their neighbour calves in the same way. Cleaning was assumed to be imperfect between each calf because it is more difficult in such indoor housing facilities. Furthermore, contacts with fomites contaminated by adults could also occur.

In the model, outside grazing occurred from April to November for animals older than 6 months. Calves and heifers before their first calving were grouped, as they often use the same pasture. Adults were assumed to graze separately.

An all-year round calving system was represented in the model. All male calves were sold between the 2nd and 4th week after birth. All female calves were kept on premises. Sales of heifers before first calving could occur to regulate the size of the herd. The mean culling rate of adults was 35.5% irrespective of the reason for culling, but varying between parities (27, 25, 31, 31 and 62% for parity 1, 2, 3, 4 and above 5, respectively). Endemic infected herds were represented, in which no *Map* control measures were implemented. There was no additional

culling for subclinically infected cows. The mean time spent by a clinically infected adult on the farm was 6 months.

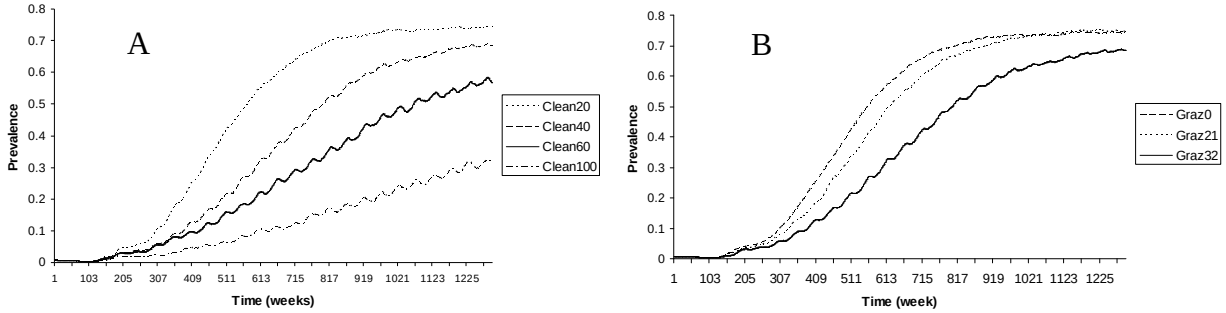
Herd management scenarios

Three scenarios were defined based on the contact structure within a herd. First, the impact of adult-to-calf contact was explored by studying several levels of exposure of calves to any environment contaminated by adults (from on-site to off-site rearing of calves). Grazing was allowed or not allowed during the pasture season resulting in variable exposure of calves to adult faeces. Second, the impact of calf-to-calf contact structure before weaning was studied when off-site rearing was performed until weaning (no exposure of calves to any environment contaminated by adults until weaning). Several levels of hygiene were studied to decrease the exposure when existing. The efficacy of hygiene measures was modelled through the percentage of bacteria removed from the environment. Third, the option of isolating calves in hutches for different periods of time after separation from the dam was studied. This last option combined several control measures at calf-to-calf and adult-to-calf contact structure levels.

RESULTS

Adult-to-calf indirect contacts

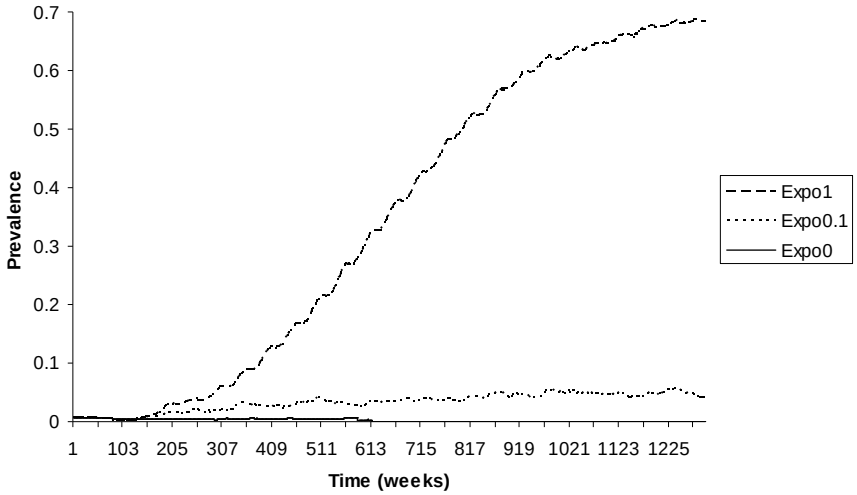
Increasing the percentage of bacteria removed from the farm every week led to a lower prevalence of infectious adults over time (Fig. 2A). The lower the quantity of *Map* remaining in the farm environment, the less infection occurred. Similarly, allowing cattle above 6 months to graze led to a lower prevalence, the longer cattle could spend outside, the fewer calves less than 6 months were exposed to any environment contaminated by adults (Fig. 2B).



Legend: Clean20 to Clean100 = Cleaning of 20% to 100% of the global environment of the farm; Graz0 to Graz32 = time spent by cattle older than 6 months grazing (from 0 to 32 weeks per year)

Fig. 2 Mean prevalence of infectious adults in a herd of 110 cows depending on A: the percentage of bacteria removed from the farm every week, B: the length of time spent by adults grazing

However, a small reduction of the exposure of susceptible calves to any environment contaminated by adults had a larger impact on *Map* prevalence than hygiene measures (higher decrease in prevalence) and could lead to the fadeout of infection (Fig. 3).

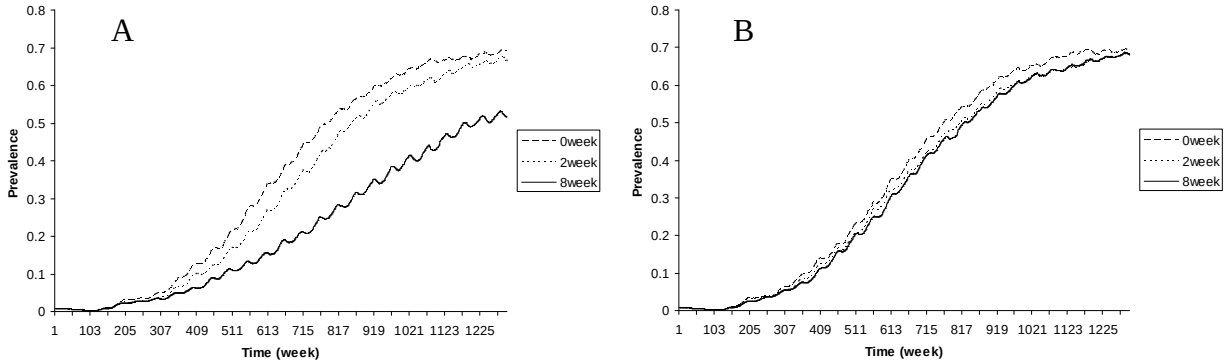


Legend: Expo0 to Expo1 = level of exposure of susceptible cattle (until 1 year old) to the global environment contaminated by adults (Expo1 = 100%, Expo0.1 = 10%, Expo0 = no exposure)

Fig. 3 Mean prevalence of infectious adults in a herd of 110 cows depending on the exposure of calves to adult faeces

Calf-to-calf contacts

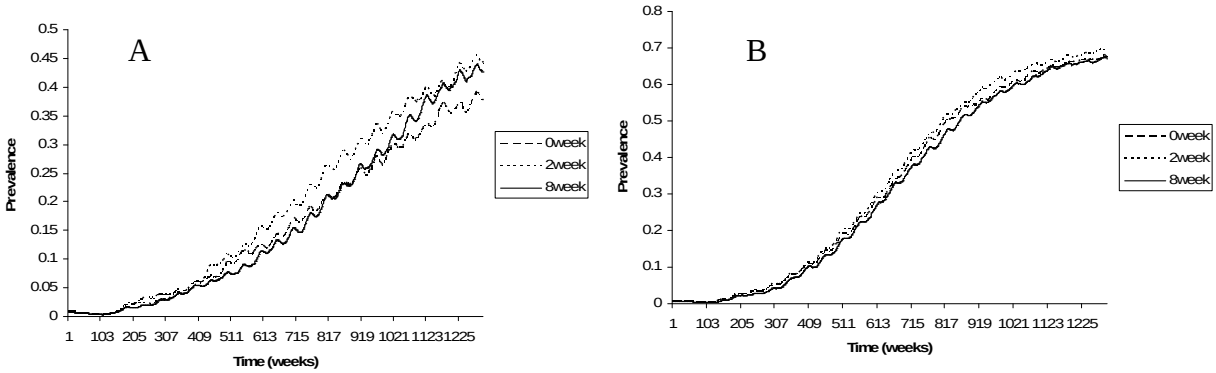
When exposure of calves to adults was delayed until moving into group pens, the longer a calf stayed in individual pen, the lower the mean prevalence at the end of the simulation (Fig. 4A). A similar effect was not observed when calves were exposed to adults while in individual pen (Fig. 4B).



Legend: 0week to 8week: time spent in weeks (0 to 8) by a calf in an individual pen

Fig. 4 Mean annual prevalence of infectious adults in a herd of 110 cows depending on the time spent in individual pen when A: there is no exposure of calves to adult faeces while in individual pen, B: there is always exposure of calves to adult faeces

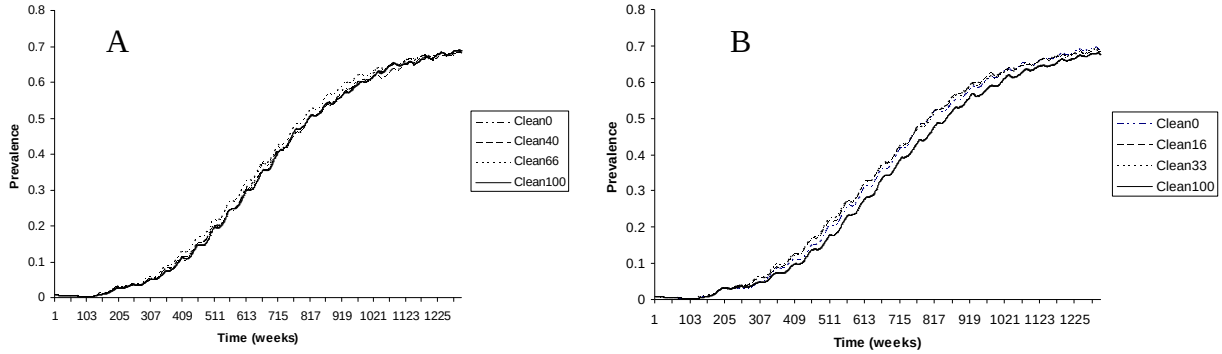
Exposure of calves to adults was assumed to either be not possible before weaning or possible from birth until weaning, independent of their housing facilities. In both cases, the length of the stay in an individual pen (separation from other calves) did not seem to influence the final mean prevalence (Fig. 5).



Legend: 0week to 8week: time spent in weeks (0 to 8) by a calf in an individual pen

Fig. 5 Mean prevalence of infectious adults in a herd of 110 cows depending on the time spent in individual pen when A: there is no exposure of calves to adult faeces until weaning, B: there is always exposure of calves to adult faeces

The impact of hygiene measures in individual and group pens of calves was studied by varying the percentage of *Map* removed from individual or group pens when animals leave the pen. No effect on prevalence of infectious adults was observed (Fig. 6).



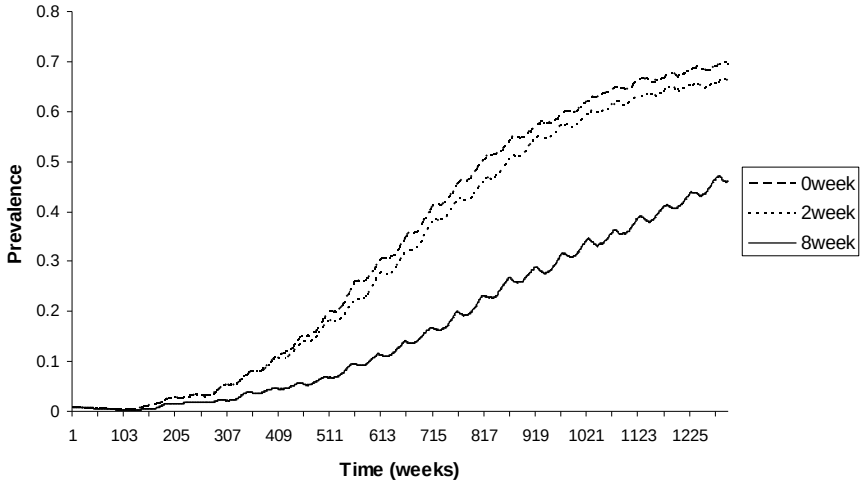
Legend: Clean0 to Clean100 = Removal of *Map* in 0 to 100% of the environment of A: individual pens, B: group pens

Fig. 6 Mean prevalence of infectious adults in a herd of 110 cows depending on A: the cleaning of individual pens when empty, B: the cleaning of group pens when empty

Combination of control measures

Housing a calf in a hutch combined 3 control measures: no exposure of calves to adults until moving to group pens, no contact with neighbour calves while in a hutch, and perfect cleaning of the hutch between calves.

The longer a calf spent in a hutch (from 1 to 8 weeks), the lower the mean prevalence of infectious adults (Fig. 7).



Legend: 0week to 8week: time spent in weeks (0 to 8) by a calf in an individual hutch

Fig. 7 Mean prevalence of infectious adults in a herd of 110 cows depending on the time spent by calves in individual hutches

DISCUSSION

The management system of a typical dairy farm results in structuring the population in groups with relatively little direct interaction amongst groups. How animals encounter or share an environment may affect disease spread and persistence, especially if animals are not all equally susceptible or infectious. Here a systematic analysis of contact structure that could be applied to diseases other than paratuberculosis was undertaken, developing realistic models to support decision making. To perform this analysis, knowledge on the disease and management of dairy cattle herds to identify all factors influencing contact structure and therefore *Map* transmission was summarised. Then, a simulation model representing the hypothesis identified in the previous step was developed. Finally, the model behaviour was explored using simulation of scenarios.

The mathematical model used here is novel in that it is the only one amongst available within-herd *Map* transmission models which specifies the contamination of both the whole environment of a farm and calf housing facilities explicitly. It was possible to study different calf management (individual *versus* group pens) and herd management (grazing, herd size).

The study of adult-to-calf contact structure showed that preventing exposure of calves to adult faeces is essential to control *Map* transmission within a dairy herd. The study of calf-to-calf contact structure before weaning when no exposure to adults is possible showed that calf contact structure did not play a significant role in *Map* prevalence within the herd. In contrast, delaying the exposure of calves to adults from 1 week old until weaning reduced the prevalence of infection. The combination of several control measures as implemented when hutches are used showed that the use of hutches (as defined in this study) for a long period of time (maximum of 8 weeks) decreased *Map* transmission within a dairy herd. In persistently infected herds, calf-to-calf transmission appeared to be a minor route of transmission. Environmental contamination by adults was the main issue. Hygiene was more important than calf housing facilities management when it comes to paratuberculosis control in practice.

An interaction between herd size and calf management could exist. Notable, in larger herds, raising all the calves in one group pen or several group pens of a smaller size could have an impact on *Map* transmission. The next step would be to study the influence of herd size on *Map* transmission, and then to study different contact structures among calves. However, as adult-to-calf indirect transmission was by far the predominant infection route, it is probable that studying different contact structure among calves would lead to similar conclusions.

Other modelling studies evaluating the impact of contact structure on disease transmission have been performed. For other pathogens, such as bovine BVD virus (Ezanno et al., 2008), raising adults separately to calves has been advised. However, why such a separation is needed was different. Susceptible and infectious animals do not belong to the same age category as in the case of paratuberculosis. Indirect transmission can occur whatever the age, but the risk of being infected is more important for pregnant cattle. Decreasing the risk of exposure for susceptible cattle can thus be performed by limiting the exposure of adults to calves; while for paratuberculosis, calves have to be protected mainly from adults and also from other calves. In the present study, the novelty was related to the fact that calf housing facilities and different calf management strategies (which was the susceptible population here) were modelled. The basic model structure could be adapted to represent other diseases, particularly ones for which calves are potentially susceptible. In that case, calf management can be important in the control of disease.

The study of the effect of contact structure in a dairy farm on *Map* transmission leads to advising farmers to delay exposure of calves to adults. Off-site rearing could be useful for controlling *Map* transmission provided the location or facility is kept biosecure from locations and facilities where cows are raised. However, off-site rearing is not a common practice in Europe. An alternative to this type of calf management is to raise calves in a delimited part of the farm with strict hygiene measures and organisation rules: always taking care of calves before adults, changing clothes and boots before going to the calf part of the farm as is done in poultry industries. The use of hutches as defined in this study for 8 weeks in association with strict hygiene measures is another way to reduce the potential for indirect transmission as it postpones exposure of calves to adults. An advantage of such housing facilities is based on the fact that hutches could be moved and kept far away from adult rearing. However, all changes of housing facilities within a farm (structural changes) have a cost and cannot always be carried out.

REFERENCES

Antognoli, M.C., Hirst, H.L., Garry, F.B. and Salman, M.D. (2007). Immune response to and faecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* in young dairy calves, and the association between test results in the calves and the infection status of their dams. Zoonoses Public Health 54, 152-159.

Bolton, M.W., Grooms, D.L. and Kaneene, J.B. (2005). Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in calves: implications for disease control and management. In, 8th ICP, Copenhagen, Denmark, pp. 1-9.

Chiodini, R.J., Van Kruiningen, H.J. and Merkal, R.S. (1984). Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet. 74, 218-262.

Ezanno, P., Fourichon, C. and Seegers, H. (2008). Influence of herd structure and type of virus introduction on the spread of bovine viral diarrhoea virus (BVDV) on the spread of bovine viral diarrhoea virus (BVDV) within a dairy herd. Vet. Res. 39:39.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D.U. and Fourichon, C. (2010a). Modelling the spread of paratuberculosis within an initially susceptible dairy herd: when endemic infection can be prevented without any control measures implementation. Submitted. Vet Res.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D.U. and Fourichon, C. (2010b). Modelling within-herd transmission of *Mycobacterium avium paratuberculosis* in dairy cattle: a review. Submitted. J. Dairy Sci.

Streeter, R.N., Hoffsis, G.F., Bech-Nielsen, S., Shulaw, W.P. and Rings, M. (1995). Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am. J. Vet. Res. 56, 1322-1324.

Taylor, T.K., Wilks, C.R. and McQueen, D.S. (1981). Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. Vet. Rec. 109, 532-533.

Turner, J., Begon, M., Bowers, R.G. and French, N.P. (2003). A model appropriate to the transmission of a human food-borne pathogen in a multigroup managed herd. Prev. Vet. Med. 57, 175-198.

Van Roermund, H.J.W., Bakker, D., Willemsen, P.T.J. and De Jong, M.C.M. (2007). Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves. Vet. Microbiol. 122, 270-279.

ESTIMATE OF BASIC REPRODUCTION NUMBER (R_0) OF LOW PATHOGENICITY AVIAN INFLUENZA OUTBREAKS USING A BAYESIAN APPROACH

A. COMIN*, D. KLINKENBERG, A.J. STEGEMAN, L. BUSANI AND S. MARANGON

SUMMARY

Low pathogenicity avian influenza (LPAI) is a mild disease in poultry with low mortality. Certain virus subtypes may evolve into the highly pathogenic forms, with devastating consequences. Characterising the epidemiological determinants of LPAI is critical to facilitate effective control measures. The basic reproduction number (R_0, the average number of new cases generated by one infectious individual in a fully susceptible population) is an important epidemiological parameter which summarises the transmission potential of a disease. Ideally, R_0 is estimated from the complete course of an outbreak, taking into account the infection times of each bird and the length of the infectious period. Unfortunately, onset and duration of LPAI infections are difficult to establish in the field because of the mainly subclinical course of the disease and the high number of birds per flock. Using serosurveillance data, a Bayesian hierarchical model was implemented to estimate R_0 of LPAI outbreaks in meat turkey flocks from the final size p (the proportion of a population that had been infected by the end of an outbreak) through the equation $p = 1 - \exp(-p\,R_0)$. Two different probability distributions for R_0 (gamma and lognormal) were compared and test sensitivity (as a fixed value or a distribution) was further included into the model. A lower sensitivity increased the estimates of R_0, but better allowed for the fact that the data came from a serosurveillance programme, the results of which depend on both the true infectious status of the flock and the accuracy of the diagnostic assays. Based on the deviance information criterion, the best model had a gamma-distributed R_0 with mean 5.7 (95% CI: 3.3-20.5), and a posterior median sensitivity of 97.6%. The estimated variance of 12.6 (95% CI: 1.8-382.8) indicates that R_0 in the population of infected flock is possibly highly variable, an argument in strong favour of the use of field data to estimate transmission parameters.

INTRODUCTION

Low pathogenicity avian influenza (LPAI) infections caused by H5 and H7 subtypes are widespread, leading to outbreaks in domestic birds in several countries (OIE, 2009). Although LPAI strains do not impose a serious concern for animal health, some subtypes may evolve, under favourable conditions, into highly pathogenic strains (HPAI). HPAI outbreaks not only lead to enormous economic losses due to high mortality rates and costs of control measures (Lupiani & Reddy, 2009) but also threaten human health (WHO, 2009).

* Arianna Comin, Veterinary Epidemiology Departiment, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy. Email: crev.acomin@izsvenezie.it

It is likely that mutation of H5 or H7 LPAI viruses into HPAI forms happened in the epidemics occurring in the USA in 1983 (Bean et al., 1985), Mexico in 1994 (Garcia et al., 1996), Italy from 1999 to 2000 (Marangon et al., 2005), Chile in 2002 (Suarez et al., 2004), The Netherlands in 2003 (Elbers et al., 2004), Canada in 2004 (Bowes et al., 2004) and the UK in 2008 (Defra, 2008), increasing the importance of control and eradication of not only HPAI but also LPAI viruses of H5 and H7 subtypes (OIE, 2005). Characterising the epidemiological determinants of LPAI is important to optimise surveillance systems and to determine the most effective control strategies.

The basic reproduction number (R_0), representing the average number of new cases generated by one infectious individual in a fully susceptible population, is an important epidemiological parameter which summarises the transmission potential of a disease in a given population (Matthews & Woolhouse, 2005). If $R_0>1$ then, on average, the number of new infections will grow, whereas if $R_0<1$, new infections will, on average, decline and a major outbreak cannot occur. An estimate of R_0 together with a model of the effects of control measures can help decision makers drive public health interventions.

Estimates of R_0 can be obtained by several methods depending on the data available and the characteristics of the epidemic (Heffernan et al., 2005). Ideally, R_0 is estimated from the entire course of an epidemic, taking into account the times that animals are infected and the lengths of the latent and infectious periods. In the case of experimental infections, these parameters can be estimated by monitoring the challenged and not-challenged animals on a daily basis. However, experimental settings are usually very different from field conditions and the number of infected animals is often too low to obtain reliable estimates. In contrast, outbreak data reflect reality, but they are usually more difficult to collect, manage and interpret.

Estimates of R_0 have been obtained for several HPAI strains using outbreak data (e.g. Tiensin et al., 2007; Bos et al, 2009). In these examples, generalised linear models have been applied, using mortality data to extrapolate the time of virus introduction. This method can not, however, be applied to LPAI outbreaks, because LPAI infections result in mild symptoms and low mortality. Van der Goot et al. (2003) estimated R_0 for LPAI and HPAI strains from small scale experiments, applying both generalised linear modelling and final size analysis. The latter is a robust method that does not require knowledge of the exact time of initial infection or the length of the latent and infectious periods. It is based on the relationship between R_0 and the final size of the epidemic (i.e. the number of susceptible animals that have been infected when the infection chain has ended), through the final size equation and a maximum likelihood function. However, in that study, estimates of R_0 for LPAI were associated with a confidence interval ranging from 0.0 to 2.5, making it impossible to establish if R_0 was significantly different from the critical value of 1.

In the period 2000-2005, Italy experienced four epidemics of LPAI, mainly involving meat turkeys. Data from these epidemics provided the opportunity to estimate R_0 for LPAI in turkey flocks, and the variation in R_0 between flocks. In this paper, a Bayesian method is used to estimate the (within-flock) R_0 of LPAI outbreaks in meat turkey flocks, using serosurveillance data.

MATERIALS AND METHODS

Data source

Data came from the Italian intensive surveillance system run during the LPAI epidemics that occurred in 2000-2001, 2002-2003, 2004 and 2005. During that period, a total of 6,102 poultry farms were routinely visited, identifying 495 infected premises (i.e. outbreaks), 88% of which were rearing meat turkeys. From the 429 outbreaks in turkey farms, only unvaccinated flocks were included (n=204). Although it would have been interesting to investigate the disease dynamics in vaccinated birds, data were incompatible because only unvaccinated sentinels were sampled from the vaccinated flocks. Furthermore, only those farms housing birds in a single shed were included, in order to fulfil the assumption of homogeneous mixing as required for the analysis.

Inclusion criteria

The data editing process led to 64 available outbreaks, for which multiple samplings had been carried out. The earliest sample associated with a positive serological finding was used to calculate the corresponding seroprevalence (i.e. the number of birds that tested positive divided by the number of sampled birds). The results of virus isolation in proximity to the serological testing, were then observed, when available. If the flock tested negative to virus isolation ±5 days from the positive serological testing, then the outbreak was assumed to be over and the seroprevalence was considered to represent the proportion of the population that had been infected by the end of the outbreak, i.e. the *final size* (*p*). In comparison, the selected seroprevalences were compared to those with a positive virus isolation ±5 days from the positive serological testing.

Model building

The key assumption of this model is the relationship between the final size of an epidemic (*p*, the proportion seropositive) and the basic reproduction number, expressed by the final size equation given in Eq. (1)

$$p = 1 - e^{-pR_0} \tag{1}$$

which is considered to be valid under very general circumstances (Ma & Earn, 2006).

The serosurveillance data were fitted to a hierarchical model (Fig. 1) assuming that R_0 in the population of infected flocks followed a probability distribution with mean m and variance s^2. Each R_{0i} of flock i corresponds to a final size p_i, backcalculated from the final size equation Eq. (1) by means of numerical solution. The observed number of positive samples x_i in each flock is then considered to be a sample from a binomial distribution with $n = n_i$ (sample size) and $p = p_i$ (final size).

The model was implemented in WinBUGS software. Uninformative prior distributions were used for the parameters determining the probability distribution of R_0. Posterior inferences were based on 30 000 iterations with a sampling lag of 10, after a burn-in of 15 000 iterations were discarded. Convergence was assessed by running multiple chains from dispersed starting values and using the Gelman-Rubin statistic.

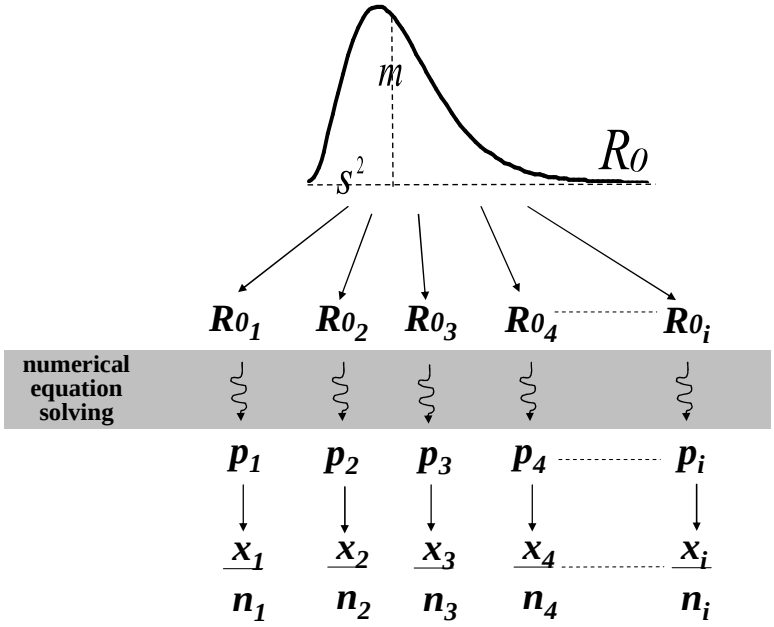


Fig. 1 Hierarchical model linking serosurveillance data with R_0 in the population of infected flocks, through the final size equation

Sensitivity analysis

A sensitivity analysis was performed by fitting six models with different assumptions relating to the probability distribution of R_0 and test sensitivity. Two probability distributions for R_0 in the population of infected flocks were considered.

In Models 1,2 and 3

$$R_0 \sim \text{Gamma}(\kappa,\rho),$$

with $\kappa > 0$ (scale parameter) and $\rho > 0$ (rate parameter). The prior distributions for κ and ρ were given by, $\kappa \sim \text{Gamma}(0.01,0.01)$ and $\rho \sim \text{Gamma}(0.01,0.01)$ and the mean and variance defined by

$$m = \frac{\kappa}{\rho}$$

$$s^2 = \frac{\kappa}{\rho^2}$$

In Models 4, 5 and 6

$$R_0 \sim \text{Lognormal}(\mu,\sigma)$$

with $-\infty < \mu < \infty$ and $\sigma > 0$. The prior distributions for μ and σ were given by, μ ~ N(0,0.001) and σ ~ Gamma(0.01,0.01)

$$m = e^{\mu+\sigma^2/2}$$

$$s^2 = (e^{\sigma^2} - 1)e^{2\mu+\sigma^2}$$

In models 1 and 4, the test sensitivity was assumed to be 100%, in models 2 and 5, the sensitivity was 98% based on lab experience, and in models 3 and 6, the sensitivity was estimated from the data, with an uninformative prior probability distribution (i.e. Beta(1,1)). The six models were compared by means of the deviance information criterion (DIC) where the smaller the value the better.

RESULTS

Data description

Based on the inclusion criteria, 36 seropositive meat turkey flocks negative to virus isolation were included in the study. Of these 36 flocks, 31 were tested for virus isolation and antibody detection on the same day. One was tested for virus isolation 5 days before the serological testing, and 4 were tested 3 to 5 days after the serological testing. The mean detected seroprevalence was 89.3% [85.7;92.2] (in square brackets the exact Fisher's 95% confidence interval).

Six more flocks were tested for virus isolation on the same day as a positive serological test result. These flocks were positive. The mean detected seroprevalence in these flocks was 61.7% [50.3;72.3], which was significantly lower than in the virus negative flocks (*t*-test, P < 0.001).

Models and sensitivity analysis

Inference was based on the marginal posterior densities of the parameters m and s^2, representing the mean and the variance of R_0, respectively (Table 1). For all six models, the probability distributions of R_0 for the population of infected flocks were plotted using the medians of the marginal posterior densities of the parameters κ and ρ (under the gamma distribution assumption), or μ and σ (under the lognormal distribution assumption) (Fig. 2).

Assuming that animals which tested positive were truly infected (models 1 and 4), the mean value of R_0 (i.e. the median of the marginal posterior distribution of the parameter m) was estimated to be 3.7 or 4.2, for the gamma and lognormal distributions for R_0, respectively. The variance of R_0 (i.e. the median of the marginal posterior distribution of the parameter s^2) was higher for model 4, indicating little more variability under the lognormal assumption. Figure 2 shows that models 1 and 4 gave similar results, even if values from the lognormal distribution of R_0 were slightly more dispersed towards the right.

Table 1. Posterior estimates of the mean and variance of R_0, under different assumptions. In brackets the 95% credible intervals of the estimates.

	MODEL OPTIONS		R_0 ESTIMATES		DIC
	R_0 distribution	test sensitivity	mean* (m)	variance* (s^2)	
model 1	gamma	Se=100%	3.7 [2.9;5.4]	3.1 [1.0;12.9]	79.04
model 2	gamma	Se=98%	5.2 [3.4;14.4]	9.5 [1.8;177.0]	69.01
model 3	gamma	Se~Beta(1,1)	5.7 [3.3;20.5]	12.6 [1.8;382.8]	62.40
model 4	lognormal	Se=100%	4.2 [3.1;7.4]	6.8 [1.6;71.5]	78.68
model 5	lognormal	Se=98%	6.6 [3.7;18.7]	34.4 [3.5;1228]	66.48
model 6	lognormal	Se~Beta(1,1)	7.0 [3.6;21.8]	42.1 [3.1;1676]	64.74

*****median of the marginal posterior distribution of the parameters determining the mean and the variance of R_0

Models 2 and 5 assumed that a diagnostic test with 98% sensitivity was applied to detect infected birds. The mean and variance of R_0 were 5.2 and 9.5 for the gamma model, and 6.6 and 34.4 for the lognormal model. The higher variance compared to models 1 and 4 resulted in slightly flatter distributions, as shown in Fig. 2.

Finally, a diagnostic test with unknown sensitivity was assumed in the detection of infected animals in models 3 and 6. The estimates of the mean R_0 were quite close to those of the previous assumption of test sensitivity being equal to 98%: 5.7 for model 3, and 7.0 for model 6. The uncertainty related to unknown test sensitivity was reflected in the higher estimates of the variance of R_0 resulting in flatter R_0 distributions (Fig. 2). In models 3 and 6, estimates of test sensitivity were derived directly from the input data and appeared consistent between the two models and very close to the value of sensitivity chosen for models 2 and 5. The medians of the marginal posterior distributions of test sensitivity were 97.6 (95% CI: 93.8 – 99.8) for model 3 and 97.6 (95% CI: 94.1 – 99.8) for model 6.

Model 3 (gamma distribution, sensitivity estimated from data) had the lowest DIC, indicating the best fit. According to this model, the final estimates are a mean R_0 of 5.7 (95% CI: 3.3 – 20.5), with a variance of 12.6 (95% CI: 1.8 – 382.8).

DISCUSSION

For estimation of R_0 of LPAI in turkeys by the final size equation Eq. (1), some conditions had to be met. First, data needed to reflect a single population of well-mixed animals. Most farms had more than one shed, so data from these farms could have come from different sheds violating the condition of a single population. Only 64 flocks consisted of only one shed. Second, the seroprevalence in the samples should be representative of the entire flocks. That was ensured by implementing a hierarchical model in which the seroprevalence in each flock was considered a sample from a binomial distribution depending on the final outbreak size and the sample size of every flock. Furthermore, according to the surveillance plan, sampled animals were randomly selected within each flock. Third, the outbreak in the flocks should have been over, i.e. no virus should still be circulating. This condition was the reason for only including flocks with a negative virus test ± 5 days from the day of serological testing. The lower seroprevalence in virus positive flocks indicates the validity of this inclusion criterion, although

absolute certainty about the final size status of the flocks can never be obtained. If virus were still circulating in some flocks, R_0 would have been underestimated.

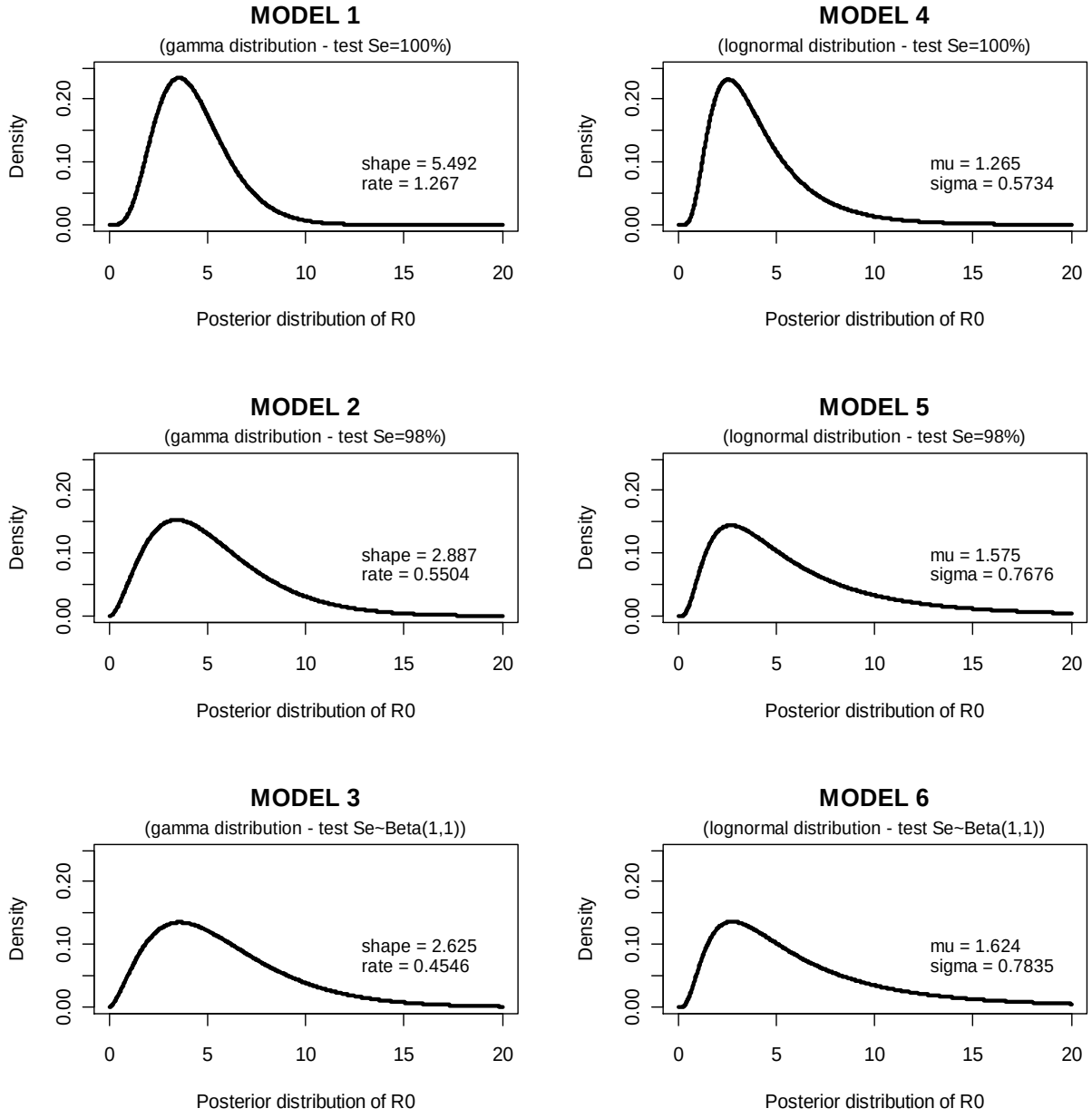


Fig. 2 Posterior distributions of R_0 modelled under different assumptions.

The DICs indicate that model 3 fits the data best. In that model, test sensitivity was estimated along with R_0, resulting in a median estimate of 97.6%, which is close to the value suggested by lab experience (98%). Incorporating the uncertainty associated with test performance accounted for the fact that the data came from a serosurveillance programme, the results of which depend on the true infectious status of the flock, the sampling scheme and the accuracy of the diagnostic assays. In model 3, R_0 was assumed to follow a gamma distribution, although Fig. 2 indicates that the differences between the gamma and lognormal distributions are not very large. The median estimates of the mean and variance of R_0 were 5.7 and 12,

respectively. This would imply that $R_0 < 1$ in approximately 2% of the flocks, and that $R_0 > 10$ in 12% of the flocks. Thus, there is a large variability between flocks.

In earlier analyses of AI from outbreak data (Tiensin et al, 2007; Bos et al., 2009) only a single value of R_0 was estimated, under the assumption that there is an unique value of R_0 common to all flocks. This means that the transmission potential of the infection would be constant among the flocks. However, what can be observed in the field is a wide range of effects produced by the same LPAI virus among different flocks. Some variation could have been due to differences between the viruses in different flocks, but transmission variability of the virus could also have been due to characteristics of the farms (i.e. management, housing, animal density, humidity, etc.) and the age at which outbreak occurred. For example, the density of birds, which may differ from one flock to another, affects the contact rate between birds. The time at which the virus enters into a flock may instead influence the infectivity and/or susceptibility of the birds, in relation to their age, immunological competence and eventual stress due to the intensive production cycle. The approach used here took this variability into account by modelling R_0 as a probability distribution, and thus letting the transmission vary from flock to flock.

In conclusion, this paper presents a new tool for estimating R_0 for LPAI using outbreak data. Since LPAI infections are associated with mild symptoms and low mortality rates, an alternative approach alternative to compartmental models based on mortality data was necessary to make use of field data. In addition, the heterogeneity of disease manifestations in the field suggested that the transmission potential of the disease may differ from flock to flock, and this should be taken into account. This study showed that R_0 is possibly highly variable, indicating that proper use of field data is strongly recommended to obtain reliable estimates of transmission parameters such as R_0.

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REFERENCES

Bean, W.J., Kawaoaka, Y., Wood, J.M., Pearson, J.E. and Webster, R.G. (1985). Characterization of virulent and avirulent A/Chicken/Pennsylvania/83 influenza A viruses: potential role of defective interfering RNA's in Nature. J. Virol. 54,151-160.

Bowes, V.A., Ritchie, S.J., Byrne, S., Sojonky, K., Bidulka, J.J. and Robinson, J.H. (2004). Virus characterization, clinical presentation, and pathology associated with H7N3 avian influenza in British Columbia broiler breeder chickens in 2004. Avian Dis. 48(4), 928-934.

Bos, M.H.E., Nielen, M., Koch, G., Bouma, A., De Jong, M.C.M. and Stegeman, A. (2009). Back-calculation method shows that within-flock transmission of higly pathogenic avian influenza (H7N7) virus in the Netherlands is not influenced by housing risk factors. Prev. Vet. Med. 88, 278–285.

Defra (2008). Highly Pathogenic Avian Influenza H7N7, Oxfordshire, June 2008. Situation at 12.30pm Wednesday 11th June. http://www.cvm.ncsu.edu/dphp/phm/documents/defraEpiReportHPAIOxfordshire2008.pdf (accessed on 30 November 2009).

Elbers, A.R.W., Fabri, T.H.F., de Vries, T.S., Pijpers, A. and Koch, G. (2004). The highly pathogenic avian influenza A virus (H7N7) epidemic in the Netherlands in 2003: lessons learned from the First five outbreaks. Avian Dis. 47, 914-920.

Garcia, M., J.M. C, Latimer, J.W., Rivera-Cruz, E. and Perdue, M.L. (1996). Heterogenecity in the haemagglutinin gene and emergence of the highly pathogenic phenotype among recent H5N2 avian influenza viruses from Mexico. J. Gen. Virol. 77, 1493-1504.

Heffernan, J.M., Smith, R.J.and Wahl, L.M. (2005). Perspective on the basic reproductive ratio. J. R. Soc. Interface 2, 281–293.

Lupiani, B. and Reddy, S.M. (2009). The history of avian influenza. Comp. Immunol. Microb. 4, 311-323.

Ma, J. and Earn, D.J.D. (2006). Generality of the final size formula for an epidemic of a newly invading infectious disease. B. Math. Biol. 68, 679-702.

Marangon, S., Capua, I., Rossi, E., Ferrè, N., Dalla Pozza, M., Bonfanti, L. and Mannelli, A. (2005). Avian Influenza: prevention and control. Kluwer Academic Publishers, p. 33.

Mattews, L. and Woolhouse, M. (2005). New approaches to quantifying the spread of infection. Nat. Rev. Microbiol. 3, 529–536.

OIE – World Organization for Animal Health, (2005). Chapter 2.7.12 Avian Influenza. In: Terrestrial Animal Health Code, 14th Edition. OIE, Paris, France.

OIE – World Organization for Animal Health (2009). WAHID-Interface, Summary of Immediate notifications and Follow-ups - 2007. World Organization of Animal Health. http://www.oie.int/wahis/public.php?page=disease_immediate_summary (accessed on 09 December 2009).

Suarez, D. L., Senne, D.A., Banks, J., Brown, I.H., Essen, S.C., Lee, C.W., Manvell, R.J., Mathieu-Benson, C., Moreno, V., Pedersen, J.C., Panigrahy, B., Rojas, H., Spackman, E. and Alexander, D.J. (2004). Recombination resulting in virulence shift in avian influenza outbreak, Chile. Emerg. Infect. Dis. 10, 693-699.

Tiensin, T., Nielen, M., Vernooij, H., Songserm, T., Kalpravidh, W., Chotiprasatintara, S., Chaisingh, A., Wongkasemjit, S., Chanachai, K., Thanapongtham, W., Srisuvan, T. and Stegeman, J.A. (2007). Transmission of the highly pathogenic avian influenza H5N1 virus within flocks during the 2004 epidemic in Thailand. J. Infect. Dis. 196: 1–6.

Van der Goot, J.A., De Jong, M.C.M., Koch, G. and Van Boven, M. (2003). Comparison of the transmission charachteristics of low and hig pathogenicity avian influenza A virus (H5N2). Epimiol. Infect, 131: 1003-1013.

WHO – World Health Organization (2009). H5N1 avian influenza: Timeline of major events. Available at http://www.who.int/csr/disease/avian_influenza/Timeline090727.pdf (accessed on 30 September 2009).

ZOONOSES

DEVELOPMENT OF A SYSTEMATIC METHODOLOGY BASED ON MOLECULAR EPIDEMIOLOGY TO ASSESS RISK FACTORS FOR BOVINE TUBERCULOSIS: THE EXAMPLE OF BELGIUM DURING THE 1995 TO 2006 PERIOD

M.-F. HUMBLET, M. GILBERT, K. WALRAVENS, M. GOVAERTS, M. FAUVILLE-DUFAUX AND C. SAEGERMAN*

SUMMARY

The aims of the present study were to elaborate a model of bTB space-time dynamics analysis and to identify potential risk factors for the disease in order to suggest a re-orientation of surveillance measures. A database including *Mycobacterium bovis* strains isolated during the 1995-2006 period was compiled using three molecular typing techniques. *Mycobacterium bovis* strains were classified into twelve lineages (I to XII): lineage VII appeared as being predominant in the country. Several potential risk factors (n=49) were tested thanks to a multiple stepwise logistic regression. Two approaches were undertaken: all *M. bovis* strains were considered in the first step, while the second approach only included the predominant lineage. The first approach identified a bTB antecedent in the herd ($P<0.001$), the distance to an outbreak (neighbouring effect) ($P<0.001$) and cattle density ($P<0.001$) as significant risk factors. The second approach highlighted the proportion of movements from an infected area during the current year as a main risk factor ($P=0.007$). Several risk factors for bTB were identified in Belgium for the first time thanks to the present study.

INTRODUCTION

The situation of bovine tuberculosis (bTB) remains preoccupant in the European Union. Some Member States (MSs) even reported recently a re-emergence of the disease (EFSA, 2006). Control measures were successful for the eradication of bTB in some MSs, while other countries Officially Tuberculosis-Free (OTF) still notify several outbreaks every year (EFSA, 2006). Belgium was indeed declared as OTF in 2003; nevertheless, between 5 and 10 outbreaks are notified every year (FASFC, 2008). In 2008, the number of reported outbreaks increased to 14 (FASFC, 2008).

Risk factors for bTB, including a variety of parameters such as wildlife, contacts between animals, movements, animal density, etc. were identified around the world (review in Humblet et al., 2009). The failure to eradicate *M. bovis*-tuberculosis in cattle could be explained by

* Research Unit in Epidemiology and Risk Analysis applied to Veterinary Sciences (UREAR), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, B42, Boulevard de Colonster 20, B-4000 Liège, Belgium. Email: claude.saegerman@ulg.ac.be

factors such as inadequate control measures, agro-environmental factors, wildlife reservoirs and movement of infected animals (Gilbert et al., 2005). A reduction of control measures, by suppressing testing at purchase and reducing herd testing, was recently suggested, partly because bTB control programmes are economically forcing. Nevertheless, no study investigated to date the potential risk factors for bTB in Belgium to date. Animal movements were indeed previously identified as a major risk factor for bTB in the UK (Gilbert et al., 2005, Gopal et al., 2006).

A database including all *M. bovis* strains isolated between 1995 and 2006 in the country was compiled thanks to molecular typing. This database was first used to analyse the bTB space-time dynamics in Belgium during the period of interest. A complete review of the literature on bTB risk factors allowed to identify potential risk factors to be tested in the country. A statistical model developed for the situation in the UK was applied to the Belgian context in order to test these potential risk factors; their identification would be valuable for sanitary authorities to re-evaluate and adapt current bTB control measures.

MATERIALS AND METHODS

Databases

The database of all *M. bovis* strains isolated in Belgium between the 1st of January 1995 and the 31st of December 2006 compiled the results of samples analysed by the national reference laboratory for bTB. The strain was identified by using three molecular techniques in parallel: spoligotyping, RFLP (Restriction Fragment Length Polymorphism) and MIRU (Mycobacterial Interspersed Repetitive Unit) - VNTR (Variable-Number Tandem-Repeat) (Allix et al., 2006; Durr et al., 2000; Roring et al., 2000). After classifying these strains into XII lineages according to their molecular profile, Lineage VII appeared to be predominant between 1995 and 2006 (Walravens et al., 2006).

The national list of cattle holdings as well as their annual inventory (number of cattle heads per herd, as defined on the 31st of December of the civil year, data available from 2000 and after) were provided by the Federal Agency for the Safety of the Food Chain (FASFC). Data on cattle movements between 1995 and 2006 were extracted from the National Cattle Tracing System (SANITEL).

Annual estimated populations of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), wild boars (*Sus scrofa*), fallow deer (*Dama dama*) and mouflons (*Ovis orientalis*) were provided by the Nature and Forest Division (NFD) and included in the model.

Land use and land cover data were also included in the model (pasture, crops, forests, water and urban areas) as well as the length of forest-pasture edge.

The collection of remotely sensed climate data included in the model was previously described by Hay and collaborators (Hay et al., 2006). Elevation was an additional parameter used in the model.

Predictors

A complete revision of literature on bTB risk factors allowed to select the potential risk factors to be investigated in Belgium. A total of 49 parameters, named predictors and derived from the databases mentioned above, were included in the statistical model and considered as

potential risk factors for bTB. All predictors were compiled into a unique database. For the analysis of bTB dynamics, the temporal unit was annual, and the spatial unit was defined as follows: the territory was divided into 5km x 5km cells identified by their *X* and *Y* Lambert coordinates. The following predictors were then re-sampled at the 5km resolution: distance to the centre of an infected cell (short-distance spread), wild population densities, land use and land cover as well as climatic data, cattle movements and density. Land cover data included in the model were expressed as the percentage of a cell occupied by the different types of vegetations (equivalent to ha/km^2). BTB persistence (PBTB) was included in the model as follows: a score was allocated to each cell, for each year of the period of interest according to the presence/absence of bTB outbreaks. No bTB outbreak corresponded to a score of 0, while a score of 1 meant the presence of bTB. When the presence of bTB was confirmed, the *M. bovis* strain was specified.

Two records were registered for each movement of cattle: the location the animal has moved off and the location it has moved onto. The treatment of cattle movement data involved pairing of 'on' and 'off' movements, which provided three categories of variables for each cell: the total number of inward movements, the total number of movements from infected areas and the proportion of movements from infected areas. The influence of movements that occurred the year before a bTB outbreak was first tested. The impact of movements taking place during the year of occurrence of a bTB outbreak in a given cell was further investigated. The whole process led to the building of a unique multi-annual database including all informations on the 49 predictors, per cell and per year.

Statistical analyses

A stepwise multiple logistic regression was applied to analyse the relationship between the occurrence of bTB and the 49 predictors. Gilbert and collaborators originally built up this regression model to study the impact of animal movements on bTB transmission in Great Britain (Gilbert et al., 2005). The model was further adapted to Belgium, the results of molecular typing being as well included. Each predictor Wald's statistics quantified its contribution to the model. The following predictors were first entered in the model: PBTB and short-distance spread (number of infected cells in the previous year in a 5km in radius window). A positive relationship between these two predictors and the presence of bTB was highlighted. Other predictors were added to the model using a standard-entry stepwise procedure. The model was restricted to variables with the highest predictive power, and only those presenting more than 1% of log-likelihood change after removal were retained. The best predictors were systematically tested with others families of predictors (wildlife, climatic, land cover), in a descending approach. At each step of the process, the predictor with the lowest *z* value was discarded. The final step consisted in testing together all predictors showing a positive or a negative relationship with the presence of bTB. In fact, the 49 predictors could not be initially tested together at the same time because some of them were correlated. A predictor was considered as being a significant risk factor when presenting a positive association with $P<0.05$.

Two statistical approaches were performed. The first approach included all *M. bovis* strains identified in Belgium between 1995 and 2006 while the second approach focused on strains belonging to the predominant Lineage VII (Walravens et al., 2006).

R software was used to carry out the whole statistical process (R Development Core Team, 2009).

RESULTS

All *M. bovis* strains

PBTB, the distance to the centre of an infected cell and cattle density were positively associated with the presence of bTB, as shown in table 1, and were thus systematically tested with other families of predictors. None of the three categories of movements (total number of inward movements, total number of movements from infected areas and proportion of movements from infected areas) was associated with the presence of bTB.

Wildlife predictors were tested in parallel with the best predictors (PBTB, short-distance spread and cattle density) in a descending approach, to finally conserve predictors showing a significant association. Red deer and roe deer densities happened to be negatively associated with the presence of bTB. The same approach was carried out for land cover predictors: forest density was negatively correlated with the risk of bTB.

A descending approach involving climatic predictors and elevation revealed that annual amplitude of mean middle-infrared (MIR) temperature was positively associated with the presence of bTB. On the other hand, bi-annual amplitude of mean MIR temperature, normalized difference vegetation index (NDVI) phase of annual cycle and elevation were negatively associated with the presence of bTB, as shown in Table 1.

Once all the families of predictors had been tested separately with the three best predictors, the variables presenting a significant association with the presence of bTB were tested all together. Some predictors then lost their significant effect as for example, red deer and roe deer densities, percentage of forest cover per pixel and elevation.

M. bovis strains strictly belonging to lineage VII

Only data linked to lineage VII were included in this approach. Table 2 resumes the statistical results of this approach. PBTB and short-distance spread were still positively associated with the presence of bTB contrary to cattle density, which was not associated anymore, except for the year 2001. Thus, only PBTB and short-distance spread were tested with other families of predictors. The proportion of movements from infected areas during the current year presented a significantly positive relationship with the presence of bTB, as well as crop surface (percentage of a cell cover). Wild species densities, especially roe deer, were negatively associated with the presence of bTB. Regarding climatic variables, annual amplitude of MIR temperature was positively associated with the occurrence of bTB while Bi-annual amplitude of mean MIR temperature and NDVI phase of annual cycle were negatively associated with the presence of bTB (Table 2).

After testing all families of predictors separately, a model including all variable significantly associated with the presence of bTB was applied. The same predictors as in the first approach (all strains) lost their significant effect. The proportion of movements from infected areas during the current year was positively associated while bi-annual amplitude of mean MIR temperature was negatively associated with the occurrence of bTB (Table 2).

Table 1. Summary of the predictors significantly associated with the occurrence of bTB (first approach including all M. bovis strains)

Predictor	***P* value**	**Significance**
Persistence of bTB	$< 2^{e-16}$	***
Distance to the centre of an infected cell	$< 2^{e-16}$	***
Cattle density in 2003	7.01^{e-09}	***
Red deer density	0.007	**
Roe deer density	0.002	**
Surface of the cell occupied by forests (km^2)	0.00015	***
Land surface temperature annual amplitude (°C)	2.23^{e-05}	***
Land surface temperature bi-annual amplitude (°C)	0.03	*
Normalised difference vegetation index phase of annual cycle	0.0005	***
Elevation	0.002	**

* = $P<0.05$, ** = $P<0.01$ and *** = $P<0.001$

Table 2. Summary of the predictors significantly associated with the occurrence of bTB (second approach exclusively including M. bovis strains belonging to Lineage VII)

Predictor	***P* value**	**Significance**
Persistence of bTB	2.86^{e-07}	***
Distance to the centre of an infected cell	$< 2^{e-16}$	***
Proportion of movements from infected areas[#]	0.007	**
Roe deer density	0.01	*
Surface of the pixel covered by crops (km^2)	0.002	**
Land surface temperature annual amplitude (°C)	0.004	**
Land surface temperature bi-annual amplitude (°C)	0.007	**
Normalised difference vegetation index phase of annual cycle	0.002	**

* = $P<0.05$, ** = $P<0.01$ and *** = $P<0.001$; [#]from the current year

DISCUSSION

Several predictors presented a significant associated with the presence of bTB in Belgium, and can thus be considered as risk factors, identified for the first time in Belgium.

The persistence of bTB was shown to be a significant risk factor, as previously observed in other countries: English scientists demonstrated that bTB outbreaks occur repeatedly in the same areas (White and Benhin, 2004). Two hypotheses can be assumed to explain such observation: the source of infection is not eradicated and/or permanent factors may support the re-emergence of bTB in a particular area.

The distance to an infected cell is a significant risk factor for bTB in Belgium, which confirms the importance of bTB short-distance spread. Griffin and collaborators already noticed in the Republic of Ireland that, in a short period of time, bTB outbreaks affect most frequently several holdings at the same time rather than a sole herd (Griffin et al., 1996); they concluded the contiguity with an infected herd was a risk factor. More recently, Denny and Wilesmith showed in the same country that an outbreak in a particular herd was associated with an infected

nearby herd (Denny and Wilesmith, 1999). BTB infection in contiguous herds could find its origin in a common source of infection. Contacts between infected and healthy animals over fences were shown to play a key role in the transmission of *M. bovis* in North America (Kaneene et al., 2002; Munroe et al., 1999).

Cattle density was identified as a risk factor in the first approach (inclusion of all *M. bovis* strains in the regression model), but only for the year 2001 when the statistical analysis focused on Lineage VII. Intensive farming, involving closer contacts between animals, has gained ground during the last decades (Thoen et al., 2006). As Airborne transmission is indeed the principle route of infection in cattle, the risk of spreading the disease increased in such conditions; aerogenic transmission of *M. bovis* predominates under intensive conditions (Menzies and Neill, 2000; Gannon et al., 2007). Cosivi and collaborators previously observed the highest incidence of bTB was generally noticed in areas of intensive farming (Cosivi et al., 1998).

When all *M. bovis* strains were included in the model, animal movements had no significant effect on the risk of bTB. Nevertheless, considering Lineage VII-strains, the proportions of movements from infected cells from the current year were positively associated with the presence of bTB. Gilbert and collaborators had previously identified animal movements as a major risk factor of bTB occurrence in Great Britain (Gilbert et al., 2005). Recently, an additional study carried out in the UK and focusing on the analysis of cattle movements between 1985 and 2003, also highlighted the major impact of animal movements from an endemic zone to a free-area on the risk of bTB (Gopal et al. 2006).

Wildlife reservoirs would play an important role in the transmission of *M. bovis* to domestic cattle, e.g. badgers in the UK and Republic of Ireland (Cheeseman et al, 1988; Denny and Wilesmith, 1999; Griffin et al., 1993) and brush-tail possums in New Zealand (Morris and Pfeiffer, 1995). Cervids are implicated in the transmission of the disease in North America (Kaneene et al., 2002), Spain (Aranaz et al., 1996) and France (Zanella et al., 2008). Infected wild boar are frequently reported in Western Europe especially in France, Spain and Italy (Parra et al., 2003; Serraino et al., 1999; Zanella et al., 2008). *M. bovis* has not been isolated from Belgian wildlife so far, but the risk of infection should not be left aside. In the present study, wild species densities showed a negative relationship with the presence of bTB. These predictors were in fact correlated with land covered by forests. Wildlife densities and forests are also located in highest areas of the country, which explains that elevation was also negatively associated with the risk of bTB. Indeed, few outbreaks have been reported in these areas.

Crop density was identified as a significant risk factor in the approach focusing on Lineage VII-strains, which could be explained by the fact that farms are generally concentrated around culture areas for fodder supply.

The environmental survival of *M. bovis* is influenced by climate (Phillips et al., 2003). The present study identified annual amplitude of temperature on the earth surface as a significant risk factor for bTB. Scientists from New Zealand suggested the environmental survival of *M. bovis* would be inversely proportional to mean daily temperatures (Jackson et al., 1995). Favourable conditions for *M. bovis* survival are temperatures just above 0°C coupled with a strong hygrometry, conditions frequently observed in Western Europe in the wintertime (Artois et al., 2004). Seasonality and climate changes from one year to another would also be related to the occurrence of bTB, as shown in Wales and Scotland (Wint et al., 2002). Climatic conditions may be associated with elevation, as they are more severe at higher elevations and temperatures

are lower in the wintertime. Nevertheless, the influence of climate on the environmental survival of *M. bovis* still needs to be further investigated.

This is the first study to investigate bTB risk factors in Belgium: antecedents of bTB in a herd or in a small area, the proximity with an outbreak and cattle density were shown to be significant risk factors for the occurrence of bTB. Thus, it does not seem judicious to consider suppressing skin testing at purchase and herd testing, as animal movements from infected areas were shown to be at risk for the predominating lineage of strains circulating in the country. Epidemiological surveillance of wildlife must not be slackened as the situation in France or in the UK got worse lately. The importance of environment and climate on the survival of *M. bovis* should be given additional attention. Particular strains of *M. bovis* could possibly behave differently, as suggested by the results obtained in the present study. Molecular epidemiology is thus crucial to deepen possible differences of virulence between *M. bovis* strains.

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REFERENCES

Allix, C., Walravens, K., Saegerman, C., Godfroid, J., Supply, P. and Fauville-Dufaux, M. (2006). Evaluation of the epidemiological relevance of variable-number tandem-repeat genotyping of *Mycobacterium bovis* and comparison of the method with IS6110 restriction fragment length polymorphism analysis and spoligotyping, J. Clin. Microbiol. 44 (6), 1951-62.

Aranaz, A., Lichana, E., Mateos, A., Dominguez, L., Vidal, D.K., Domingo, M., Gonzolez, O., RodriguezFerri, E.F., Bunschoten, A.E., VanEmbden, J.D.A. and Cousins, D. (1996). Spacer oligonucleotide typing of *Mycobacterium bovis* strains from cattle and other animals: a tool for studying epidemiology of tuberculosis. J. Clin. Microbiol. 34, 2734-2740.

Artois, M., Loukiadis, E., Garin-Bastuji, B., Thorel, M.F. and Hars J. (2004). Infection des mammifères sauvages par *Mycobacterium bovis* – Risque de transmission aux bovins domestiques. Bulletin Epidémiologique, Agence Française de Sécurité Sanitaire des Aliments 13, 1-3.

Cheeseman, C.L., Wilesmith, J.W., Stuart, F.A. and Mallinson, P.J. (1988). Dynamics of tuberculosis in a naturally infected badger population. Mammal Rev. 18, 16-71.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F.A.K., de Kantor, I. and Meslin, F.X. (1998). Zoonotic Tuberculosis due to *Mycobacterium bovis* in developing countries. Emer. Infect. Dis. 4, 59-70.

Denny G.O. and Wilesmith J.W. (1999). Bovine tuberculosis in Northern Ireland: a case-control study of herd risk factors. Vet. Rec. 144, 305-310.

Durr, P.A., Clifton-Hadley, R.S. and Hewinson, R.G. (2000). Molecular epidemiology of bovine tuberculosis. II. Applications of genotyping. Rev. Sci. Tech. 19, 689-701.

European Food Safety Authority (2006). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2005. The EFSA Journal. [on line] 94, 136-143. http://www.efsa.europa.eu/EFSA/DocumentSet/Zoonoses_Report_EU_en_2005.pdf.

Federal Agency for the Safety of the Food Chain. Annual Activity Report (2008) [on line] p.130. http://www.favv-afsca.fgov.be/rapportsannuels/_documents/2009-07-07_RA08_fr.pdf. (consulted on August 21, 2009).

Gannon, B.W., Hayes, C.M. and Roe, J.M. (2007). Survival rate of airborne *Mycobacterium bovis*. Res. Vet. Sci. 82, 169-72.

Gilbert, M., Mitchell, A., Bourn, D., Mawdsley, J., Clifton-Hadley, R. and Wint W. (2005). Cattle movements and bovine tuberculosis in Great Britain. Nature 435, 491-6.

Gopal, R., Goodchild, A., Hewinson, G., De la Rua-Domenech, R. and Clifton-Hadley, R. (2006). Introduction of bovine tuberculosis to north-east England by bought- in cattle. Vet. Rec. 159, 265-271.

Griffin, J.M., Hahesy, T., Lynch, K., Salman, M.D., McCarthy, J. and Hurley, T. (1993). The association of cattle husbandry characteristics, 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.M., Martin, S.W., Thorburn, M.A., Eves, J.A. and Hammond, R.F. (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, 75-87.

Humblet, M.F. Boschiroli, M.L. and Saegerman C. (2009). Classification of worldwide bovine tuberculosis risk factors in cattle: a stratified approach. Vet. Res. 40, 50.

Jackson, R., De Lisle, G.W. and Morris, R.S. (1995). A study of the environmental survival of *Mycobacterium bovis* on a farm in New Zealand. N. Z. Vet. J. 43, 346-35.

Kaneene, J.B., Bruning-Fann, C.S., Granger, L.M., Miller, R. and Porter-Spalding A. (2002). Environmental and farm management factors associated with tuberculosis on cattle farms in northeastern Michigan. J.A.V.M.A. 221, 837-842.

Menzies, F.D. and Neill, S.D. (2000). Cattle-to-cattle transmission of bovine tuberculosis. Vet. J. 160, 92-106.

Morris, R.S., Pfeiffer, D.U., and Jackson, R. (1994). The epidemiology of *Mycobacterium bovis* infections. Vet Microbiol. 40, 153-177.

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, 119-133.

Parra, A., Fernández-Llario, P., Tato, A., Larrasa, J., García, A., Alonso and J.M., de Mendoza, J.H. (2003). Epidemiology of *Mycobacterium bovis* infections of pigs and wild boar using a molecular approach. Vet. Microbiol. 97, 123-133.

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

R Development Core Team, (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-90005-07-0, URL http://www.R-project.org.

Roring, S., Hugues, M.S., Skuce, R.A. and Neill, S.D. (2000). Simultaneous detection and strain differentiation of *Mycobacterium bovis* directly from bovine tissue specimens by spoligotyping. Vet. Microbiol. 74, 227-236.

Serraino, A., Marchetti, G., Sanguinetti, V., Rossi, M.C., Zanoni, R.G., Catozzi, L., Bandera, A., Mignone, W., Franzetti, F. And Gori, A. (1999). Monitoring of transmission of tuberculosis between wild boars and cattle: genotypical analysis of strains by molecular epidemiology technique. J. Clin. Microbiol. 37, 2766-2771.

Thoen C., LoBue P. and de Kantor I. (2006). The importance of *Mycobacterium bovis* as a zoonosis. Vet. Microbiol. 112, 339-345.

Walravens, K., Allix, C., Supply, P., Rigouts, L., Godfroid, J., Govaerts, M., Portaels, F., Dufey, J., Vanholme, L., Fauville-Dufaux, M., Saegerman, C. (2006). Dix année d'épidémiologie moléculaire de la tuberculose bovine en Belgique. Epidémiol. et santé anim. 49, 103-111.

White, P.C.L. and Benhin, J.K.A. (2004). Factors influencing the incidence and scale of bovine tuberculosis in cattle in southwest England. Prev. Vet. Med. 63, 1-7.

Wint, G.R.W., Robinson, T.P., Bourn, D.M., Durr, P.A., Hay, S.I., Randolph, S.E. and Rogers, D.J. (2002). Mapping bovine tuberculosis in Great Britain using environmental data. Trends Microbiol. 10, 441-444.

Zanella, G., Durand, B., Hars, J., Moutou, F., Garin-Bastuji, B., Duvauchelle A., Ferme, M., Karoul, C. and Boschiroli, M.L. (2008). *Mycobacterium bovis* in wildlife in France. J. Wildl. Dis. 44, 99-108.

SHIFTING THE TB-CONTROL PARADIGM: AN EPIDEMIOLOGICAL CRITIQUE OF THE BOVINE TUBERCULOSIS ERADICATION SCHEME

D.A. ABERNETHY*, D. GRAHAM, R. SKUCE, A. GORDON, F. MENZIES, P. ROBINSON, R. HARWOOD AND N. CLARKE

SUMMARY

Bovine tuberculosis (BTB) remains an intractable problem in parts of Europe despite long-term eradication programmes, extensive research and substantial financial investment. EEC Directive 64/432 (as amended), a trade Directive, provides the legislative basis for eradication and stipulates livestock identification and movement control requirements, the nature of BTB surveillance employed and requisite control measures following disclosure of infection.

The epidemiological basis of Directive 64/432 was assessed using results from historical and concurrent studies in Northern Ireland. Descriptive analyses evaluated the "one-size-fits-all" approach of the Directive. An observational study assessed the epidemiological value of confirming infection in test reactors, a key Directive criterion for determining the intensity of control measures. A retrospective cohort study explored the long-term risks of BTB exposure associated with putative latency.

The studies demonstrated that BTB is strongly clustered at strain level, within-herd prevalence is generally low (median number of reactors is two, quartiles = one and four) and major risk factors for recurrent infection were location, a history of BTB and multiple reactors at the disclosure test. For example, herds with BTB disclosed between 2005 and 2007 were three times more likely to have an outbreak in 2008 than those with a clear history (Relative Risk = 3.6; 95% C.I. = 3.31 to 3.92; $p<0.001$). Similarly, the odds of disclosing infection at post-outbreak tests were doubled in herds with multiple reactors compared to those with no further positives (Odds Ratio = 2.15; 95% C.I. = 1.88 to 2.46; $p<0.001$).

These studies suggest EC 64/432 is an inadequate framework for the eradication of TB as it fails to address key risk factors and constrains attempts to target measures in a more efficient and cost-effective manner. An alternative approach is proposed that will require shifting the control paradigm from trade to eradication.

INTRODUCTION

Bovine tuberculosis (BTB) was once a disease of significant animal health and public importance in member states (MSs) of the European Community but has since been eliminated from most, usually through surveillance and eradication programmes. In 1964, legislation (EEC

* Epidemiology Unit, Veterinary Service, Department of Agriculture and Rural Development, Northern Ireland. E-mail: darrell.abernethy@dardni.gov.uk

64/432) was introduced to govern intra-Community trade in livestock and its prerequisites for BTB have formed the basis for BTB eradication programmes. Although the Directive has been altered extensively over the years, the core BTB measures have remained largely unchanged, including test intervals (annual if herd prevalence exceeds 1%), movement restrictions for herds with BTB, test methods and standards, compulsory removal of test reactors and follow-up requirements (at least two negative tests 42 to 60 days apart if infection is confirmed or one further test after 42 days if unconfirmed).

Northern Ireland (NI) has a population of approximately 1.6 million cattle among 25000 active, predominantly beef-cow herds. A BTB eradication programme commenced in 1959 but despite early successes, elimination of the disease has proved elusive. Putative risk factors include substantial between-herd and within-herd cattle movement, farm fragmentation and renting of pasture, high cattle and herd densities and BTB in the Eurasian badger (*Meles meles*). This paper describes recent trends and epidemiological features of BTB in NI. Fundamental issues of epidemiology and control are highlighted that must still be addressed if progress towards eradication is to be achieved and an argument is made for a more flexible, epidemiology-driven approach to the disease.

MATERIALS AND METHODS

Testing, movement and farm data were extracted from the Department of Agriculture's Animal and Public Health Information System while data on BTB strains were obtained from the Northern Ireland Agrifood and Bioscience Institute, a non-Departmental Government Body. Descriptive statistics and univariate analyses were performed using data from 1995. Clustering of the ten most prevalent strains identified by VNTR was explored with SatScan (Version 3.0.4; Kulldorff M and Information Management Services Inc.) using the location of the main farm premises as point data with maximum cluster size set at the default 50% of the population at risk. Logistic regression was used to explore the effect of selected risk factors on recrudescence of BTB in breakdown herds recertified after completing their statutory regimen of short-interval testing whilst under movement restrictions. The six-month post-outbreak test was used as the outcome variable with a positive case being any reactor to the test. The sampling frame excluded chronic breakdowns, defined for this study as herds with repeated restrictions and no post-outbreak test for two or more years, and herds where the post-outbreak test was superseded by testing due to additional risk factors. The herd identifier was included as a random effect in the model as herds may have experienced several breakdowns during the study period.

A retrospective cohort study was undertaken to explore the long-term risks of putative exposure to BTB. The exposed cohort was identified using two-stage sampling of herds with more than 20 cattle, confirmed infection and multiple reactors at their disclosure test between 1995 and 2003. Exposed cattle were those present and negative at both the disclosure test and on recertification. The first stage sample of unexposed herds was selected from a sampling frame of herds with a proven three-year freedom of infection and located within three kilometres of exposed herds. Two unexposed herds were selected within each year for each exposed herd and the first herd test and date within the year used as the reference test and date respectively. The unexposed cohort thus comprised cattle present at the reference test. Both exposed and unexposed cohorts were divided into those that were retained in, or slaughtered directly from their herd of birth ("retained" sub-cohort) and those that were sold on ("moved out" sub-cohort).

RESULTS

Northern Ireland experienced a significant increase in BTB herd prevalence and incidence between 1995 and 2002, from 5.4% to 13.8% and from 4.2% to 11.9% respectively. Both decreased until 2007 (to 8.4% and 6.8% resp.) then remained constant the following year (Figure 1).

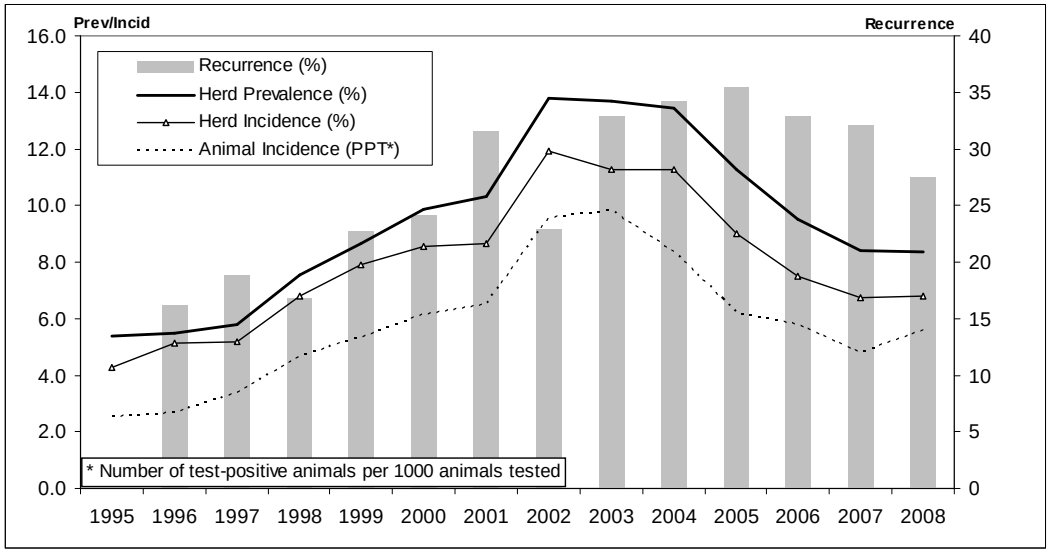


Fig. 1 BTB Trends in Northern Ireland, 1995 to 2008

The rise was observed in most (22 of 29) rural district council districts (RDC) and was independent of underlying BTB prevalence at the start of the period. The annual increases were similar across most regions but unusually high increases lasting 2 to 4 years and resembling localised epidemics were observed in four. The total number of tests performed increased from an annual mean of 2.2 million in the five years preceding 2001, or 1.32 tests per animal in the population, to 2.7 million (1.52 tests per animal) in the five years after 2001. However, repeat testing is biased to certain herds and regions: in 2008, 66.3% of animals were tested once (72.4% of herds), 21.2% twice (17.4% herds) and 12.5% on three to six occasions (10.2% of herds). The percentage of recurrent breakdowns (TB-positive herds within each year that were positive the previous calendar year) increased in line with the disease trends (2001 and 2002 were affected by test changes associated with the FMD epidemic), but reduced at a slower rate when incidence declined (Fig. 1).

Between 1990 and 2008, 45.3% of BTB herds experienced a single outbreak, 23.1% had breakdowns in two separate years while the remaining 31.6% had three or more incidents. The within-herd prevalence (WHP) increased strongly between 1996 and 2002 but the overall increase across the study period was slight, affected by herd size and the disease-status of the test. Thus, the regression equation for the WHP trend in the middle tercile of herds (45 to 103 cattle) was $y = 0.029x + 3.82$ for routine tests, $y = -0.042 = 5.52$ for risk-derived tests and $y = 0.123x + 3.78$ for restricted tests. The median number of reactors for test-positive herds in 2008 was two (upper and lower quartiles = one and four resp.). The cumulative prevalence of confirmed infection among test reactors was 39.9% (range 31.4% in 2004 to 48.6% in 1997).

In an analysis of the 10 most prevalent VNTR strains, spatial clustering was observed in all, with a mean radius of 27.7km in the primary clusters (range: 15.9 km to 46.3 km). Reactor cattle disclosed with these strains in 2006 or 2007, which moved once between herds in different rural council districts (and from which fifty BTB isolates had been obtained), were more likely to

have a strain consistent with that identified in the RDC of the destination herd than the herd of origin, irrespective of the duration spent in either the birth or destination herds (65% of 445 cattle; $\chi 2$ test; $p < 0.001$).

Table 1. Multivariate Model of Risk Factors

Variable	Category	Odds Ratio	95% C.I.
Outbreak within previous 2 years	n/a	1.191	1.065 - 1.332
Number of reactors at disclosure test	1	1.000	-
	2	1.175	0.990 - 1.393
	3-5	1.485	1.269 - 1.739
	>5	1.766	1.482 – 2.103
Number of reactors at follow-up tests	0	1.000	-
	1	1.209	0.928 - 1.574
	>1	1.840	1.420 - 2.382
Number of Follow-up Tests	0	1.000	-
	1	1.258	1.095 – 1.444
	>1	0.937	0.713 – 1.230
BTB in district council area	<7%	1.000	-
	7 to 11%	1.402	1.213 – 1.621
	>11%	2.037	1.775 – 2.338
Test Herd Size at Disclosure	<37	1.000	-
	37 – 74	1.582	1.317 – 1.901
	75 – 137	1.972	1.649 – 2.358
	>137	2.698	2.264 – 3.215
Number Cattle Purchased During Post-outbreak Interval	0	1.000	-
	1 – 10	1.122	0.982 – 1.280
	11 – 30	1.227	1.038 – 1.450
	>30	1.228	1.027 – 1.468

The study of risk factors for repeat breakdowns included 19871 incidents (12545 herds), about 70% of all breakdowns in the period. In the final random effects model, herds with more than two reactors at the disclosure test or more than one reactor at a follow-up test were at a significantly higher risk of a further breakdown than herds with singleton reactors, as were those with only one further follow-up test. Other significant variables were herd size, a history of BTB, an increased level of BTB in the RDC and acquisition of more than 10 cattle during the post-outbreak interval (Table 1). Neither confirmation of infection at the disclosure test nor

subsequently in the breakdown was statistically significant. In the cohort study, the crude relative risks of failure were 1.87 in the retained sub-cohort (95% C.I. = 1.84 – 1.91) and 1.35 in the moved-out sub-cohort (moved out within 180 days; 95% C.I. = 1.25 – 1.45).

DISCUSSION

Eradication of BTB has proved elusive in some European MSs, most notably the United Kingdom, Ireland and Spain, where potential wildlife reservoirs are present. Despite extensive research programmes, fundamental questions remain concerning transmission routes, the relative roles of cattle versus wildlife and methods of control. For example, most outbreaks in the Republic of Ireland are attributed to badgers and reactive culling is the principal control method (More, 2009). By contrast, culling in England was judged to be ineffective unless undertaken repeatedly, over large areas (Donnelly et al., 2007) and efforts have thus concentrated on mitigating the risk from infected cattle.

In Northern Ireland, cattle-cattle transmission is still viewed by many to be the most significant means of spread and badger culling is not employed. The nature of farming in the province with high stock densities, high cattle movement and extensive contact at pasture supports the role attributed to the infected bovine. The increase in BTB between 1995 and 2002 is still not fully understood but a resource-associated delayed response was postulated to be a contributory factor, supporting the role of cattle-based programme management (Abernethy et al., 2006). A concomitant increase in bovine brucellosis was similarly attributed to reduced programme measures and field investigations demonstrated the role of local cattle-cattle contact in the spread of that disease (Abernethy, 2008). After 2002, the reduction in BTB in the absence of badger-related measures appeared to support the value of bovine-related measures, raising the question as to whether badger-related controls are necessary.

Caution is needed however, before assuming that BTB can be addressed solely through enhanced bovine testing and control measures; the data point equally to a substantial role for a wildlife source. Both the observational and cohort studies indicate an ongoing risk in or around infected herds, but significant lateral spread despite low within-herd spread is paradoxical. The consistent, localised clustering of BTB strains despite substantial cattle movement and the significant proportion of moved reactor cattle with strains matching the areas of destination suggests a significant role for a localised, non-bovine source. It is likely that BTB in NI results from a complex mixture of both cattle-cattle and badger-cattle transmission pathways. Thus, it is possible that the rise and fall of BTB between 1995 and 2007 reflects programme-related, cattle-associated issues, superimposed on an underlying, increasing trend present since 1986 (D. Abernethy, unpublished observations). It is interesting to observe that such a trend in NI is similar to that in Great Britain rather than in Ireland (where the level of BTB has remained more-or-less constant), despite greater similarities in farming and control measures within the island of Ireland than between NI and Great Britain.

BTB eradication within the European Community is governed by EEC Directive 64/432 and compliance is monitored by the European Food and Veterinary Office and through co-financing arrangements. MSs can exceed the legislative requirements but may be constrained by stakeholders who question the legal basis or equitable application of any targeted proposals. MSs may not, however, apply measures less stringent than those stipulated by the Directive, implying that the legislation must be epidemiologically sound or it will be inefficient, failing to enhance eradication or unnecessarily increasing eradication costs. The Directive however, has

certain inherent weaknesses. It is standardised across the EC and thus does not allow for differences in risk factors between MSs and consequently, in the measures employed to address them. Thus, for example, contiguous spread has historically been considered to be more important in Northern Ireland than in Great Britain (Wilesmith & Williams, 1986) while premovement testing was introduced in certain regions in the latter but deemed to have limited value in the Republic of Ireland (Clegg et al., 2008). These differences are not problematic as the associated measures do not conflict with the Directive, although failure to implement pre-movement testing among other issues of compliance in 2005 resulted in a five-year withdrawal of EC funding for the programme in NI. A more fundamental issue arises however, when risk factors in the Directive are not supported by the epidemiological evidence. Thus, for example, the results of the observational study suggest that multiple reactors are a significant risk factor for further disease in infected herds rather than confirmation of infection. These findings, albeit preliminary, are consistent with those of Olea-Popelka and colleagues (2004), who found the future hazard of a breakdown in Ireland to increase significantly with number of reactors, not confirmation of infection. On this basis it would be prudent to use reactor numbers rather than confirmation to determine the follow-up strategy in outbreaks but this is not permissible within current regulations. As a consequence, some infected herds may be permitted to trade prematurely, as the Directive permits recertification after one negative test in culture-negative herds, irrespective of the number of test reactors. This is likely to increase the risk of spread through sale of infected cattle. Conversely, herds with singleton, culture-positive reactors present little risk and thus do not merit the same follow-up procedures as larger outbreaks. In the observational study, almost eight percent of herds contained reactors at their six-month post-outbreak test and recurrent infection has been an increasing phenomenon in recent years. Such outbreaks may arise due to residual infection, suggesting the restricted period was too short, or from ongoing exposure suggesting biosecurity measures were inadequate. In either case, these herds present an ongoing risk to other herds through sale of cattle or contact at pasture. It would therefore seem prudent to prolong the restricted period in such herds and either limit movement to slaughter for extended periods or permit trade between herds of similar status.

Using EEC 64/432 as the sole basis for BTB programmes creates, by default, a regulatory, trade-based paradigm that views success in terms of freedom to trade. However, it is unable to deal effectively with the complexities of BTB as it takes no cognisance of wildlife factors, defines a narrow range of risk factors and imposes a uniform approach that does not reflect epidemiological differences between regions or over time.

A new strategy is needed, one that is epidemiologically driven and evidence-based, with time-bound objectives tailored to the needs of the MS or region. Such a paradigm shift was seen as crucial to the success of the BTB eradication programme in New Zealand (Ryan et al., 2006). At herd-level, this will mean defining the epidemiological unit to reflect equivalent risk at pasture, housing or even locality, rather than at herd “number” level which, as currently deployed, does not always reflect that risk adequately. It will involve assigning a risk status to each herd based on its farming type, history, locality and outbreak characteristics, and managing the risk appropriately. Twenty years ago this would have been administratively impossible but modern computational technology, disease modelling tools, strain typing and GIS systems now remove much of the administrative burden. At regional level it will necessitate identification of high-risk areas - and ensure the risk is managed - or emerging areas where surveillance and control measures are intensified to prevent formation of endemic foci. At a strategic level, concepts of “control” versus “eradication” must be explicitly defined, with surveillance and interventions tailored to each. The experience from Northern Ireland suggests that the intensity and quality of testing plays a role in determining BTB prevalence but it is increasingly apparent

that merely increasing testing is not the answer, as was the Irish experience in the 1980s. Indeed, repeated testing may even be counter-productive as it may result in desensitisation of skin responses in infected cattle (Coad et al., 2010). Conversely, testing needs to be optimised, efficiently conducted and using the full range of facilities available, including parallel testing and reduced cut-points, but where interpretation is dependent on the epidemiological context rather than legislative requirement.

BTB eradication is unlikely to be achieved in the United Kingdom or Ireland within the short-term, even with introduction of an efficient vaccine. Fundamental questions remain regarding the source of BTB outbreaks and their associated risk and these must be addressed as they determine whether an eradication strategy is feasible and if so, how intervention measures can be best deployed. Addressing these challenges will require long-term vision, optimisation of measures in a resource-constrained world and international cooperation.

REFERENCES

Abernethy D.A. (2008). The Epidemiology and Management of Bovine Brucellosis in Northern Ireland. PhD Thesis, Royal Veterinary College, University of London, England.

Abernethy D.A., Denny GO., Menzies FD., McGuckian P., Honhold N. and Roberts A.R. (2006). The Northern Ireland programme for the control and eradication of *Mycobacterium bovis.* Vet. Mic. 112, 231-237

Coad M., Clifford D., Rhodes S.G., Hewinson R.G., Vordermeier H.M. and Whelan A.O. (2010). Repeat tuberculin skin testing leads to desensitisation in naturally infected tuberculous cattle which is associated with elevated interleukin-10 and decreased interleukin-1 beta responses. Vet. Res. 41, 14. [doi:10.1051/vetres/2009062].

Donnelly C.A., Wei G., Johnston W.T, Cox D.R., Woodroffe R., Bourne F.J., Cheeseman C.L., Clifton-Hadley R.S, Gettinby G., Gilks P., Jenkins H.E., Le Fevre A.M., McInerney J.P. and Morrison W.I (2007). Impacts of widespread badger culling on cattle tuberculosis: concluding analyses from a large-scale field trial. Int. J. Inf. Dis. 11, 300-308

Clegg T.A., More S.J., Higgins I.M., Good M., Blake M. and Williams D.H (2008). Potential infection-control benefit for Ireland from pre-movement testing of cattle for tuberculosis. Prev. Vet. Med. 84, 94-111

Olea-Popelka F.J., White P.W., Collins J.D., O'Keeffe J., Kelton D.F. and Martin S.W. (2004). Breakdown severity during a bovine tuberculosis episode as a predictor of future herd breakdowns in Ireland. Prev. Vet. Med. 63, 163–172

More S.J. (2009). What is needed to eradicate bovine tuberculosis successfully: an Irish perspective. Vet. J. 180, 275–278

Ryan T.J., Livingstone P.G., Ramsey D.S.L., de Lisle G.W., Nugent G., Collins D.M., and Buddle B.M. (2006). Advances in understanding disease epidemiology and implications for control and eradication of tuberculosis in livestock: The experience from New Zealand. Vet. Microbiol. 112, 211–219

Wilesmith J.W. and Williams D.R. (1986). Tuberculosis lesions in reactor cows. Vet. Rec., (Letter) July 12, 1986.

EPIDEMIOLOGY AND CONTROL OF LEPTOSPIROSIS IN NEW ZEALAND

C. HEUER*, A. DREYFUS, P.R. WILSON, J. BENSCHOP, S. SUBHARAT, A.M. AYANEGUI-ALCÉRRECA, F. FANG, J.M. COLLINS-EMERSON AND A.C. MIDWINTER

SUMMARY

Leptospirosis is an occupational disease and currently the most important zoonosis in New Zealand. Vaccination of dairy cattle against leptospirosis in the 1990s coincided with a substantial decline of notified human cases. A new increase of cases in 2002 was partially attributed to workers in sheep-only slaughter workers. This paper briefly reviews epidemiological features of the disease and reports about three studies designed to quantify the risk of human exposure associated with slaughtering sheep, to determine prevalence and incidence of human infection of workers at a sheep-only abattoir, and to estimate the duration of antibody titres for informing occupational safety and public health authorities about the risk of workers to multiple infections over time.

Abattoir sampling and stochastic risk modelling demonstrated that workers at a sheep-only abattoir were exposed to 5-25 carcasses/day that were kidney-culture positive for *Leptospira borgpetersenii* serovars Hardjo or *L. interrogans* serovar Pomona. Another sheep-only abattoir had 9.5% workers with serum antibody ('infected') in the microscopic agglutination test (MAT) for Hardjo or Pomona. The risk of infection was higher for workers at the first stage of slaughter (bleeding, pelting) than at the point of handling kidneys or at meat inspection. Home slaughter was also a significant risk factor for exposure. A preliminary 14 months follow-up of 129 seronegative workers resulted in an adjusted annual cumulative incidence of 7.9% (95%CI 3.1-12.9%) seroconversion to either Hardjo or Pomona. However, none of the individuals experiencing seroconversion reported severe signs of disease. State transition modelling revealed that MAT titres would last about 16 months for Pomona and 8 months for Hardjo. As MAT antibody was reported to correlate with protective capacity of human sera in an animal model, the observed titre duration may be an indication for the time that individuals might return to being susceptible again after experiencing infection. Observing higher prevalence in farm animals and shorter titre duration, both associated with Hardjo, is consistent with more frequent infection with Hardjo than Pomona in notified human cases.

We conclude that sheep carcasses constitute a significant leptospire exposure risk for workers, especially those positioned at the slaughter board, that exposure was associated with 8% annual incidence of infection by Hardjo or Pomona, and that MAT titres lasted about 8-16 months and less than 2 years.

* EpiCentre, Institute of Veterinary, Animal and Biomedical Sciences, Wool Building, Massey University, Priv. Bag 11222, Palmerston North 4442, New Zealand. E-mail: c.heuer@massey.ac.nz

INTRODUCTION

Notified human cases of leptospirosis in New Zealand increased significantly in 2002 alerting public health authorities and meat industries alike, and raised considerable public awareness. It was realised that too little was known about the distribution of the disease and sources of infection, thus triggering a number of ad-hoc investigations and scientific studies.

Leptospirosis occurs at a higher frequency in New Zealand than in countries where the disease is notifiable. In New Zealand, it is primarily a disease of domestic and wild mammals. Humans are infected by direct contact or indirectly through environmental contamination whereas human-to-human transmission is considered to be extremely rare. Human leptospirosis is therefore predominantly an occupationally acquired zoonosis (Keenan, 2007[1]). The annual human incidence in the general population declined from 17.0/100,000 in 1978 to 2.9/100,000 in 1996-98. However, it was 40-fold higher in meat workers, 20-fold higher in farmers, and 10-fold higher in forestry workers. It was argued that the rate of illness among meat processing workers remained as high as previously: at constant exposure a meat processing worker carries a risk of 1:20 over a service career of 30 years for contracting leptospirosis of sufficient severity to seek medical attention. Adjusting the denominator for true exposure to dairy cows, the incidence of leptospirosis among male dairy farm workers aged 15-64 years was suspected to be as high as 233.8/100,000 equivalent to a 1:14 risk of severe illness over 30 years of occupational exposure (Thornley et al., 2002; Wilson PR, 1998). However, no information is available about the risk of disease in other occupational groups, especially deer, sheep and beef farmers, veterinarians and other farm service personnel.

Public health surveillance data demonstrated that most human infection was caused by *Leptospira borgpetersenii* serovars Hardjo and Ballum, and *L. interrogans* serovar Pomona. Hardjo and Pomona were frequently found in sheep, beef cattle, and deer: 85% deer herds, 70% beef cattle herds and 44% sheep flocks had evidence of infection with these serovars (Ayanegui-Alcerreca et al., 2007; Dorjee et al., 2008; Heuer, 2009). These observations and the occupational association suggested that humans get primarily infected by domestic livestock. Also important but less frequent serovars are *L. borgpetersenii* sv. Ballum in humans and rodents, and *L. interrogans* sv. Copenhageni and sv. Tarassovi in deer (Population and Environmental Health Group, 2008; Thornley et al., 2002; Wilson PR, 1998). Tarassovi formerly occurred in pigs, but its finding in humans has declined and is now regarded as unlikely to be prevalent at any significant endemic level after the pig industry obliged pig farmers to control leptospirosis by vaccination. Importantly, it appeared that the role of sheep as a source for human infection has previously been underestimated (Blackmore et al., 1982). Serological evidence has also been found in rodents and wildlife (Marshall and Manktelow, 2002).

The decrease of the initially extremely high human leptospirosis incidence in the 1970s is generally believed to be attributable to vaccinating dairy cows. Vaccination has been recommended widely and was adopted by about 85% dairy farmers to date after research demonstrated high infection rates of dairy cows and an associated high risk of infection primarily through splashing urine during milking. However, while vaccination of dairy cattle and pigs appeared to have reduced the human case incidence until around 1980, there is strong evidence suggesting that substantial opportunity remains for occupational and possibly non-occupational human exposure to infected sheep, beef cattle and deer.

[1] Report available at: http://www.dol.govt.nz/PDFs/leptospirosis2007.pdf

Figure 1 indicates that the population incidence declined further after 1980 and settled at an endemic level of around 2-3 per 100,000, followed by an almost two-fold increase in 2002, a further slow decline until 2008 when another small but significant increase was reported. Up to 2006, not all laboratory identified cases were notified but surveillance reports since 2007 indicate that this reporting constraint has been resolved recently.

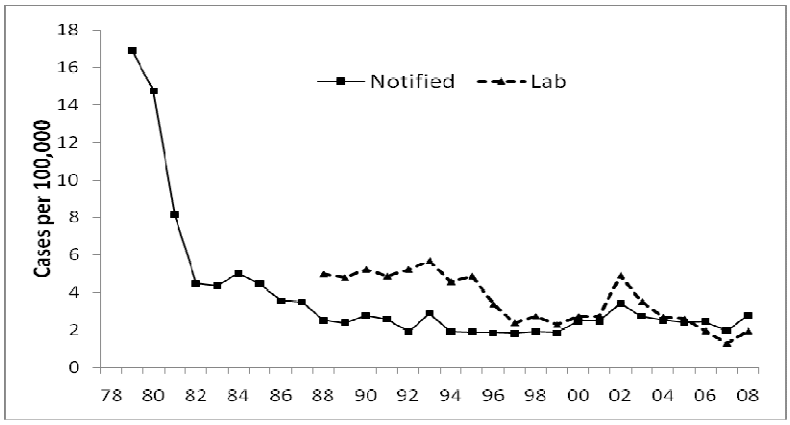


Fig. 1 Annual case incidence of human leptospirosis in New Zealand (1974 - 2008)

Occupational physicians believe that an unknown number of individuals with leptospirosis will not seek medical attention or remain undiagnosed because the symptoms are mild, of short duration, due to difficulties accessing medical services or due to doctors not suspecting leptospirosis if an exposure to animals was not explicitly stated (Thornley et al., 2002). Moreover, the laboratory confirmation of a clinical suspect diagnosis may lack sensitivity because serologic tests are often negative in the acute stage of disease and testing during recovery may not likely be undertaken.

This paper therefore reports findings from recent and ongoing studies about the extent of occupational exposure and the prevalence and incidence of infection in abattoir workers processing sheep. It uses these findings to estimate the duration of antibody titres through mathematical modelling with the intention of informing public health authorities, occupational physicians and meat industries about how soon already infected meat workers might return to becoming susceptible again to new infection. Moreover, the knowledge of titre duration would allow estimating incidence rates from relatively low-cost prevalence studies.

MATERIALS AND METHODS

Study 1: Exposure of abattoir workers to infected sheep carcasses

The aim of this study was to estimate the leptospire exposure risk for workers in a sheep-only abattoir. Sampling and testing procedures (Dorjee et al., 2008) and risk analysis methods (Dorjee et al., 2009) were described. Briefly, 95 slaughter lines and 2,758 individual sheep carcasses were randomly selected and tested for serum antibody against *Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Pomona by the Microscopic Agglutination Test (MAT). Kidneys were culture tested for the presence of leptospires. The study lasted 14 months and covered two periods, a high-risk period (May-Nov 2004) after an extensive flood had occurred in summer (Feb 2004), and a low-risk period in the subsequent season with typical, low rainfall (Dec 2004 - Jun 2005). A stochastic spreadsheet model was

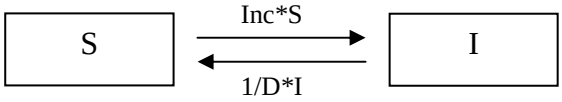
developed in @RISK[1] to assess the daily number of infected carcasses processed by eviscerators, meat inspectors and offal-handlers during these two seasons.

Study 2: Prevalence and incidence of infection in abattoir workers processing sheep

To estimate prevalence and associated risk factors, a cross-sectional study was conducted in meat workers at an abattoir slaughtering and processing sheep in February-March 2008. Sera were tested by MAT with a titre cut off of 1:24, using serovars Pomona and Hardjo as antigens. Age, sex, previous clinical episodes of leptospirosis, details of occupational and ex-factory exposure were recorded. The descriptive results of this study have recently been reported (Benschop et al., 2009). A follow-up study tested workers, who had been seronegative to Pomona or Hardjo in 2008, again in April-May 2009 following identical procedures. It was intended to estimate the annual infection incidence for these two serovars. The incidence for this 14-months period was standardised to an annual incidence risk by multiplication with 12/14.

Study 3: Titre duration of abattoir workers

Estimates of prevalence and annual incidence of infection from study 2 were used to estimate the length of time that MAT antibody titres were maintained by the host. It was assumed that infection could not occur while titres were above a cut off of 1:24 or 1:48. Thus, this study aimed to approximate the time after which infected abattoir workers would return to being susceptible for re-infection. Two states were defined, antibody negative = 'susceptible (S)', and antibody positive = 'infected (I)'. Individuals changed from S to I by the incidence of seroconversion measured in study 2. The rate of new infections was assumed to be independent of the density of already infected individuals because direct transmission between workers was highly unlikely. Infected individuals would become susceptible again by the rate of titre loss calculated as the inverse of the titre duration (D). A range of D was modelled to determine the duration that best reproduced the observed prevalence and incidence. The prevalence at endemic equilibrium was the one measured at the first sampling event in 2008 (study 2). As seasonal rainfall and temperatures were reasonably typical during the past four years, prevalence was assumed to have been constant over time. A schematic representation of the transitions at equilibrium is:



At equilibrium, Inc*S = 1/D*I, and since P=I/N and 1–P = S/N, it follows that:

$$D = P/[Inc*(1–P)]$$

This approximation works well when prevalence and incidence are small. However, when they are not small, such as when the risk period is long (as in this study), the approximation is

[1] Software version 5.5.0, 2009 (Palisade Corporation, New York, USA)

biased. In that case, the use of derivatives provides more accurate estimates. We therefore expressed the endemic state by the following differential equations:

$$dS/dt = -Inc*S + 1/D *I$$

$$dI/dt = -1/D *I + Inc*S$$

Titre duration was modified such that the observed prevalence at equilibrium was reproduced by the observed incidence. As incidence was measured with error and titre duration is known to be subject to considerable individual variation, the rates of new prevalent and new susceptible individuals were assumed to follow a poisson distribution with means of observed incidence and inverse of titre duration, respectively. The stochastic form of the two differential equations for each of 50 simulations (i = 1… 50) was expressed as:

$$newP[i] = dt*poisson(inc)*S[i] – dt*poisson(1/D)*P[i]$$

$$newS[i] = dt*poisson(1/D)*P[i] – dt*poisson(inc)*S[i]$$

where ‘newP’is the number of new prevalent cases, ‘newS’ is the number of new susceptible individuals, dt is the time interval at risk for transition (year/10,000), ‘poisson(inc)’ is the distribution of the observed incidence, ‘poisson(1/D)’ is the distribution of the rate of newly susceptible, S[i] and P[i] are the numbers susceptible and prevalent, respectively, at simulation [i].

The prevalence resulting from a range of titre durations was plotted and mean and confidence interval for titre duration were interpolated from adjacent, corresponding values of observed prevalence. This was repeated for both serovars, Pomona and Hardjo, at two MAT titre cut offs, 1:24 and 1:48.

RESULTS

Study 1: Exposure of abattoir workers to infected sheep carcasses

The abattoir processed on average 25–40 lines and 9,416–21,728 sheep per week. Median daily exposure risks during high and low risk periods were 11 (95%CI 5–19) and 3 (95%CI 1–8) kidney culture positive carcasses/day for eviscerators, 18 (9–29) and 6 (2–12) for meat inspectors, and 54 (32–83) and 18 (8–31) for offal handlers (Figure 1), respectively. Stochastic risk modelling provided strong evidence that processing of sheep carcasses exposed meat workers regularly to live leptospires which, in extreme seasons, could be about four fold above the level of a season with typical summer rains.

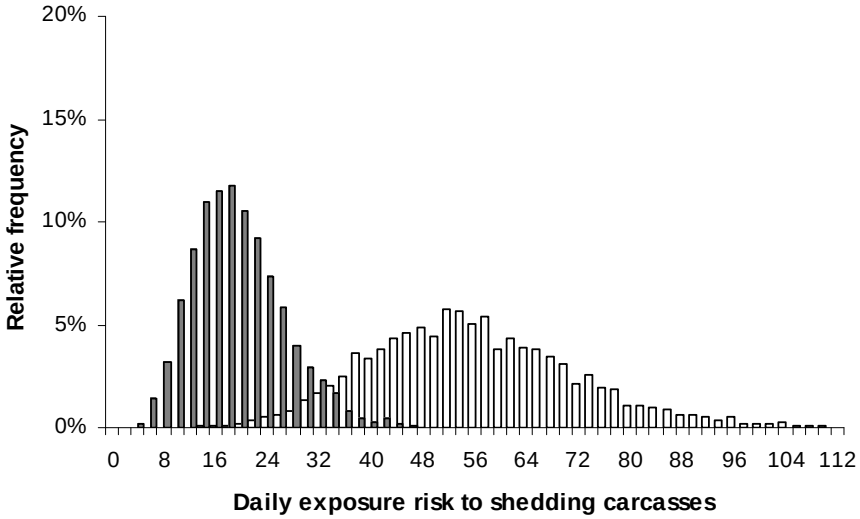


Fig. 2 Frequency distributions of 10,000 simulation runs of the daily risk of exposure of an offal worker to carcasses potentially shedding live leptospires during 18 May - 30 June 2004 (high risk season, empty bars) and 01 November 2004 - 04 June 2005 (low risk season, solid bars)

Study 2: Prevalence and incidence of infection in abattoir workers processing sheep

The overall seroprevalence to either serovars of 242 participants was 9.5% (95%CI 5.8–13.2%). At a titre cut offs of 1:24 and 1:48, 10 and 9 workers were positive to serovar Hardjo (titres 1:24–1:192), and 14 and 12 were positive to serovar Pomona (titres 1:24–1:768), respectively (Table 1). Seroprevalence was 13.1% and 4.1% in men and women, respectively. The median age for seropositive workers was 54 years while that for seronegative workers was 48 years; 23 workers (9.5%) reported a leptospirosis disease episode 1–35 years previously, and 14 (60.9%) of these were seropositive in the study, whereas 219 could not remember a disease episode including 9 (4.1%) seropositive workers. Five of 23 workers with a history of clinical leptospirosis stated that they had been ill twice at intervals of 3-5 years. A preliminary risk analysis (Heuer et al., 2009) revealed that workers at the beginning of the slaughter board (bleeding, pelting) were more likely to be seropositive than workers further down the line (popping kidneys, meat inspection), but any worker positioned at the slaughter board had substantially higher odds of being seropositive compared to workers in separate rooms (boning, cutting, chilling, freezing, rendering). Home slaughter was the strongest off-work risk factor.

A total of 129 and 124 workers who were seronegative for serovars Hardjo and Pomona in 2008 participated again in 2009. The 12-months adjusted incidence risks of seroconversion to Hardjo and Pomona is shown in Table 1. Of 119 workers who were negative to both serovars in 2008, 11 (7.9%; 95%CI 3.1–12.8%) converted to either serovar at a titre cut off of 1:24, and 8 (5.7%; 95%CI 1.5–9.8%) to either serovars at cut off 1:48. Overall, the data indicated that the average annual risk of infection to either Hardjo or Pomona was about 6-8% in this sheep-only abattoir, and that this incidence established an endemic prevalence level of about 8-10%. Few workers reported mild, flu-like symptoms typical for leptospirosis during the 14 months between samplings.

Table 1. Seroprevalence (Prevalence) to leptospirosis serovars Hardjo and Pomona in 2008 and annual incidence risk (Incidence) of seroconversion from 2008 to 2009 at titre cutoffs 1:24 and 1:48, respectively

	N	MEAN	95% CONFIDENCE INTERVAL
PREVALENCE			
Hardjo 1:24	242	0.041	0.016 – 0.066
Hardjo 1:48	242	0.037	0.013 – 0.061
Pomona 1:24	242	0.058	0.028 – 0.087
Pomona 1:48	242	0.050	0.022 – 0.077
INCIDENCE			
Hardjo 1:24	129	0.066	0.024 – 0.109
Hardjo 1:48	129	0.047	0.010 – 0.083
Pomona 1:24	126	0.048	0.011 – 0.086
Pomona 1:48	126	0.041	0.006 – 0.075

Study 3: Titre duration of abattoir workers

To maintain the endemic prevalence for serovars Harjo and Pomona as shown in Table 1, titres were required to last about 8-10 months for Hardjo and 15-16 months for Pomona (Table 2). The uncertainty about these estimates was primarily dependent on the relatively wide confidence intervals around endemic prevalence, and to a lesser extent on the selected titre cut off or stochastic variation (Figure 3).

Table 2. Estimated titre duration (months) for serovars Hardjo and Pomona and cutoff titres of 1:24 and 1:48

	MEDIAN	95% CONFIDENCE INTERVAL
Hardjo		
1:24	7.8	2.9 – 12.8
1:48	10.1	3.5 – 16.8
Pomona		
1:24	16.2	7.2 – 23.8
1:48	15.3	6.6 – 24.3

DISCUSSION

In presenting these three studies, we intended to explore the role of sheep as a potential reservoir for human infection. Sheep had not been considered an important species with regard to the zoonotic risk of leptospirosis in the 1980s (Blackmore et al., 1982). But in recent years, meat industries got increasingly concerned about human leptospirosis in workers at sheep only abattoirs, especially in Gisborne and Hawkes Bay regions (Keenan, 2007).

Two previous studies had investigated the distribution of leptospires in sheep with almost identical methods and findings, albeit presenting squarely contradicting inferences. The first one was carried out in 19882 at 3 abattoirs and reported a farm prevalence of 46% for Hardjo and 51% for Pomona including all age groups, and carcass prevalence among lambs of 2.9% for Hardjo and 2.5% for Pomona at titre cut off 1:48. The prevalence substantially increased with age. It concluded that sheep were unlikely to constitute a significant reservoir for infection of other species. A more recent study of slaughter lambs found 45% farms to be positive for Hardjo or Pomona at cut off 1:24, and 5% and 1% positive carcasses for Hardjo and Pomona, respectively. Despite similar results, it was now concluded that the study demonstrated the presence of a definite risk of occupational exposure of meat workers in a sheep-only slaughterhouse. Using the data of Dorjee et al. (2008), we developed a stochastic risk model to quantify the daily exposure of workers at different positions at the slaughter board (study 1). The result showed that the daily number of infected carcasses was clearly greater than one. On most slaughter days of an 'average' season, each worker was in contact with several infected carcasses. The risk model also suggested that seasonal variation could increase this contact rate 4-5 fold.

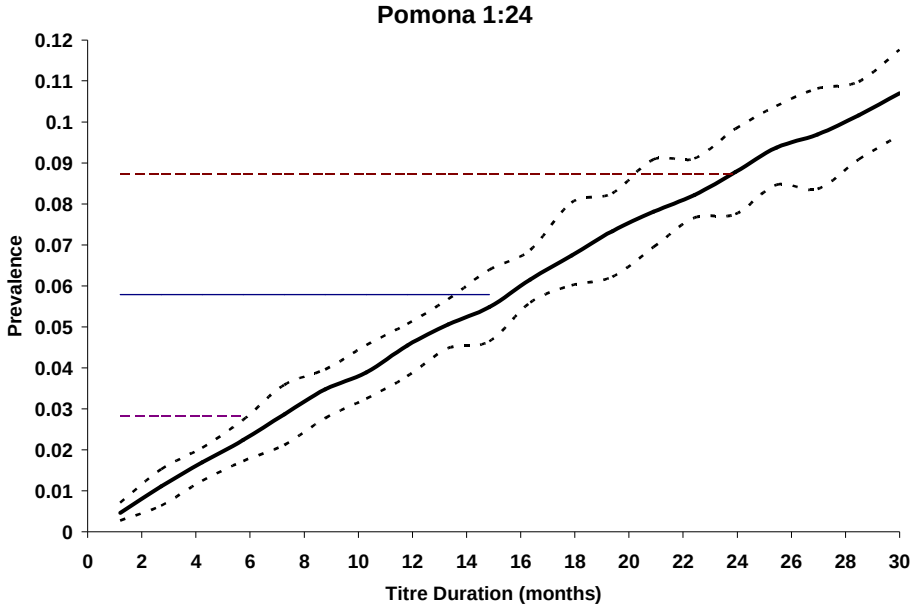


Fig. 3 Relationship between titre duration and prevalence within the stochastic envelop of 50 simulations (dotted line) at estimated mean incidence risk of 0.045 for leptospira serovar Pomona at a cutoff of 1:24. Horizontal lines indicate the prevalence at equilibrium (solid line = mean; dashed lines = lower and upper 95%CI).

These findings consequently raised the question to what extent the observed exposure rate would result in infection (study 2). Infection was initially measured as prevalence of workers at an abattoir that almost exclusively processed sheep. When it turned out that 9.5% of the workers showed evidence of past infection, the question about the rate of new infections over the course of a slaughter season was raised by the plant management. It was initially attempted to explore this by estimating incidence from assumed titre duration and observed prevalence. This resulted in incidence estimates ranging from 2-15%. It was evident that this range was too large to draw any useful inference. Reported MAT titre durations were highly variable ranging from 6 months to 5 years and were based on repeatedly tested subjects who were initially seropositive and lived in environments where leptospires were endemic. Moreover, different tests were employed measuring different antibody fractions or cellular immune response (Cumberland et al., 2001).

Hence, an estimate of actual incidence was required. In the face of economic risks associated with export trade and potential negative implications for the meat industries, further studies appeared unlikely to be politically feasible at first. However, the widely publicised death of a meat worker in mid 2007 raised considerable media interest and eventually triggered the request for investigating incidence and associations with potential infection sources. This study is currently ongoing and involves eight abattoirs processing sheep, cattle and/or deer. Study 2 presents the first result from a sheep processing plant, the same plant that gave rise to the reported prevalence. Results were available one year ahead of the other plants because over 200 workers were known to be serologically negative from the previous prevalence study. The annual incidence of infection was around 5-8% which was similar to the initial estimate but with a much smaller confidence interval. Whereas this rate supported concerns about sheep as an effective source for human infection, the fact that only few workers reported mild, flu-like signs of disease implied that the risk of severe leptospirosis might be relatively low in view of infection as frequent as this. Hence, new questions arise about specific causes and supporting factors conducive to the development of the severe clinical manifestation of humans infected with leptospires. Such factors probably include the number of leptospires at contact (i.e. dose of infection) and the level of immunity acquired through a previous contact.

A key interest is therefore in immune mechanisms determining the outcome of infection. Of particular importance herein is the duration of protective immunity after an infectious contact. The knowledge about titre duration would inform public health workers about the need for continued protection against infection, even for individuals who had experienced clinical leptospirosis or had worked in an exposed environment for long periods during which seroconversion could be assumed to have occurred. It appears that agglutinating antibodies of the MAT are serovar-specific and indicate protective immunity. This was inferred from exposure of hamsters to MAT positive sera from humans and subsequent challenge (Adler and Faine, 1978). The duration of MAT antibodies apparently depends to a great extent on the severity of leptospirosis and may last several years (Cumberland et al., 2001). This implies that the MAT titre of workers who seroconverted but did not remember any signs of disease indicative of leptospirosis might only last for short periods. Study 3 was appropriate to explore the question about titre duration of workers that were infected but did not develop any, or at least no severe disease symptoms. It was impossible to measure titre duration by repeated sampling of seropositive workers, firstly because sufficient compliance would have been hard to achieve, and secondly because it had to be assumed that repeated exposure might have occurred several times a year. In the presence of frequent exposure, titres would be expected to remain high due to natural booster. This was evident in some published studies that reported titre durations of 1-5 years (Blackmore et al., 1984; Lupidi et al., 1991) whereas one study tested 61

patients with confirmed leptospirosis of whom only 2 retained high MAT titres 13 months after the acute stage of disease (Romero eta l., 1998).

Thus, modelling the duration based on measured prevalence and incidence was the only feasible way for estimating the duration of MAT titres (study 3). The result suggested that a sub-clinical type of infection induced relatively low MAT titres (>1:100) for periods of 2-24 months. The duration was only about half as long for serovar Hardjo (8 months) as it was for Pomona (16 months). In view of such short duration, the incidence observed in study 2 might have been underestimated as some workers might have seroconverted early in the year and become seronegative before follow-up sampling. The short duration further implies that exposed workers could get infected several times and may explain why individuals can repeatedly develop clinical disease as was stated by 5 workers in study 2. Another explanation for repeated infection and/or disease is the lack of cross-protection of agglutinating antibodies to different serovars (Adler & Faine, 1978).

Consequently, workers may be well advised to maintain a high level of protection as long as they remain in an exposed environment. In addition, the knowledge of titre duration can be used to estimate incidence rates from prevalence studies. The implementation of prevalence studies is more feasible and of lower cost than incidence studies which usually suffer substantial loss to follow up. This was evident in our study 2 where 107/242 (44%) of the initial participants did not come forward for the second blood sampling or had left the work space one year after first sampling.

We conclude that workers were exposed to several infected sheep carcasses throughout the slaughter season on a daily basis, that exposure was associated with about 10% prevalence and 8% annual incidence of leptospirosis infection due to Hardjo or Pomona, and that MAT titres lasted about 8-16 months and unlikely longer than 2 years. Further research should explore the determinants for the development of clinical disease of infected individuals as well as options for effective protective measures.

More work is currently in progress: preliminary results of a cross-sectional survey of leptospirosis among 1,895 farmers in 2008 showed that the annual human leptospirosis incidence was higher on farms where leptospirosis was diagnosed in deer, and was lower when livestock were vaccinated against leptospirosis compared to farms not using vaccination. The incidence also increased with the herd size of dairy or beef cattle on farms with more than 400 animals (Verdugo and Heuer, unpublished[1]). Multi-locus-sequence-typing (MLST) and real-time PCR procedures were pre-tested and calibrated in our laboratory to be used for source attribution and shedding studies. Investigations are currently underway to serotype farmers, farm workers and animals on the same property with the intention to explore the animal-human transmission pathway and to evaluate risk factors for human and animal infections.

Vaccination of livestock appears to be the most effective means for controlling leptospirosis in animals and humans. In the absence of public subsidies for the control of human leptospirosis, vaccination of livestock needs to be financially attractive for farmers to achieve sufficient population coverage. Initial results of the efficiency of production increase through vaccination in deer suggested a highly economical effect of up to 6.5kg body weight at slaughter on farms where substantial natural challenge existed. Therefore, vaccine efficacy studies in sheep and

[1] Report available at : http://www.jdrc.co.nz/documents/EpiSurveyReport2.pdf

beef cattle are the subject of current funding proposals, whereas vaccination of dairy cattle and pigs has already been adopted by about 90% of producers.

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REFERENCES

Adler, B. and Faine, S. (1978). The antibodies involved in the human immune response to leptospiral infection. J. Med. Microbiol. 11, 387-400.

Ayanegui-Alcerreca, M.A., Wilson, P.R., Mackintosh, C.G. Collins-Emerson, J.M., Heuer, C., Midwinter, A.C. and Castillo-Alcala, F. (2007). Leptospirosis in farmed deer in New Zealand: A review. New Zealand Vet. J. 55, 102-108.

Benschop, J., Heuer, C., Jaros, P., Collins-Emerson, J., Midwinter, A. and Wilson, P.R. (2009). Sero-prevalence of leptospirosis in workers at a New Zealand slaughterhouse. New Zealand Med. J. 122, 1-9.

Blackmore, D.K., Bahaman, A.R. and Marshall, R.B. (1982). The epidemiological interpretation of serological responses to Leptospiral serovars in sheep. New Zealand Vet. J. 30, 38–42.

Blackmore, D.K., Schollum, L.M. and Moriarty, K.M. (1984). The Magnitude And Duration Of Titers Of Leptospiral Agglutinins In Human-Sera. New Zealand Med. J. 97, 83-86.

Cumberland, P., Everard, C.O., Wheeler, J.G. and Levett, P.N. (2001). Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979-1989. Eur. J. Epidemiol. 17, 601-608.

Dorjee, S., Heuer, C., Jackson, R., West, D.M., Collins-Emerson, J.M., Midwinter, A.C. and Ridler, A.L. (2008). Prevalence of pathogenic Leptospira spp. in sheep in a sheep-only abattoir in New Zealand. New Zealand Vet. J. 56 (4), 164-170.

Dorjee, S., Heuer, C., Jackson, R., West, D.M., Collins-Emerson, J.M., Midwinter, A.C. and Ridler, A.L. (2009). Assessment of occupational exposure to leptospirosis in a sheep-only abattoir in New Zealand. Epidemiology and Infection. Epidem. Infect. *(*recd. 18 Nov. 2009).

Heuer, C. (2009). Diseases limiting calving percent in beef herds. In R&D News (Wellington, Meat&Wool New Zealand), 1-4. http://www.meatnz.co.nz/main.cfm?id=44&sid=88&id2=98

Heuer, C., Dreyfus, A., Benschop, J. and Wilson, P.R. (2009). Leptospirosis in livestock and humans in New Zealand. In: Proceedings of the 6th Int. Leptospirosis Soc. Meeting, Cochin, India, p. O16.

Keenan, B.R. (2007). Leptospirosis - Reducing the impact on New Zealand workplaces (PO Box 3705, Wellington, New Zealand, Department of Labour), 1- 55. http://www.dol.govt.nz/publications/research/leptospirosis2007/index.aspLupidi, R., Cinco, M., Balanzin, D., Delprete, E., Varaldo, P.E., 1991. Serological follow-up of patients involved in a localized outbreak of leptospirosis. J. Clin. Microbiol. 29, 805-809.

Marshall, R.B. and Manktelow, B.W. (2002). Fifty years of leptospirosis research in New Zealand: a perspective. New Zealand Vet. J. 50, 61-63.

Population and Environmental Health Group, E.S.R. (2008). Notifiable and other disease in New Zealand - Annual Report 2007. Environmental Science and Research (ESR), http://www.surv.esr.cri.nz/surveillance/annual_surveillance.php?we_objectID=1987.

Thornley, C.N., Baker, M.G., Weinstein, P. and Maas, E.W. (2002). Changing epidemiology of human leptospirosis in New Zealand. Epidemiol. Infect. 128, 29-36

Wilson PR, J.M., RB Marshall, LJM Audige, J Collins-Emerson, W Quankai and MR Alley (1998). Observations of leptospirosis in farmed deer. New Zealand Vet. J. 46, 131-139.

SURVEILLANCE AND CONTROL

MANAGEMENT PRACTICES REDUCING THE TEST-PREVALENCE OF PARATUBERCULOSIS IN DANISH DAIRY HERDS

S.S. NIELSEN* AND N. TOFT

SUMMARY

A risk-based control programme on paratuberculosis in Danish dairy cattle was initiated in 2006. This study assessed the effect of different management practices on test-prevalences in the programme.

A questionnaire on management practices was distributed to all 1258 participating herds in January 2009. The questionnaire was returned from 1092 (87%) herd-managers. Repeated prevalence data from 1063 of the herds were available and included in the present analyses. A hierarchical logistic model was used to assess changes in test-prevalence from the start of interventions for six different management measures: culling of repeated antibody-positive cows; separation of cows in calving area; fast removal of calf from dam; cleaning of calving area; use of colostrum and waste milk from high-risk dams.

The overall test-prevalence was reduced from 9.7 to 5.6%. Multivariable analyses suggested that only culling of repeated antibody-positive animals and use of waste milk from specific cow groups influenced the reduction in test-prevalences.

INTRODUCTION

Paratuberculosis in cattle is a chronic infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). *Mycobacterium avium* subsp. *paratuberculosis* infections are widespread among dairy cattle in Europe, and cause significant economic losses (Ott et al., 1999). These losses are primarily due to reduced value at slaughter (Kudahl & Nielsen, 2009), reduced milk yield, premature culling (Raizman et al., 2009) and indirect effects (Kudahl et al., 2007). Therefore, several control programmes have been established to reduce spread of MAP (Benedictus et al., 2000; Kennedy & Nielsen, 2007).

Transmission of MAP is thought to occur primarily via the faecal-oral route, although infections *in utero* and via ingestion of MAP contaminated colostrum or milk are also likely routes of transmission (Sweeney, 1996). Calves are considered more susceptible to infection than adults, although animals of all ages are at risk of becoming infected (Windsor &Whittington, 2009). Therefore, efforts minimising calves exposure to faeces, colostrum and milk of MAP infectious adult animals should result in a decrease in the incidence of MAP infections. Control strategies have primarily been studied using simulation studies, and most

* Søren Saxmose Nielsen, Dept. Large Animal Sciences, University of Copenhagen, Grønnegårdsvej 8, DK-1870 Frb. C, Denmark. Email: ssn@life.ku.dk

findings suggest that focus should be on management practices to reduce transmission, possibly in combination with test-and-cull procedures. However, it has been claimed that test-and-cull procedures alone are not enough to reduce the prevalence (Groenendaal et al., 2003; Kudahl et al., 2007). Therefore, the Danish control programme on paratuberculosis focuses on both test-and-manage and test-and-cull combinations. However, it has not yet been evaluated, if the conceptual risk factors addressed through the recommended strategy in the programme actually reduce the prevalence of MAP.

The objective of this study was to assess whether within-herd test prevalences of MAP-specific antibodies were reduced with different management strategies in Danish dairy herds.

MATERIALS AND METHODS

The Danish control programme on bovine paratuberculosis

The Danish control programme on bovine paratuberculosis was initiated in February 2006. The programme is risk-based, and animals are divided into High-Risk and Low-Risk animals based on four annual herd screenings using a milk antibody ELISA (Nielsen, 2009a). High-Risk cows are defined as animals with an ELISA-positive result among the last four test-results, whereas Low-Risk cows are those without any positive results in the last four tests. Furthermore, High-Risk animals are divided into Red and Yellow cows, where Red cows have been test-positive on the last two tests, or on three of the last four tests. Low-Risk cows are also denoted Green, and considered non-infectious, whereas Red and Yellow are potentially infectious. Details about the classification scheme are given in Nielsen (2009b). An in-house ELISA (Nielsen, 2002) was used until October 15, 2008, and a commercial test (IDScreen, IDVet, Montpellier, France) was used after that date. The in-house test was considered positive at an optical density reading of 0.3, and the commercial test was considered positive at a sample-to-positive ratio of 0.2. These cut-offs were chosen to make the tests as sensitive as possible, thereby potentially resulting in more false-positives.

The number of participants in the programme has been as follows: July 31, 2006: 621 (12% of all Danish dairy herds); Dec. 31, 2006: 1002 (20%); July 31, 2007: 1,136 (23%); Dec. 31, 2007: 1,195 (25%); Dec. 31, 2008: 1,261 (28%) (Nielsen, 2009b). Participating farmers are advised to reduce the risk of transmission through the following recommendations:

- Red cows should be slaughtered prior to next calving so they do not enter the calving area;
- High- and Low-Risk cows should calve in separate calving areas, and the calving area should be cleaned after the calving of High-Risk cows;
- Calves born to High-Risk cows should be removed as soon as possible and preferably within 2 hours after birth;
- Milk and colostrum from High-Risk cows should not be used for feeding of heifer calves staying in the production system for more than 1 year.

Questionnaire data

Mailed questionnaires were sent to the 1,261 herds participating by Dec. 23, 2008. Reminders were sent twice to the non-responders, resulting in a total of 1092 responders (87%). The questionnaire included one page of questions regarding how the farmer followed the recommendations to reduce transmission and regarding culling practices. The responses to the questions were re-organised into six categorical variables used as independent variables in the further analyses. These were:

1. Culling of Red cows: a) No Red cows calve again; b) Some Red cows calve again; or c) Most Red cows calve again.

2. Fast removal of calves from dam: a) Calves separated from Red and Yellow dams within 2 hours; b) Calves separated from Red dams, but not Yellow dams, within 2 hours; or c) Calves not separated from High-Risk dams within 2 hours after birth.

3. Calving area cleaning : a) Calving area cleaning after Red and Yellow cows' calving; b) Calving area cleaned after Red, but not Yellow, cows' calving; or c) Calving area not cleaned after High-Risk cows' calving.

4. Cows' separation in calving area: a) Red and Yellow cows separated from Green in calving area; b) Red, but not Yellow cows, separated from Green cows in calving area; or c) No separation of High- and Low-Risk cows in calving area.

5. Colostrum usage: Colostrum for feeding of heifer calves used form: a) Green cows only; b) Yellow and Green cows, but not Red cows; or c) all cows.

6. Waste milk usage: Waste milk used for feeding of heifer calves occurs from: a) Green cows only; b) Yellow and Green cows, but not Red cows; or c) all cows.

A total of 1092 herds (87%) provided responses, but of these, 31 had ceased production since the questionnaire data were obtained. Therefore, 1063 herds provided both questionnaire and prevalence data. Some farmers responded "Do not know" to some questions and were considered not having established procedures to avoid transmission (i.e. were classified as worst-case). The distribution of responses in each category among the 1063 herds is shown in Table 1.

Prevalence estimation

ELISA data for prevalence estimation were obtained from the Danish Cattle Database by December 1, 2009, covering the entire programme period. Unfortunately, initial explorative descriptive statistics examining test-prevalences as a function of time since start in the programme (TIME), overall and stratified by tests, showed that the apparent prevalence of the two tests differed to an extent, which discouraged further modelling of the full dataset. Therefore, only test results of the second test (IDScreen®) were used for further analyses. Data were not obtained from herds, which had ceased production. Herds in the control scheme were tested by antibody ELISA through the milk recording scheme, where milk samples are obtained 6 or 11 times per year in each herd. Four of these test rounds were used for prevalence estimation at each sampling point. Because milk samples were used, only lactating animals were tested. At each test-date, the prevalence was determined as the number of ELISA-positive among animals tested.

Table 1. Results of univariable logistic analyses of the probability of testing positive in *Mycobacterium avium* subsp. *paratuberculosis* specific antibody ELISA for Danish dairy cows tested at different time points (TIME) after first test-results were available in herds with different management practices.

Risk factor[a]	N_{Herds}	Estimate[b]	Standard error	P-value[c]
Red cows calve again				
Intercept		-2.25	0.06	
Most	208	-0.07	0.03	0.002
Some	707	-0.15	0.02	
None	148	-0.21	0.05	
Separation of cows in calving area				
Intercept		-2.24	0.06	
No separation	470	-0.15	0.03	0.761
Red from Green	281	-0.15	0.03	
Red & Yellow from Green	312	-0.13	0.03	
Calves removed within 2 hours from				
Intercept		-2.24	0.06	
No cows	158	-0.13	0.03	0.098
Red cows	187	-0.189	0.03	
Yellow & Red cows	718	-0.134	0.02	
Calving area cleaned after				
Intercept		-2.24	0.06	
No cows	582	-0.13	0.02	0.112
Red cows	211	-0.19	0.04	
Yellow & Red cows	270	-0.14	0.03	
Colostrum used from				
Intercept		-2.24	0.06	
Red, Yellow & Green cows	109	-0.10	0.04	0.244
Yellow & Green cows	273	-0.17	0.03	
Only Green cows	681	-0.14	0.02	
Waste milk used from				
Intercept		-2.23	0.06	
Red, Yellow & Green cows	137	-0.14	0.04	0.013
Yellow & Green cows	211	-0.21	0.03	
Only Green cows	715	-0.13	0.02	

[a]"Red", "Yellow" and "Green" cows are categories of animals based on their test-results, where Red cows were repeated antibody positive, Green cows antibody-negative on up to last three tests, and Yellow cows with other test-combinations based on repeated results.

[b] The estimate for each stratum represents the slope of TIME variable within that stratum.

[c]The P-value gives the significance of the interaction (i.e. different slopes between strata), the main effect of TIME was highly significant in all analyses.

The 1,063 herds contributed a total of 3,774 herd-examinations, with 551,591 cows tested using the IDScreen® test. Thirty-eight herds were tested one time, 68 herds two times, 333 herds three times, 519 herds four times, and 105 herds five times. Among the 3,774 herd-examinations, the median number of animals tested at each herd-examination was128 (range 11-1087).

Descriptive statistics

A semi-parametric analysis was performed using the GAM procedure in SAS version 9.1 (SAS Institute, Cary, NC, USA), with the test-prevalence as the outcome and TIME, stratified by each of the six risk factors (one at a time), as a covariate modelled with a univariate smoothing spline with 4 degrees of freedom. These analyses were used to explore the relationship between test-prevalence and TIME. The analyses suggested, based on visual inspections that an approximate linear relationship was appropriate for all risk factors.

Furthermore, two-way cross-tabulations of the six risk factors were made. Some combinations of outcomes were not possible, e.g. feeding colostrum from Red dams if these did not calve again. Therefore, it was decided not to examine all possible interactions between risk factors as data did not permit this.

Statistical analyses

To account for the clustering within herd, a logistic analysis using a generalised linear mixed model approach was carried out. Essentially the initial model for each of the 6 risk factors was as shown in Eq. (1).

$$\text{logit}(p) = a + RF + TIME + TIME \times RF + Herd \quad (1)$$

where p is the probability of testing positive in a herd (modelled using number of positive/number tested per herd per test round); **a** is the common intercept; RF is the fixed effect of one of the 6 potential risk factors; TIME is the time since start in the programme; and Herd is a first order auto-regressive AR(1) correlation structure used for the working correlation of the Generalized Estimating Equations (GEE) analysis to account for clustering of repeated measures within farm. For each analysis, the main effect of the risk factor was assessed and removed if non-significant ($p>0.05$), whereas the interaction term was kept even if it was deemed non-significant and results were reported as estimates of the slope of the linear function of TIME for each of the strata of the risk factor, with a P-value reflecting the test of significance of the interaction.

The analyses were carried out using the GENMOD procedure in SAS version 9.1. First, univariable analyses were carried out. Subsequently, a forward selection procedure using the significant univariable effects was carried out to establish a multivariable model.

RESULTS

Results of the logistic analysis showed that overall, the test-prevalence decreased from 9.7% to 5.6%, as measured by the IDScreen® test (Fig. 1).

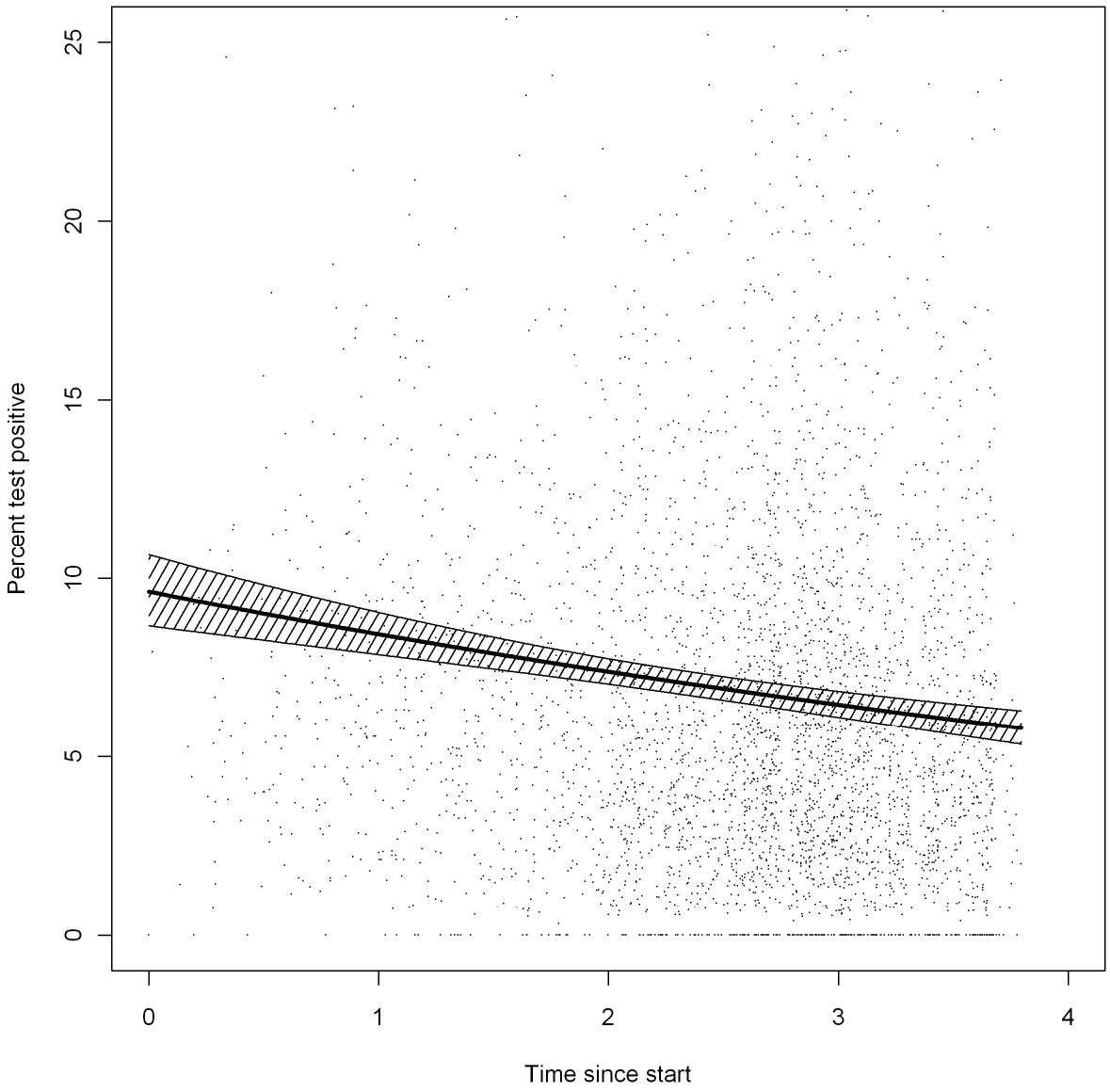

Fig. 1 Estimated overall prevalence (with 95% CI limits) and individual herd prevalence estimates. Approximately 30 observations between 25 and 36% are not shown.

The estimates from the univariable analyses are given in Table 1. In Fig. 2, the association between apparent prevalence and TIME is illustrated for the univariable analyses, where a significant effect of the stratum interaction with TIME was found.

For the multi-variable analysis, only the risk factors regarding whether Red cows calve again and the use of waste milk were examined. Cross-tabulation suggested that it was possible to examine the interaction of TIME, calving and use of waste milk. The results of the multivariable analyses are shown in Table 2 and illustrated in Fig. 3. For all models, the estimated autocorrelation was approximately 0.75, suggesting that there is a strong within herd effect on the prevalence.

Table 2. Results of multivariable logistic analyses of the probability of testing positive in *Mycobacterium avium* subsp. *paratuberculosis* specific antibody ELISA for Danish dairy cows tested at different time points (TIME) after first test-results were available in herds with different management practices.

Risk factor[a]		Estimate[b]	Standard error	P-value[c]
Intercept		-2.25	0.06	
Red cows calve again	Waste milk used from			0.002
Most	Red, Yellow & Green cows	-0.06	0.05	
	Yellow & Green cows	-0.22	0.04	
	Only Green cows	-0.03	0.03	
Some	Red, Yellow & Green cows	-0.16	0.05	
	Yellow & Green cows	-0.19	0.04	
	Only Green cows	-0.14	0.02	
None	Red, Yellow & Green cows	-0.30	0.07	
	Yellow & Green cows	-0.26	0.10	
	Only Green cows	-0.18	0.06	

[a]"Red", "Yellow" and "Green" cows are categories of animals based on their test-results, where Red cows were repeated antibody positive, Green cows antibody-negative on up to last three tests, and Yellow cows with other test-combinations based on repeated results.

[b] The estimate for each stratum represents the slope of TIME variable within that stratum.

[c]The P-value gives the significance of the interaction (i.e. different slopes between strata), the main effect of TIME was highly significant in all analyses.

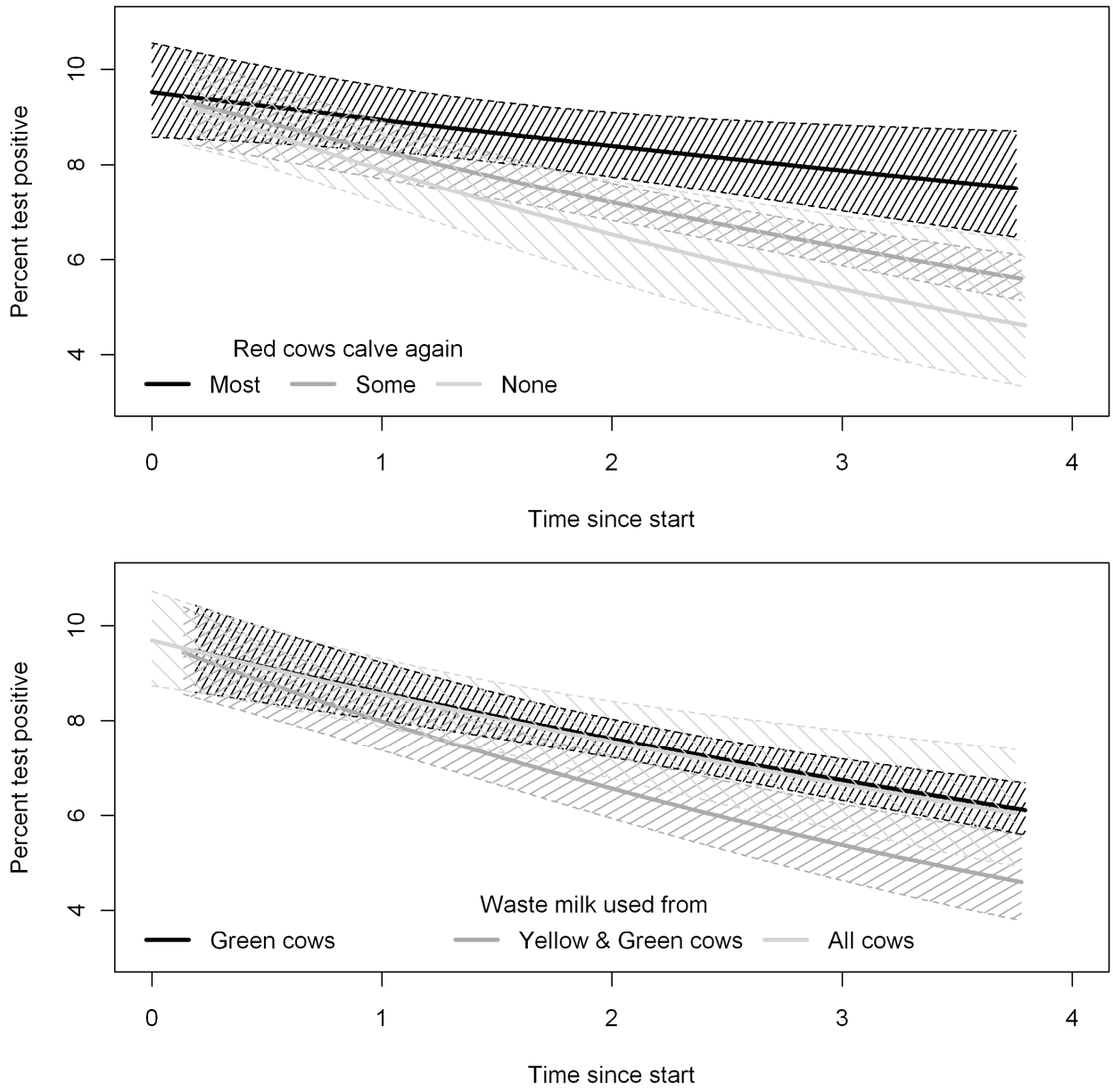

Fig. 2 Illustration of the estimated association between test-prevalence and TIME for each stratum of the 'Red cows calve again' and the 'Waste milk used from' risk factors. Shading indicates the associated 95% CI of each stratum specific predictions.

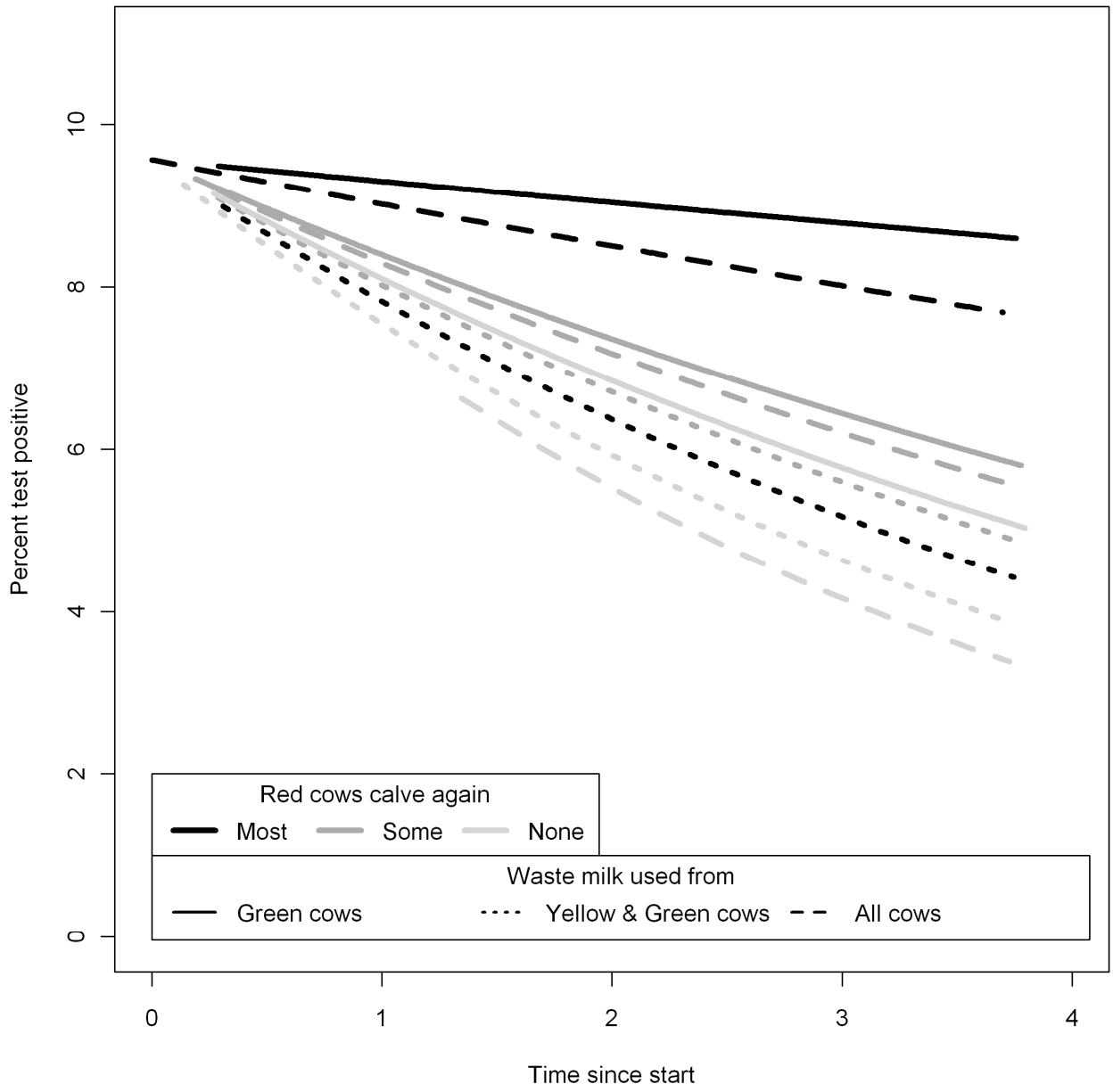

Fig. 3 Illustration of the multivariable association between test-prevalence and TIME stratified by the interaction of the waste milk and removal policies

DISCUSSION

Reduction in the prevalence of MAP infections is usually considered to occur via changes in management, and not test-and-cull alone (Groenendaal et al., 2003; Kudahl et al., 2007). The results from this study suggest that culling of repeated test-positive animals (Red cows) does contribute significantly to a reduction in the prevalence, whereas the effect of the other management factors seem to be somewhat uncertain, except for use of waste milk, which have previously been shown to have limited effect on the transmission of MAP (Nielsen et al., 2008). The results on use of waste milk also provides contradicting results, because "use of waste milk from Green cows" (considered best practice) resulted in less test-prevalence reduction than "use of waste milk from Yellow and Green cows" (considered second-best practice) (Table 2 and Fig. 2). Several potential risk factors yielded similar contradictory results in the univariable analyses (Table 1), but these findings were non-significant. However, the findings from the univariable

analyses point to an interesting result: The considered second-best practice resulted in faster prevalence reduction than the considered best practice in several instances. For example, for the risk factor "Calving area cleaned after….." yielded a lower estimate when the calving area was stated cleaned only after Red cows, compared to stated cleaning after both Red and Yellow cows. This may suggest that farmers stating that cleaning was done only after Red cows were more honest about their actual practice, or more thorough when managing Red cows, than farmers stating that they cleaned after both Red and Yellow cows. However, it could also merely be an effect of confounding.

Furthermore, these findings point to one of the major weaknesses in this study: Use of questionnaires at one time-point. Non-differential misclassification is very likely to occur, resulting in lack of association or even reversal of the hypothetical association (Rothman & Greenland, 1998). Many management practices differ from animal to animal, and over time within a herd. For example, removal of calves from a High-Risk dam within 2 hours is less likely to occur during the night than during the day, and new staff may change the management over time. We only asked about management practices at one time-point. The questionnaire was kept short to increase the proportion of responders and to reduce complexity, but at the cost of being unable to detect dynamics in management. The large sample size may to some extent correct for this on long term, when more prevalence data have been collected.

So far, prevalence data covers the period of 0 to 4 years after availability of the first test-results. Many infected animals may only become antibody-positive after reaching the age of 4 years due to the long incubation period. Therefore, some of the longer-term effects may only show within the next 5-10 years, requiring that the prevalence is monitored longer and that the analyses are repeated, when more data become available. We discarded many observations because the antibody test was replaced, and the models could not adequately take this into account, because of differences in (the partially unknown) misclassification bias provided by the two tests. This suggests that it would be beneficial to continue with the same test throughout the following period to avoid this effect or to adopt e.g. Bayesian methods which are more capable of addressing the issue of misclassification. Furthermore, as more data become available, more information about the course of the infection within the individual herds will be obtained, thus necessitating a more flexible model for the change in prevalence through time. Future modelling capturing non-linear effects might be needed to replace the current linear (on the logit scale) model, with a model that allows for a potentially new steady state prevalence within a herd when the reductions that can be obtained from the applied set of management practices has been harvested.

Although relatively few observations from each herd were available, there was still significant evidence of the clustering within herd. Furthermore, it is apparent from Fig. 1 that there are large variations in the actual within herd prevalences.

Prevalence estimation and choice of model have been a challenge, because we expected a time-lag in the decrease in the prevalence. This time-lag was expected because it would take a while before risk-factors affecting transmission would be detectable, since animals are tested as cows but are generally presumed to be infected as calves. However, the data suggested that there was an immediate drop in the prevalence, probably because of a significant effect of culling of Red cows. These cows also have a theoretical high impact on the infectious load in the herd, since antibody-positivity is a predictor of bacterial shedding of major amounts of MAP. Although a short term effect in the reduction in prevalence can be attributed to culling of Red cows, the long term effect must primarily be due to the removal of infectious animals thereby

reducing transmission rather than due to culling alone. If transmission is not broken, there will continuously be new infected cows becoming test-positive, and culling alone would not be expected to keep up with these new infections. Culling of Red cows may have a greater impact because antibody-positivity may often precede major bacterial excretion of MAP (Nielsen, 2008), thereby being an early indicator of infectiousness, allowing for the farmer to respond to the test-results prior to the cow becoming a risk to the herd.

To conclude, culling of repeated antibody-positive cows can be associated with a decrease in the test-prevalence over a period of 4 years. The prevalence reduction appears almost abolished if use of waste milk from repeated antibody-positive cows is practised when most of the repeated antibody-positive animals calve again. No other management factors could clearly be demonstrated to be associated with a reduction in the prevalence of MAP-infections, but longer-term studies are required to elucidate these effects.

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REFERENCES

Benedictus, G., Verhoeff, J., Schukken, Y.H. and Hesselink, J.W. (2000). Dutch paratuberculosis programme history, principles and development. Vet. Microbiol. 77, 399-413.

Groenendaal, H., Nielen, M. and Hesselink, J.W. (2003). Development of the Dutch Johne's disease control program supported by a simulation model. Prev. Vet. Med. 60, 69-90.

Kennedy, D.J. and Nielsen, S.S. (2007). Report from the first IDF ParaTB Forum. Bull. Int. Dairy Fed. 410, 3-7.

Kudahl, A.B. and Nielsen, S.S. (2009). Effect of paratuberculosis on slaughter weight and slaughter value of dairy cows. J. Dairy Sci. 92, 4340-4346.

Kudahl, A.B., Østergaard, S., Sørensen, J.T. and Nielsen, S.S. (2007). A stochastic model simulating paratuberculosis in a dairy herd. Prev. Vet. Med. 78, 97-117.

Nielsen, S.S. (2002). Variance components of an enzyme-linked immunosorbent assay for detection of IgG antibodies in milk samples to *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. J. Vet. Med. B, 49, 384-387.

Nielsen, S.S. (2008). Transitions in diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* infections in cattle. Vet. Microbiol. 132, 274-282.

Nielsen, S.S. (2009a). Use of diagnostics for risk-based control of paratuberculosis in dairy herds. In Practice 31, 150-154.

Nielsen, S.S. (2009b). Parameters used to assess the efforts to control paratuberculosis in Denmark. Bull. Int. Dairy Fed. 441, 14-20.

Nielsen, S.S. and Toft, N. (2008). Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-γ assay and faecal culture techniques. Vet. Microbiol. 129, 217-237.

Nielsen, S.S., Bjerre, H. and Toft, N. (2008). Colostrum and milk as risk factors for infection with *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle. J. Dairy Sci. 91, 4610-4615.

Raizman, E.A., Fetrow, J.P. and Wells, S.J., 2009. Loss of income from cows shedding *Mycobacterium avium* subspecies *paratuberculosis* prior to calving compared with cows not shedding the organism on two Minnesota dairy farms. J. Dairy Sci. 92, 4929-4936.

Rothman, K.J. and Greenland, S. (1998). Modern Epidemiology, 2nd Ed. Lippincott-Raven Publishers, Philadelphia, PA, USA, p. 127.

Sweeney, R.W. (1996). Transmission of paratuberculosis. Vet. Clin. North Am. Food Anim. Pract. 12, 305-312.

Windsor, P.A. and Whittington, R.J. (2009). Evidence for age susceptibility of cattle to Johne's disease. Vet. J. (in press, doi:10.1016/j.tvjl.2009.01.007).

USING ENTERPRISE GROSS OUTPUT AS AN ARGUMENT FOR ENDEMIC DISEASE CONTROL AT FARM LEVEL

A. G. GANSER*, F. BRÜLISAUER, B.J.J. MCCORMICK, G.J. GUNN AND A.W. STOTT

SUMMARY

A longitudinal study was used to investigate the utility of enterprise gross output analysis for disease impact assessment and provision of performance indicators to enable informed decisions on endemic disease control at the farm level. Empirical information available through the British national cattle tracing system was used to calculate five consecutive annual enterprise gross outputs for 22 beef enterprises. The economic information was then linked to bovine viral diarrhoea herd status. This approach allowed assessment of disease mortality and reduced fertility on performance within farms and benchmarking between farms. Furthermore, it can be adapted to include information on individual livestock weights thus enabling assessment of morbidity on performance. Contracting active infection with bovine viral diarrhoea virus was associated with reductions in enterprise gross output ranging from 3% to 35%. For farms with prolonged infection, production risk increased as measured by greater variation in enterprise gross output.

INTRODUCTION

Endemic infectious disease has been recognised as an integral part of animal production (McInerney, 1996) and can pose substantial constraints on livestock productivity by reducing the efficiency of input transformation (Morris, 1999). Although losses due to endemic pathogens have been assessed through both empirical case studies (Duffell et al., 1986; Barber and Nettleton, 1993; Penny et al., 1996) and where data are scarce, through models (Bennett, 2003; Stott et al., 2003; Gunn et al., 2004), there is significant resistance to uptake of best practice to prevent or minimise such losses on farms in Great Britain (GB) (Gunn et al., 2008). The reluctance to act upon such estimates might be attributable to the fact that losses derived from models and empirical observations deemed worthy of reporting mostly focus on rare, "outbreaks" of epidemic proportions. This neither reflects the range of situations producers might experience nor allows stakeholders to identify with these "worst case" scenarios (Stott et al., 2009). Another contributing factor is that many endemic diseases exhibit few characteristics or overt clinical signs and hence livestock producers are unaware of or underestimate their impact on production. This in turn can further the belief among farmers that only "unskilled" livestock keepers experience disease problems (Heffernan et al., 2008). In addition, neither consulting veterinarians nor livestock producers are convinced of the feasibility of endemic

* Antonia Ganser, Epidemiology Research Unit, SAC Research, King's Buildings, West Mains Road, Edinburgh EH9 3JG, UK. Email: antonia.ganser@sac.ac.uk

disease control and prevention at the individual farm level (Gunn et al., 2008). These observations have led us to investigate an alternative method to provide incentives for disease control through longitudinal combined assessment of empirical animal health and economic information. As tactical management decisions concerning herd health are generally taken by the livestock keeper (Wilson, 2001), the chosen economic method had to be familiar and interpretable for this target group. Secondly, as profit maximisation was assumed to be a major driver for the target audience (Rushton et al., 1999), provision of a performance measure indicative of the productivity of the livestock enterprise and its contribution to overall farm profit (Turner and Taylor, 1998) was desirable. Thirdly, the performance measure should enable longitudinal assessment of achievements from year to year within farm, as well as comparison between farms (Turner and Taylor, 1998; Wilson, 2001). Fourthly, the measure should account for important disease effects thus adequately reflecting the risk of income variation due to presence of disease (Rushton et al., 1999; Hardaker et al., 2004). Lastly, the data used to derive the performance measure should be universally available for all livestock enterprises (Upton, 1989; Favier and Dodd, 1991; Wilson, 2001).

The first postulate of familiarity has led us to explore traditional farm accounting methods used to assess the achievements of individual enterprises in relation to total farm profit such as enterprise net margins (ENM) (Barnard and Nix, 1979). Enterprise net margins however requires detailed records - rarely available for the business type considered here - to allow inclusion of overhead cost (Wilson, 2001). Overhead costs can be omitted to arrive at enterprise gross margins (EGM), a method proposed for economic disease impact assessment (Rushton et al., 1999). Again, specifically for business types with grazing livestock – such as the beef enterprises predominant in Scotland and Northern England - attribution of variable feed costs is problematic (James and Carles, 1996). Omission of variable and overhead costs leads to the basic element for both net and gross margin calculation, the enterprise gross output (GO). This is the total value of production from the enterprise over an accounting period, usually one year. As explained by Barnard and Nix (1979), it includes gross output, which is sales plus (allocatable) subsidies plus/minus any increase/decrease in valuation, less purchases of livestock. However, it also includes any produce consumed on the farm (assumed negligible here) and the value of any transfers to or from another enterprise on the farm. GO therefore ascribes financial performance to components of the farm business, making it easier to identify strengths, weakness and any changes over time thus guiding management. An important premise of this paper is that endemic disease has an important influence on GO, thus GO analysis is a potentially powerful tool for disease prevention and control at farm level. The components necessary to derive GO are recorded for legal and tax purposes, thus making them universally available for the census of livestock enterprises. Bovine viral diarrhoea (BVD) has been selected to investigate the utility of GO analysis for disease impact assessment and provision of performance measures enabling informed decisions on endemic disease control and prevention at farm level.

MATERIALS AND METHODS

The BVD status of 25 beef livestock enterprises was monitored between October 2006 and October 2009. Senior veterinary staff from contributing veterinary practices in Scotland and Northern England, who form part of the team reviewing approaches to BVD control, selected these farms from their client base. Selection was based on the veterinarians' judgement of their suitability for and likely interest in a study of this type, i.e. they were not a random sample. Disease effects on fertility and mortality of the herds were estimated through calculation of GO

using official herd register and cattle movement data. To evaluate the economic impact of BVD on individual farms, annual relative changes in GO per cow (RCGO) were associated with BVD status and state transitions. The results from individual farms were collated to assess the variability of disease effects. Presence or absence of active BVDV infection in individual herds was established and monitored using a combination of annual whole herd and sentinel group sampling (Houe, 1992). All samples were tested in accredited laboratories with commercially available ELISA tests for both antibody and antigen. Bovine viral diarrhoea herd status "BVD" (active infection) or "FREE" was assigned when conclusive test results were available. Herds were classified as "UNKNOWN" when information was insufficient to assign a state. Information was collected for the three direct effects of BVD: fertility, mortality and morbidity. Livestock keepers were asked to provide animal health information and consent to usage of information contained within the official GB cattle tracing system (CTS). The CTS records cattle identities, animal numbers present on farm, as well as livestock movements, births and deaths. Livestock keepers are legally bound to notify movements on and off the farm within days of occurrence (http://*www.bcms.gov.uk*). Data and metadata utilised in the current study were released through the Defra RADAR team with full permission of the farmers concerned. *(http://www.defra.gov.uk/foodfarm/farmanimal/diseases/vetsurveillance/radar/project.htm)*

The study population for each of the 25 herds was stratified into gender and 7 age groups using half yearly increments until the age of 36 months was reached. Animal numbers in each age category at the start and end of the defined business year, births and deaths as well as movements on and off farm occurring during the year were extracted from RADAR data files. A disease effect of economic importance is reduced growth; hence animal weight at sale was included into the analysis. Sales weights of livestock were available for the majority of farms through mart or abattoir records. A subset of farms weighed their calves at weaning and at sale. Due to the variety of records and incomplete weight information for years prior to onset and during the study, standard weights for animals up to 18 months were derived from a generic growth curve (de Behr 2001). Weights for animals older than 18 up to 30 months of age were approximated based on the SAC Farm Management Handbook 2009/10 (Anon, 2009). Animals older than 30 months were assumed to be breeding stock and valued at a fixed current market price, again derived from the SAC Farm Management Handbook 2009/10 (Anon, 2009). Gross output was subsequently calculated for all age groups and five consecutive business years from 1st April 2004, to 31st March 2009 (Eq. (1)). (Note that all age groups were ultimately combined into one GO for the purposes of our analysis. This GO was ascribed to the 'suckler cow enterprise'. For a specific farm business, GO should be divided up into separate GOs for each additional enterprise on the farm. These might include heifer rearing, production of store cattle and production of finished beef cattle. In our case, we have applied the GO concept to age groups within an enterprise and reduced the normal time step from one year to six months in order to more accurately assemble the final GO and make best use of the CTS data available.)

$$\text{Gross output} = (\text{Number of animals sold} * \text{value of animals sold}) - (\text{Number of animals purchased} * \text{value of animals purchased}) \pm (\text{Closing valuation} - \text{opening valuation}) \quad (1)$$

Internal movements, so called "transfers" are not recorded in CTS, but need to be included in GO; hence animal numbers transferred from one age group to the next were calculated starting with age group 1 (0-6mths) for each year (Eq. (2)).

Transfers “out” of age group 1 = (Animals at start of year in age group 1 + births + movements onto farm into age group 1) - (movements off farm out of age group 1 +deaths in age group 1 +animals at end of year in age group 1)

(2)

As transfers “out” of one age group equal transfers “in” to the next higher age group, all subsequent “out” transfers could be calculated. The value of an animal within an age group at opening and closing valuation, purchase, transfer or sale up to 30 months was calculated as (Eq. (3)).

Animal value = (Weight of animal (kg) * Live weight price per kg) (3)

The live weight price per kg of £1.65 for females and £ 1.70 for males was obtained from the SAC Farm Management Handbook 2009/10 (Anon, 2009) and used for all years to minimise the effect of price fluctuation on GO. Specific animal values were used for the internal transfers at 6, 12, 18, 24, 30 and 36 months as these were based on predicted weight for age at transfer. Animal values at purchase or sale were based on average weights within the respective age group as exact age and hence weight of individual animals was not known.

The number of female breeding stock served as the denominator for calculation of the performance indicator to reflect the resource restraining production (Upton, 1989; James & Carles, 1996) (Eq. (4)).

GO per female breeding stock = (GO / ((female breeding stock at start of period + female breeding stock at end of period) / 2)

(4)

After calculation of the GO per cow and year, the RCGO compared to the previous year was calculated, allowing longitudinal performance assessment. Relative changes in GO per cow for individual farms in any one of the three possible states and BVD state transitions were explored by year and collated over the entire study period using descriptive statistical measures. RADAR data extracts were manipulated using MatLab (Version7). Gross output and summary statistics were calculated in MS Excel (Office 2007). Plots were produced in R (version 2.9.2).

RESULTS

Gross output analysis was performed for 22 of the 25 herds initially recruited. Three herds had to be withdrawn from the study as for various reasons they did not sufficiently conform to the generic farm business model implicit in our calculation of GO described above. Assignment of herd status “BVD” or “FREE” to study farms was possible through sampling and testing from the business year 2006/07 onwards. Herd status for the business year 2005/06 could be deduced for 7 of the 22 study farms as “BVD” through identification of PIs and the period of early gestation of the respective PI carrier dams, while two herds were classified as “FREE” based on sero-negative home bred replacement heifers. In 2004/05, 21 herds were classified as “UNKNOWN” and one herd through health scheme tests as “FREE”. Results for the final

business year 2008/09 were excluded from further analysis for one farm, as a confirmed outbreak of another endemic disease caused considerable losses (Fig. 1).

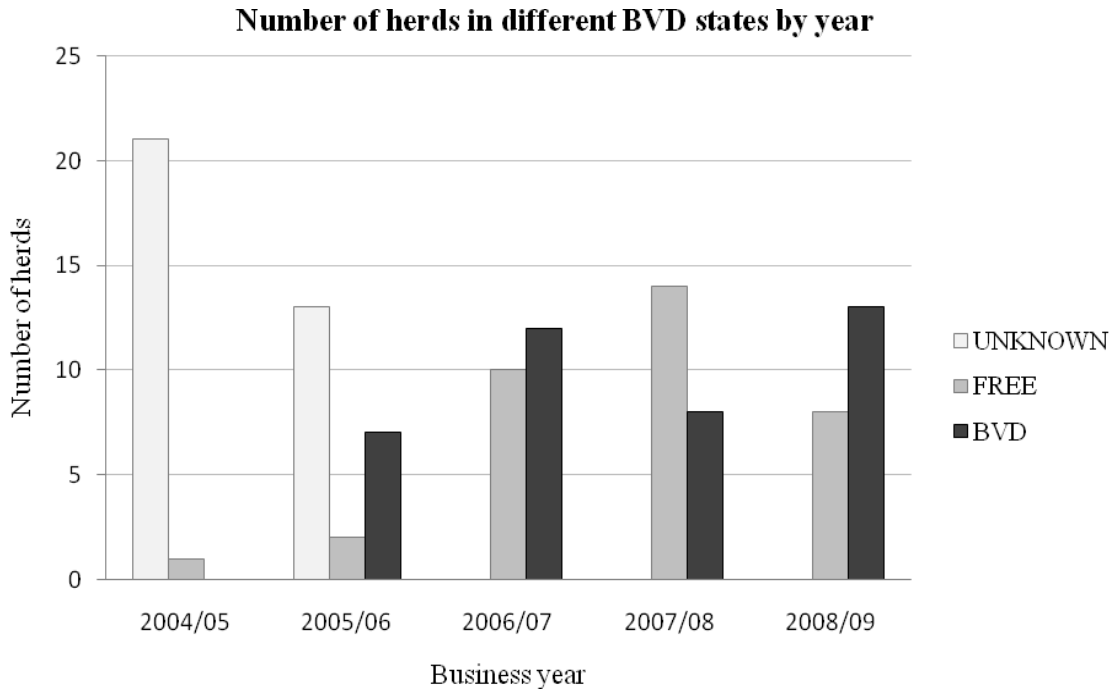

Fig. 1 Number of study herds (n=22) in BVD states “FREE”, “BVD” and “UNKNOWN” for five consecutive business years

The results obtained show that absolute GO values varied substantially over time within farm and between farms (Fig. 2 and Fig. 3).

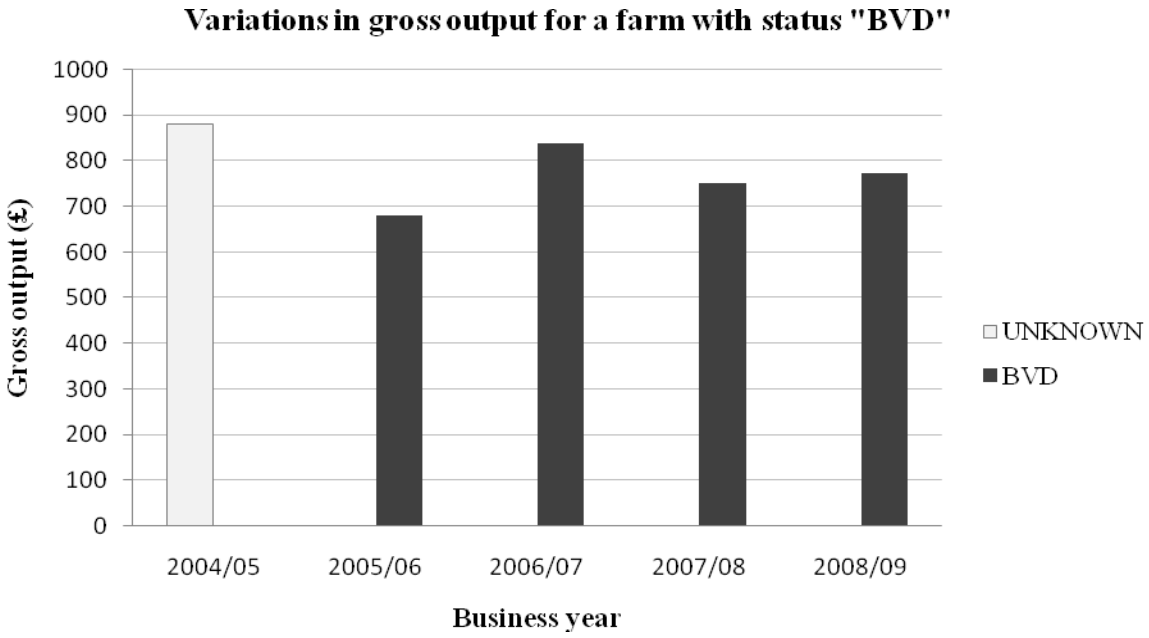

Fig. 2 Within herd variation in GO per cow for a study farm remaining actively BVD infected for 4 years

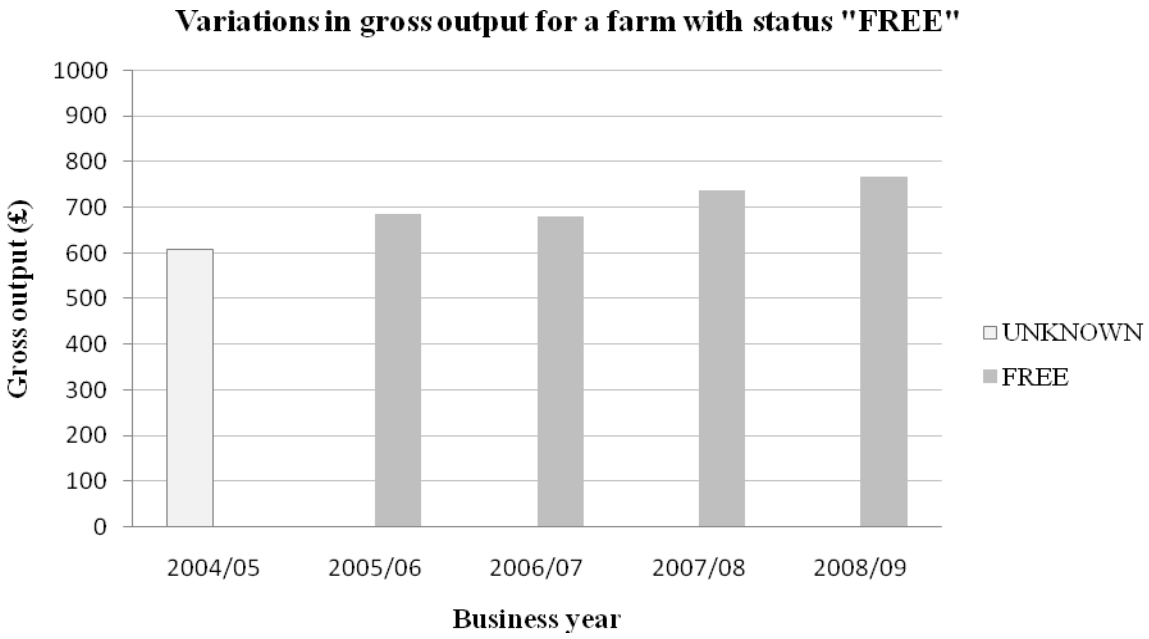

Fig. 3 Within herd variation in GO per cow for a study farm remaining “FREE” from BVD

Relative changes in GO per cow were calculated from 2005/06 onwards and evaluated for the three BVD states (Fig. 4). Furthermore, RCGO was associated with BVD state transitions (Fig. 5).

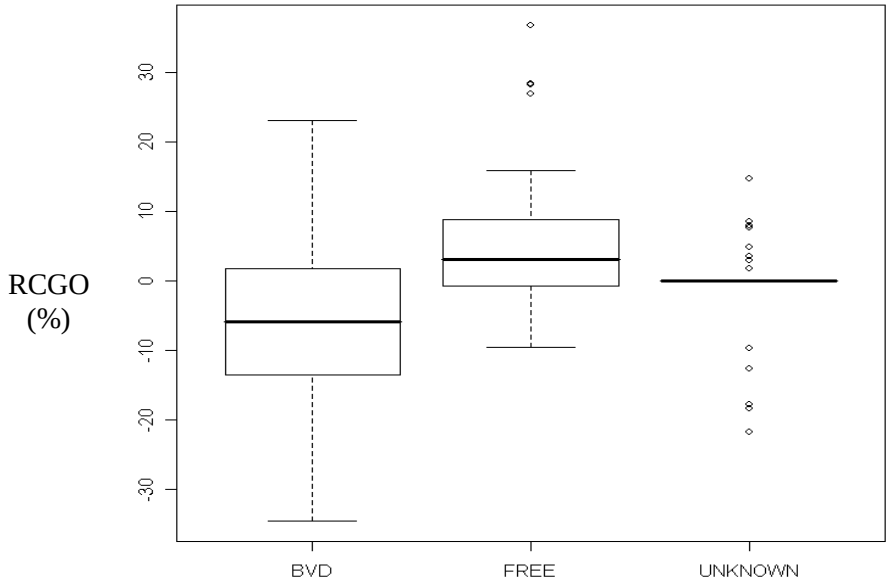

Fig.4 RCGO box plots associated with the states “BVD”, “FREE” and “UNKNOWN” for all years

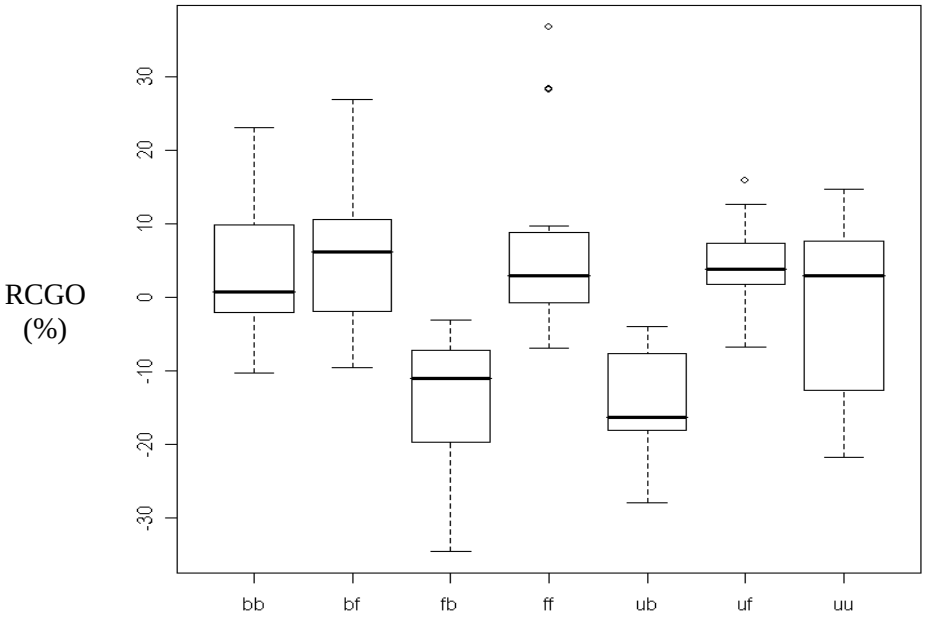

Fig.5 Box plots visualising RCGO associated with BVD state transitions for all years
bb: BVD → BVD, bf: BVD → FREE, fb: FREE → BVD, ff: FREE → FREE, ub: UNKNOWN → BVD, uf: UNKNOWN → FREE and uu: UNKNOWN → UNKNOWN

DISCUSSION

The purpose of this study was to validate a robust method for monitoring the economic impact of endemic disease by using existing, reliable data sources and providing performance indicators to enable informed decisions on endemic disease control and prevention at the farm level. Data available across all study farms consisted of official herd register and animal movement data recorded through the GB CTS. The GO derived from CTS data presented here primarily served as an indicator for herd fertility and mortality; two important disease effects of BVD (Houe, 2003). Morbidity and resulting economic losses due to reduced growth have not been evaluated in the current study, as necessary data were not available for all farms. Therefore, standardised animal weights were used for GO calculation. However, the presented method is designed to incorporate individual empirical animal weights, which allows inclusion of morbidity effects into the financial assessment.

In the present study, GO varied between and within farms over time. Regardless of this variability and the elementary input data, the method yielded unambiguous results for the assessed enterprises. Contracting BVDV infection was associated with negative relative changes in GO, while prolonged active infection was associated with a wider variation in GO, thus indicating increased production risk for affected farms.

Eighteen of the 22 livestock enterprises comprising the study population experienced state transitions from “UNKNOWN” or “FREE” to active BVD infection with the relative reduction in GO ranging from 3% to 35%. The variability of the output losses within and between enterprises observed in this study based on empirical data is in line with the predicted range of losses by Stott et al. (2009) using a modelling approach to investigate different epidemiological scenarios. The GO for farms remaining actively BVDV infected for consecutive years showed wide variation over time. These findings show that endemic diseases like BVD can have detrimental effects on business performance. The unpredictability of losses associated with livestock disease increase business risk and hence reduces the viability of affected farms.

In contrast herds experiencing state transitions from either “UNKNOWN” or “BVD” to “FREE” or herds free from BVDV infection for several consecutive years showed predominantly positive average relative changes in GO. Furthermore, variation in GO over time and thus production risk was lower for herds remaining free from BVDV. However, the number of “FREE” farms fluctuated from year to year. The fact that state transitions from “FREE” to “BVD” were observed despite high disease awareness and regular monitoring of disease status throughout the study period underlines that BVD is a constant threat and difficult to evade for individual farms when no systematic regional or national control is applied (Lindberg and Houe, 2005).

For the initial two business years, included to provide both insight into GO fluctuations for an extended time period and a baseline for calculation of future relative changes in GO, most farms were classified as UNKNOWN. By the third year evaluated, all herds could be classified as either “BVD” or “FREE”. When comparing the results of the RCGO in the “UNKNOWN” category to both “BVD” and “FREE”, farms classified as “UNKNOWN” were closer to “FREE” farms. These findings indicate that herd health status should be assessed and monitored by adequate veterinary measures to ensure that animal disease control is targeted and resources are invested effectively.

In the presented study, one GO was calculated for all beef cattle present on the farm, to allow for inclusive assessment of disease impact. However, the presented method lends itself to be applied to individual enterprises within farm; e.g. suckler cows, store and finishing cattle and breeding of replacements; or separation of commercial and pedigree stock. Specifically for large farms with several distinct units, one herd level BVD status and one gross enterprise output might not reflect the infection status and performance of individual age or management groups. Distinctive GOs will allow quantification of disease effects for different management groups and can hence facilitate targeted interventions while reducing expenditure on control measures.

During the course of this study, it became increasingly obvious that access to robust data allowing assessment of indirect disease effects is difficult. Hence the current performance assessment for livestock enterprises does not include expenditure on treatments and preventive actions including biosecurity and vaccination or additional feed and labour cost. The observation that consistent recording of animal health and management information in a format allowing economic analysis is not a priority for livestock keepers has previously been made (Bennett, 2003; Stott et al., 2009). Disease important effects associated with BVDV, like abortions and deaths in neo-natal calves, were hardly ever recorded or followed-up on study farms as the specific time of occurrence of the former was rarely observed, while the latter was regarded as a recurrent and unavoidable event.

In conclusion, the major advantages for the presented GO calculation through empirical herd register and movement data are, that important disease effects as described above can be captured through optimal use of existing data sources without necessitating additional record keeping. Furthermore, the method enables continuous monitoring of livestock performance and, in conjunction with information derived by regular sampling and testing - for instance within a health scheme - of disease impact on performance. The usage of relative changes in GO allows longitudinal assessment of performance within herd and benchmarking between herds. While the method can be used for a large number of farms, it also allows for inclusion of additional and more detailed information thus adapting it to individual circumstances and making it more acceptable to individual stakeholders.

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REFERENCES

Anon, (2009). The Farm Management Handbook 2009/10. Scottish Agricultural College, Edinburgh.

Barber, D.M.L. and Nettleton, P.F. (1993). Investigations into bovine viral diarrhoea in a dairy herd. Vet. Rec. 133, 549-550.

Barnard, C.S. and Nix, J.S. (1979). Farm Planning and Control. Cambridge University Press, Cambridge.

Bennett, R. (2003). The 'direct costs' of livestock disease: The development of a system of models for the analysis of 30 endemic livestock diseases in Great Britain. J. Agr. Econ. 54, 55-71.

Bitsch, V., Hansen, K.-E.L. and Ronsholt, L. (2000). Experiences from the Danish programme for eradication of bovine virus diarrhoea (BVD) 1994-1998 with special reference to legislation and causes of infection. Vet. Microbiol. 77, 137-143.

Brownlie, J., Clarke, M.C., Howard, C.J. and Pocock, D.H. (1987). Pathogenesis and Epidemiology of Bovine Virus Diarrhea Virus- Infection of Cattle. Ann. Rech. Vet. 18, 157-166.

Brownlie, J. (1990). Pathogenesis of mucosal disease and molecular aspects of bovine virus diarrhoea virus. Vet. Microbiol. 23, 371-382.

de Behr, V., Hornick, J.L., Cabaraux, J.F., Alvarez, L. and Istasse, L. (2001). Growth patterns of Belgian Blue replacement heifers and growing males in commercial farms. Livest. Prod. Sci. 71, 121-130.

de Verdier Klingenberg K. (2000). Enhancement of clinical signs in experimentally rotavirus infected calves by combined viral infections. Vet. Rec. 147, 717-719.

Duffell, S.J., Sharp, M.W. and Bates, D. (1986). Financial loss resulting from BVD-MD virus infection in a dairy herd. Vet. Rec.118, 38-39.

Favier, J.F. and Dodd, V.A. (1991). The development of a prototype computerised management information system for a mixed enterprise farm. Agr. Sys. 35, 287-311.

Ferrari, G., Scicluna, M.T., Bonvicini, D., Gobbi, C., Della Verita, F., Valentini, A. and Autorino, G.L. (1999). Bovine virus diarrhoea (BVD) control programme in an area in the Rome province (Italy). Vet. Microbiol. 64, 237-245.

Grom, J. and Barlic-Maganja, D. (1999). Bovine viral diarrhoea (BVD) infections - control and eradication programme in breeding herds in Slovenia. Vet. Microbiol. 64, 259-264.

Gunn, G.J., Heffernan, C., Hall, M., McLeod, A. and Hovi, M. (2008). Measuring and comparing constraints to improved biosecurity amongst GB farmers, veterinarians and the auxiliary industries. Prev. Vet. Med. 84, 310-323.

Gunn, G.J., Stott, A.W. and Humphry, R.W. (2004). Modelling and costing BVD outbreaks in beef herds. Vet. J. 167, 143-149.

Hardaker, J.B., Huirne, R.B.M., Anderson, J.R. and Lien, G. (2004). Coping with Risk in Agriculture. CABI, Wallingford, Oxfordshire.

Heffernan, C., Nielsen, L., Thomson, K. and Gunn, G. (2008). An exploration of the drivers to bio-security collective action among a sample of UK cattle and sheep farmers. Prev. Vet. Med. 87, 358-372.

Houe, H. (1992). Serological Analysis of a Small Herd Sample to Predict Presence or Absence of Animals Persistently Infected With Bovine Viral Diarrhea Virus (Bvdv) in Dairy Herds. Res. Vet. Sci. 53, 320-323.

Houe, H. (1999). Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. Vet. Microbiol. 64, 89-107.

Houe, H. (2003). Economic impact of BVDV infection in dairies. Biologicals 31, 137-143.

Hult, L. and Lindberg, A. (2005). Experiences from BVDV control in Sweden. Prev. Vet. Med. 72, 143-148.

James, A.D. and Carles, A.B. (1996). Measuring the productivity of grazing and foraging livestock. Agr. Sys. 52, 271-291.

Lindberg, A. and Houe, H. (2005). Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. Prev. Vet. Med. 72, 55-73.

McGowan, M.R., Kirkland, P.D., Richards, S.G. and Littlejohns, I.R. (1993). Increased Reproductive Losses in Cattle Infected With Bovine Pestivirus Around the Time of Insemination. Vet. Rec. 133, 39-43.

McInerney, J. (1996). Old economics for new problems - Livestock disease: Presidential address. J. Agr. Econ 47, 295-314.

Moennig, V., Eicken, K., Flebbe, U., Frey, H.R., Grummer, B., Haas, L., Greiser-Wilke, I. and Liess, B. (2005). Implementation of two-step vaccination in the control of bovine viral diarrhoea (BVD). Prev. Vet. Med. 72, 109-114.

Morris, R.S. (1999). The application of economics in animal health programmes: a practical guide. Rev. sci. tech. Off. int. Epiz. 18, 305-313.

Penny, C.D., Low, J.C., Nettleton, P.F., Scott, P.R., Sargison, N.D., Strachan, W.D. and Honeyman, P.C. (1996). Concurrent bovine viral diarrhoea virus and Salmonella typhimurium DT104 infection in a group of pregnant dairy heifers. Vet. Rec. 138, 485-489.

Potgieter, L.N.D., McCracken, M.D., Hopkins, F.M., Walker, R.D. and Guy, J.S. (1984). Experimental Production of Bovine Respiratory-Tract Disease With Bovine Viral Diarrhea Virus. Am. J. Vet. Res. 45, 1582-1585.

Rossmanith, W., Janacek, R. and Wilhelm, E. (2005). Control of BVDV-infection on common grassland--The key for successful BVDV-eradication in Lower Austria. Prev. Vet. Med. 72, 133-137.

Rushton, J., Thornton, P.K. and Otte, M.J. (1999). Methods of economic impact assessment. Rev. sci. tech. Off. int. Epiz. 18, 315-338.

Stott, A.W., Humphry , R.W. and Gunn, G.J. (2009). Modelling the effects of previous infection and re-infection on the costs of bovine viral diarrhoea outbreaks in beef herds. Vet. J. in Press, 10.1016/j.tvjl.2009.05.020.

Stott, A.W., Lloyd, J., Humphry, R.W. and Gunn, G.J. (2003). A linear programming approach to estimate the economic impact of bovine viral diarrhoea (BVD) at the whole-farm level in Scotland. Prev. Vet. Med. 59, 51-66.

Synge, B.A., Clark, A.M., Moar, J.A.E., Nicolson, J.T., Nettleton, P.F. and Herring, J.A. (1999). The control of bovine virus diarrhoea virus in Shetland. Vet. Microbiol. 64, 223-229.

Turner, J. and Taylor, M. (1998). Applied Farm Management. Blackwell Science Ltd, Oxford.

Upton, M. (1989). Livestock productivity assessment and herd growth models. Agr Sys 29, 149-164.

Valle, P.S., Skjerve, E., Martin, S.W., Larssen, R.B., Osteras, O., Nyberg, O. (2005). Ten years of bovine virus diarrhoea virus (BVDV) control in Norway: a cost-benefit analysis. Prev. Vet. Med. 72, 189-207.

Wilson, R.M. (2001). Measuring Farm and Enterprise Performance. IERM University of Edinburgh, Edinburgh, pp. 1-70.

FARMER ATTITUDES AND BEHAVIOURAL RESPONSES TO PRE-MOVEMENT TESTING FOR BOVINE TUBERCULOSIS IN GREAT BRITAIN

R.M. CHRISTLEY*, S.E. ROBINSON, B. MOORE, C. SETZKORN AND I. DONALD

SUMMARY

Pre-movement testing (PrMT) for bovine tuberculosis (bTB) was introduced in England and Wales in 2 phases from 2006. This study used farmer interviews and questionnaires, and analysis of national cattle movement records to investigate the impact of introduction of PrMT on specific farm management behaviours. A majority of farmers (65%) believed they had not changed their behaviour in response to PrMT; the main reported changes related to decisions regarding the selling of cattle. There was evidence of reduction in movements of cattle between farms in areas that must undertake PrMT. Some farmers believed that others might be more willing to purchase animals from high risk areas as a result of increased confidence due to PrMT. However, there was little evidence of this in the movement data. Following PrMT there was an increased movement of single animals and a decreased movement of large batches (>10) of animals.

INTRODUCTION

Bovine tuberculosis (bTB) is a major biological, economic and political issue in Great Britain; herd incidence is increasing at around 18% per annum (Anon, 2004) and the disease is emerging in previously unaffected areas. Government expenditure on bTB is estimated to reach £1 billion for the period 2004-2012 and costs to industry might increase 4-fold over this period (Anon, 2004). The mainstay of control in GB involves surveillance and culling. The frequency of testing is determined by local risk and may be at 1 (highest risk) 2, 3 or 4 (lowest risk) yearly intervals. These are called Parish Test Intervals (PTI) because they are determined at the Parish level and affect most farms in the parish although some farms might require more frequent testing. To reduce bTB spread via cattle movements, pre-movement testing (PrMT) of cattle in areas with a 1 or 2-yearly PTI was introduced in England and Wales from 2006 (Anon 2006b, c). Initially, Phase I applied to cattle over 15 months of age, but in 2007 Phase II extended the requirement to all cattle over 42 days (with exemptions) in areas with PTI of 1 or 2 years. Scotland introduced a separate and distinct regimen of pre- and post-movement testing in 2005 (Anon, 2005b).

By August 2009 pre-movement testing had been undertaken on 116,523 occasions in England and Wales resulting in testing of almost 1.5M cattle (Anon, 2009). There is evidence that PrMT

* Robert Christley, Epidemiology and Public Health, School of Veterinary Science, University of Liverpool, Leahurst Campus, Neston, CH64 7TE, UK. robc@liv.ac.uk

imposes additional costs to farmers. The Department for environment, food and rural affairs (Defra) have estimated the veterinary cost of testing to be £5.50 to £9.00 per animal, with a most likely cost of £6.70 (Anon, 2006a). Another survey of 60 farmers reported an average cost per animal of £8.84 due to veterinary, labour and other costs, but with wide variation and almost a third of farmers reporting costs per animal of over £20 (Bennett, 2009). However, neither estimation included other potential costs which have been identified by farmers, such as disruption to farm business or missed market opportunities (Bennett, 2009) and there is little information regarding the adaptations farmers might make in response to introduction of PrMT (Anon, 2006a). The cost per animal might decrease as more animals are tested, particularly up to approximately 30 animals (our estimate from data provided in Bennett, 2009). If more generally true, this might encourage farmers to avoid testing and moving small batches of animals resulting in movements of larger groups of animals. In contrast, there is anecdotal evidence that some farmers may prefer to test (and move) small batches of animals, in order to reduce the chance of a positive test result.

Introduction of PrMT could affect a range of decisions regarding movement of cattle. For example, PrMT may reassure farmers in low bTB-risk areas that cattle from high-risk areas are disease-free and hence increase their willingness to buy cattle from these areas. In contrast, the need to test may highlight the distinction between high- and low-risk areas and enhance trading within the low-risk areas. The relative importance of effects such as these may relate to many factors, including farmer trust in the efficacy of PrMT.

This study investigated farmer attitudes to PrMT and changes in decisions relating to animal movements. Several approaches were used; qualitative farmer interviews, a postal questionnaire to farmers and time-series analysis of cattle movement data from the RADAR database. As PrMT applies to movements from herds requiring ≤2-yearly testing, the impact of different testing intervals was of particular interest.

MATERIALS AND METHODS

Qualitative Elicitation Interviews

Twenty-one farmers (identified through local veterinary practices) from 3 areas participated in qualitative interviews designed to explore farmers' beliefs and perceptions about bTB and PrMT and to elicit salient beliefs about specific behaviours relating to cattle management. These areas included areas of relatively high (Devon, n=8) and low (Lancashire, n=7) bTB risk and an area in transition from low to high risk due to an increasing bTB incidence (Shropshire, n=6). Farmers were asked what they thought were the advantages or disadvantages of specific behaviours relating to cattle management, any obstacles or facilitators to these and about other actors that may influence their behaviours and who they would trust for advice or information. They were also asked if there was anything they thought they had changed or other farmers had changed in relation to their farm management since the introduction of PrMT.

Thirteen (62%) participants were dairy farmers, one reared heifers and 7 (33%) were beef enterprises. The mean herd size was 128 (s.d.= 66.4). At the time of the interview 10 (47%) reported a bTB breakdown either ongoing or in the last 5 years.

Postal Questionnaire

The questionnaire was based on beliefs and perceptions identified in the qualitative interviews and was designed to collect data for 2 specific analyses; (a) investigation of farmer attitudes and perceived behavioural responses to the introduction of PrMT, and (b) assessment, using a Theory of Planned Behaviour (TPB) based approach, of factors having an impact on farmers intention to purchase cattle. Only the former is reported here.

A small pilot study of the questionnaire was carried out at local livestock markets and with local farmers and the questions were modified as a result of the feedback. The questionnaire consisted of some demographic variables followed by 94 questions measuring trust in government and other groups, importance of bTB, beliefs about the role of wildlife, beliefs about PrMT and aspects of farm management. Approximately half of the questions were specifically for use in the TPB-based analysis and are not reported here. All responses, with the exception of past behaviour and changes, were made on 7 point rating scales e.g. 1= strongly disagree to 7= strongly agree. Finally, farmers were asked to report (using an open question) whether they had changed any aspect of farm management since the introduction of bTB and whether they thought other farmers had changed anything.

Names and addresses of all farmers in England were obtained from the British Cattle Movement Service and questionnaires (800 in total) were sent to 200 farmers selected at random from each of 4 areas of the country (Areas 1 to 4 in Table 1).

Table 1. Country, county and PTIs selected for inclusion in the time series analysis. Areas 1-4 were also used to generate the sampling frame for the postal questionnaire.

Country	County	Area	PTIs use in analyses
England	Cornwall,Devon	1	1,2
England	Shropshire,Worcestershire,Hereforshire,Gloucestershire	2	1,2,4
England	Lancashire,Cumbria	3	4
England	North, West & South Yorkshire, Derbyshire, Nottinghamshire	4	1,2,4
Wales	Dyfed	5	1,2,4
Wales	Gwynedd,Clwyd	6	4
Scotland	Argyll,Stirling,Perth,Angus	7	4
Scotland	Dumfrieshire,Selkirkshire,Peebleshire,Roxburghshire	8	4

Time series analysis

Cattle movement data from January 1999 to February 2007 were downloaded from the RADAR database in June 2007 enabling investigation of the impact of introduction of Phase I PrMT only. The database contained information identifying animals, locations (e.g. type of location, easting and northing and county) and animal movements (including animal and location ID, and date and type of arrival and departure). Data on past and present PTI were obtained from Animal Health (a Defra executive agency). For all analyses, PTI categories 3 and 4 were merged because there are few farms in PTI 3 and these areas are both generally exempt from PrMT (this combined category is referred to as PTI 4).

Pairs of locations were considered linked in a given period if ≥1 animal was moved between them. The potential impact of the introduction of Phase I of PrMT on 3 behaviours of cattle

farmers was explored: the total number of direct or in-direct (via markets) movements of 1 or more animals between farms; the number of movements of 1 or more animals between farms in different PTIs, and; the number of batches of animals moved between farms, or to market.

Poisson regression time series analyses were performed using datasets generated from the cattle movement data. The model for each analysis included variables representing: underlying linear trend; annual, 6 monthly and quarterly cycles; bank holidays and the Christmas-New Year period; introduction of PrMT (I_{PrMT}: 0 before and 1 after introduction); a linear trend following introduction of PrMT ($PrMT_{slope}$: 0 before and 1,2,3...n after introduction); introduction of Single Farm Payments (I_{SFP}: 0 before 1/1/2005 and 1 after) and; a linear trend following introduction of Single Farm Payments (SFP_{slope}: 0 before and 1,2,3...n after introduction). The week of introduction of PrMT was specified for each country (England 27/3/2006; Wales 2/5/2006). Scotland was given the English date; we expected no *direct* effect in Scotland of PrMT introduction in England and Wales.

The analysis of movement of animals between farms used an approach based on the multiple baseline time series method (Biglan *et al.*, 2000) with population subsets defined by PTI +/- region. Eight regions of GB covering a range of bTB risk were selected (Table 1). This approach enabled exploration of the consistency of the apparent impact of PrMT across regions and by PTI of the farms from which the movement originated; for the purposes of analysis, data were aggregated to the weekly level. Finding that farms in areas undertaking PrMT (those in PTI 1 and 2) responded differently to those that do not (in PTI 3 and 4) would enable more confident conclusions that an observed effect was due to PrMT, rather than to other unmeasured factors. Analysis was performed on 15/24 (63%) possible region-PTI combinations (some regions contained very few parishes, and hence farms, with certain PTIs). In this model, interaction terms between I_{PrMT} and *region-PTI*, and between $PrMT_{slope}$ and *region-PTI* were used to explore the potential for variable impact of PrMT in different regions and PTIs.

The analysis of movement among PTIs used datasets representing the number of movements (per week) between farms in each combination of PTI (1→1, 2→2, 4→4, 1→2, 2→1, 1→4, 4→1, 2→4, 4→2) whereas the analysis of movements of different batch sizes was performed using datasets representing the number of movements of a particular batch size range per day originating from farms under each PTI.

For each analysis, the number of events in a given period was likely to be a function of the maximum possible number of such events (for example, the number of farms in a particular region that sold animals in a time period is related to the total number of farms present in that area). To account for this, the appropriate totals were included as offset variables in the regression analyses. As the data were over-dispersed the models were fitted using a quasi-Poisson link function. The impact of introduction of PrMT (ie I_{PrMT}) was assessed by calculating the relative rate of movements after introduction of PrMT, compared to before, whilst holding all other variables in the model constant. Similarly, the relative rate of movements per week after introduction (ie $PrMT_{slope}$) was assessed; for ease of presentation this was converted to the relative rate per quarter year (13 weeks). Model fit for all models was checked against the data. As well as investigating the numbers of movements, the effect of PrMT on the number of animals moved was also explored. These analyses lead to the same conclusions as the analysis of movements and are not reported here.

RESULTS

Qualitative Elicitation Interviews

In low bTB prevalence areas there was a general lack of saliency of bTB. Foot and Mouth Disease was more salient and was suggested to be likely to affect current behaviour. Farmers also mentioned other diseases e.g. brucellosis. This was not true of transitional and high bTB areas where bTB was highly salient and farmers express feelings of helplessness and of being "under siege". Farmers in these areas reported a high level of distress and problems associated with PrMT, mainly as a result of bTB breakdowns and movement restrictions. Pre-movement testing was generally seen as effective in all areas (although farmers in low bTB areas are not directly subject to this measure) despite doubts about the effectiveness of the skin test. However it was often seen as "damage limitation" rather than a cure because many farmers identified badgers as the main source of infection. Some interviewees reported that other farmers may show too much faith in the skin test which could lead to a false sense of security when purchasing cattle; indeed, some interviewed farmers stated that buying cattle should not pose a risk since introduction of PrMT. Routine bTB testing was also generally seen as an important control measure in all areas, despite being seen as causing problems. Some farmers in high and moderate bTB risk areas wished to see annual testing extended to all areas (notably this was not suggested by farmers in low bTB areas). However both routine and pre-movement testing was seen as stressful and dangerous for both cattle and farmers and a potential source of accidents to farmers. Movement controls were also seen overall as quite effective, although less so. Most farmers reported movement controls to be a "hassle" but had incorporated them into routine and believed they are helpful in preventing disease spread. Farmers mentioned lack of flexibility in regulations as being unreasonable and causing difficulty; some farmers also mentioned ways of getting around the standstill policy. Most farmers reported little if any change in either their own or other farmers' management practices since the introduction of PrMT. Changes that farmers mentioned largely mirrored the reports of changes on the questionnaires which are reported below.

Questionnaire survey

The results presented here are based on the first 250 questionnaires returned, representing a response rate of 31%. The respondents were broadly representative of cattle farmers in the United Kingdom (Anon 2005a). The mean age of respondents was 55.5±12.1 yrs (mean±s.d.); 87% were male and 13% female. A majority (56%) kept a 'closed herd', 44% did not. The mean number of cattle was 140±151 but this varied considerably; 58.5% were in a PTI 1 area, 10.7% in a PTI 2 area, 2.7% in PTI 3, 28.1% in PTI 4. One-third (32%) had experienced a bTB breakdown on their own farm in the last 5 yrs, and 72% knew someone who had had a breakdown; 25% were dairy cattle farmers, the remainder were beef or mixed farmers. A comparison between the 250 respondents and the study population showed no substantive differences between the two in terms of farm PTI, size and type, apart from the smallest, 'hobby' farms being under-represented.

The questionnaire included items to measure trust in government and PrMT. Trust in government (Defra) and the measures being taken to control bTB was low in all four areas (2.1±1.8, 1.6±1.2, 2.5±1.8, 1.6±1.1 for areas 1, 2, 3 and 4 respectively) but was significantly higher in area 3 (Lancashire and Cumbria) than in the other three areas ($F_{3,245}$=6.1, P=0.005). There were no significant differences between PTIs in level of trust. Confidence in the efficacy

of the skin test for bTB was low in all areas (2.5±1.3, 2.5±1.4, 3.7±1.6, 3.3±1.4) but was higher in areas 3 and 4 than in areas 1 and 2 ($F_{3,242}$=12.0, P<0.001). There were similar differences between PTIs in confidence in the skin test with confidence being lower in PTI 1 than in the other PTIs ($F_{2,218}$=15.7, P<0.001).

Ninety-eight farmers (41%) agreed that moving cattle will not spread bTB since they are tested prior to moving, with another 20% unsure. There was no significant difference between areas ($F_{3,236}$ =1.8, P=0.1) but farmers in PTI1 (4.3±2.0) were more likely to agree than those in PTI 2 or 4, 3.1±1.9 and 3.4±2.0 ($F_{2,212}$=5.8, P=0.004). Farmers who agreed that moving cattle will not spread bTB since PrMT had a more favourable attitude towards buying-in (r=0.3, p<0.001) and intended to buy-in cattle (r=0.3, P<0.001). The belief that buying-in cattle was likely to bring bTB into the herd was, perhaps surprisingly, not significantly related to either area or PTI (area $F_{3,241}$=1.4, p=0.3; PTI $F_{2,217}$=0.8, P=0.4). However, farmers who thought that buying-in would bring bTB had a less favourable attitude to buying-in (r= -0.4, P<0.001) and lower intention to buy-in cattle (r=-0.4, P<0.001).

To examine the importance of bTB to farmers the questionnaire included the item 'I don't worry about bovine TB much until a test is due'. A high score represents agreement i.e. low saliency. The level of importance was not significantly different between PTIs ($F_{2,219}$=1.0, P=0.4) or areas ($F_{3,243}$=2.423, P=0.07), although there was a trend toward greater importance in area 1 and lesser importance in area 3. Low bTBimportance was related to a more favourable attitude towards buying-in (r=0.2, P<0.005) and with the intention to buy-in cattle (r=0.2, P<0.005).

The majority of farmers (90%) agreed that 'culling diseased cattle to control bTB is useless unless wildlife are controlled', 74% strongly agreed, only 8% disagreed. Similarly, 73% agreed that 'it is pointless trying to control bTB because wildlife can spread it', 52% strongly agreed and 23% disagreed. Agreement with these two statements did not differ by either area (χ^2_3=3.0, p=0.4) or PTI (χ^2_3=0.9, p=0.8) but did relate to trust in government measures (r=-0.2, p<0.001) with farmers having lower trust also tending to believe that control in cattle, without control in wildlife, is useless.

Farmers were asked if they preferred to buy locally, whether they planned to buy cattle from an area with a PTI of 1 or 2 and whether they would only buy cattle they were certain had come from a PTI 4 (Table 2). Not surprisingly most farmers preferred to buy locally. However farmers in Area 3 (Lancashire and Cumbria) had a higher preference for buying locally than farmers in the other areas ($F_{3,241}$=5.8, P<0.001). There were no significant differences between PTIs in preference for buying locally ($F_{2,217}$=2.3, P=0.1). This preference for buying locally was reflected in the differences between area and PTIs in where they planned to buy, with farmers in areas 1 and 2 and in a PTI 1 or 2 agreeing that they planned to buy from an area with a PTI of 1 (area $F_{3,230}$=6.9, P<0.001; PTI $F_{2,208}$=24.8, P<0.001). Similarly farmers in areas 3 and 4 and PTIs 3 with 4 agreed more strongly that they would only buy from a PTI 4 area ($F_{3,234}$=21.7, P<0.001; PTI $F_{2,211}$=42.1, P<0.001).

Farmers were asked to report on any changes in their farm management since introduction of PrMT and any perceived changes in other farmers' management. The changes reported on questionnaires largely confirmed those found in the interviews. Sixty five percent of farmers reported no changes in themselves or others (53%, 51%, 77%, 84% in areas 1,2,3 and 4, respectively). The main changes reported included: 1) selling cattle in larger batches (e.g. following annual test to avoid extra costs of PrMT, n=18); these were all in PTI 1 (nine farmers

in area 1, 6 in area 2 and 3 in area 4 reported these changes); 2) Selling cattle in smaller batches – only 2 farmers reported this, both in PTI 1 and area 1 (Devon & Cornwall); 3) changes in farm enterprise (n=27), which included selling cattle fat rather than as stores (n=10), all in PTI 1 (areas 1 & 2), reducing stock numbers (n=6), all PTI 1 and areas 1 and 2, rearing own/breeding own/keeping a closed herd (n=5), exit from cattle farming (n=3), other enterprise changes (n=3), and; 4) other management changes such as earlier sale of bull calves (n=1), reduced cattle movements (n=3), buying in to restock (n=2), changes in where stock are kept (n=3) and raised awareness of bTB (n=13).

Table 2. Mean (s.d.) of scores for preferred buying behaviour by area and parish test interval (PTI). High scores (maximum possible = 7) indicates strong preference for that behaviour.

	Buy local	Buy from PTI 1	Buy from PTI 4	Buy from farm	Buy at market
Area 1	5.55 (1.61)	4.13 (2.12)	3.22 (2.01)	3.49 (2.28)	3.05 (2.34)
Area 2	4.89 (2.18)	4.18 (2.08)	3.13 (2.12)	3.54 (2.19)	3.30 (2.47)
Area 3	6.24 (1.58)	2.89 (2.43)	5.32 (2.19)	3.16 (2.21)	3.83 (2.35)
Area 4	5.66 (1.74)	2.82 (2.03)	5.35 (1.78)	3.50 (2.20)	3.27 (2.36)
PTI 1	5.36 (1.87)	4.14 (2.15)	3.10 (2.05)	3.73 (2.28)	3.25 (2.45)
PTI 2	5.65 (1.87)	4.50 (1.94)	4.00 (2.34)	3.04 (2.07)	3.26 (2.15)
PTI 3 & 4	5.94 (1.72)	2.07 (1.87)	5.84 (1.91)	3.07 (2.09)	3.37 (2.29)

Time series analyses

Movements between farms:

Analysis of the time series of the weekly total number of movements between farms (i.e. the number of direct movements between farms plus the number occurring through markets) found a significant impact of PTI, with farms in PTI 4 significantly more likely to move cattle, compared to those in PTI 1 ($P=0.04$), whereas there was no difference between PTI 2 and 1 ($P>0.9$; Fig. 1a). This effect of PrMT was not constant over time following introduction. In all PTIs the relative rate decreased over time ($P=0.03$; Fig. 1c), but the decrease was significantly greater in PTI 1 and 2 compared to PTI 4 ($P<0.001$). The difference in the decrease over time between PTI 1 and 2 was not significant ($P>0.9$).

However, inclusion of an interaction term suggested that the impact of PrMT was affected by both the PTI and the area in which a farm was found. Generally, farms in PTIs 1 and 2 (those areas which must undertake PrMT) were less likely (or equally likely) to move cattle in a particular week following introduction of PrMT (compared to prior to introduction of PrMT), whereas farms in PTI 4 were generally more (or equally) likely to move cattle following introduction of PrMT (Fig. 1b) and this may be suggestive of an impact of PrMT. However, there are notable exceptions; for example, the high risk parishes in area 5 (Dyfed) were more likely to move cattle following introduction of PrMT. Also the magnitude of the change varied considerably between areas, for a given PTI. The variable effect of PrMT across the different areas suggests that any real impact was tempered by other local conditions. For most area-PTI combinations the rate of farm-to-farm movements decreased over time following introduction of PrMT (Fig. 1d); the notable exception was area 4 (Yorkshire, Nottinghamshire and Derbyshire) where there was a greater rate of movement from farms in each of the PTIs. The model appeared

to account for much of the linear and seasonal variation in the data and the model fit the data reasonably well.

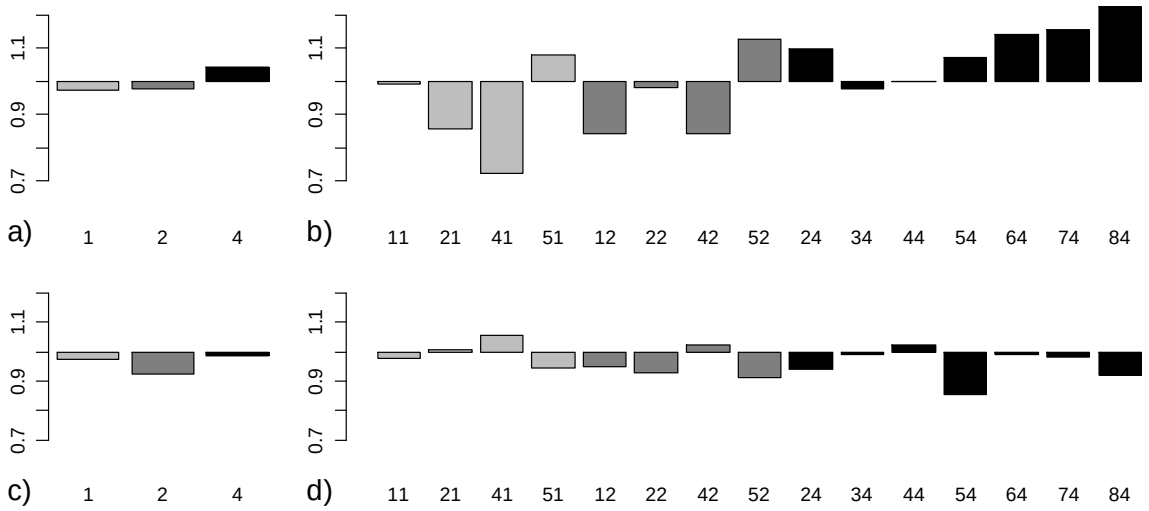

Fig. 1. Relative rates (y-axis) of movement of animals between farms (both directly and via a market) following introduction of PrMT: a) and b) step change associated with introduction (I_{PrMT}); c) and d) change per quarter (i.e. 13 weeks) following introduction of PrMT ($PrMT_{slope}$). a) and c) indicate impact of PrMT on movements originating on farms in PTI 1, 2 and 4 (ignoring variation between regions). b) and d) indicate the impact of PrMT on movements originating on farms in each area-PTI combination. The codes on the x-axis indicate the region-PTI combination, e.g."21" refers to area 2, PTI 1.

Movements between PTIs:

The movement of animals between farms in each combination of PTIs was investigated, as a function of the total number of movements possible between those PTIs (i.e., an offset term was used to account for the total number of potential dyads in each PTI combinations). Initially, all farm-to-farm movements between and within PTIs were considered together (i.e. those occurring directly between farms, plus those occurring via a market). For most PTI origin-destination combinations there was a significant decrease $P<0.001$) in the relative rate of movements following introduction of PrMT. However, this effect was reversed for movements from PTI 2 to 4, 4 to 2 and 4 to 4, for which the relative rate was greater than 1 ($P<0.001$ for each, Fig. 2a). Following introduction of PrMT, there was a change in trend with a decreased rate of movement into the high risk areas over time (irrespective of the PTI of origin; Fig. 2b).

Pre-movement testing resulted in a reduced probability of movements originating on farms in PTI 1. In terms of the actual number of movements the predominant effect was a reduction in the number of movements to other farms in PTI 1. The reduction in the number of movements to farms in other PTIs is lower, because fewer such movements would have occurred anyway. This may reflect a general decrease in movement activity of farms in PTI 1 after the introduction of PrMT as reflected by a marked decrease in farm to farm movements originating in PTI 1 of some areas and the decreased movement to farms in PTI 1, 2 and 4 indicated here. There did appear to be an increase in movements within PTI 4's, with approximately 150 more farm to farm movements per week within PTI 4 (on average) following introduction of PrMT.

Overall, when separately considering direct farm-to-farm movements and movements via a market, the impact of PrMT appeared similar to that for the composite analysis, but suggested some variation in the relative impact of changes to direct and market movements for the different PTI origin-destination combinations.

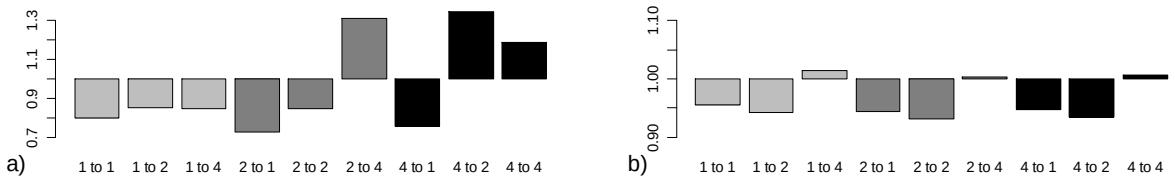

Fig 2. Relative rate of farm-to-farm movements between PTIs (both directly and via a market) following introduction of PrMT: a) step change associated with introduction (I_{PrMT}); b) change per quarter (i.e. 13 weeks) following introduction of PrMT ($PrMT_{slope}$). The x-axis indicates the PTI origin-destination combination, e.g. "1 to 2" refers to movements from PTI 1 to PTI 2.

Movement batch size:

The impact of introduction of PrMT on the movements of various batch sizes directly between farms and between farms via a market was investigated. There was no evidence of change in movement of batches of size 2-9; results for batch size 1 (i.e. single animals) and batches of greater than 10 are presented here. In order to control for the total number of movements the total number of movements of any size was included in the models as an offset. Direct movements between farms and movements between farms via a market were considered separately.

Direct farm-to-farm movements:

There was an increase in the relative rate of single-animal shipments and a decrease in the shipments of greater than 10 around the time of introduction of PrMT (both $P<0.001$; Fig. 3). However, in both models there was a significant interaction between PrMT and PTI, with the increase in movements of single animals and decrease in movements of ≥10 animals following introduction of PrMT, most evident in PTI1 and 2.

There was no significant effect of PrMT on the slope of the times series for movements of single animals (Fig. 3) suggesting that introduction of PrMT caused a sudden perturbation, with increased rate of movement of single animals, particularly in PTI 1 and 2 areas, and that this increased level persisted relatively unchanged in the available data series. In contrast, there was a significant effect of PrMT on the slope for movement of batches of more than 10 animals. The main effect of this appears to be "compensation" to the immediate effect which occurred with introduction of PrMT, with an increasing trend in PTIs 1 and 2 and a decreasing trend in PTI 4.

Movements between farms via a market:

There was no significant change in the rate of movements of single animals between farms via a market at the time of introduction of PrMT in any PTI. The rate of movement of single animals increased per quarter in the period after introduction ($p=0.047$) and despite evidence of some variation, this was not significantly different between PTIs (Fig. 3). There was also no significant effect of PrMT on either the step change, or slope in any PTI for the relative rate of movements of larger batches (>10) of animals via markets.

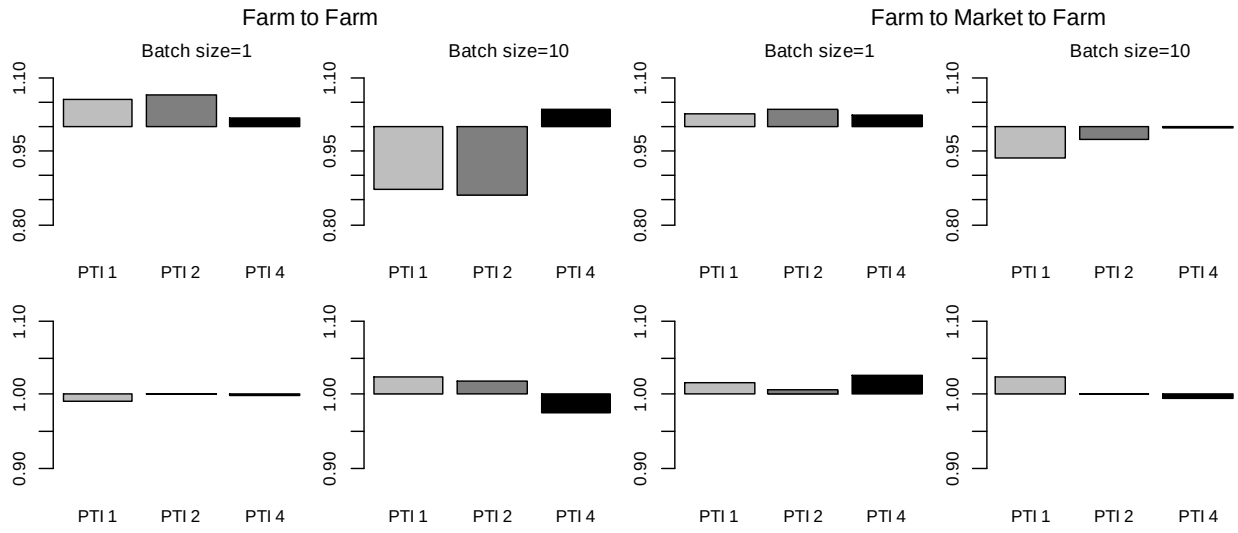

Fig. 3. Relative rates associated with shipments of batch size 1and of batch size ≥10 directly between farms (left plots) or between farms via a market (right plots) for (top) I_{PrMT} and (bottom) $PrMT_{slope}$ for each origin PTI.

DISCUSSION

To date there has been limited investigation of the impact of government animal disease control policies on the behaviour of farmers. This study used a range of approaches to explore farmers' perceived behavioural change and to identify evidence of behavioural change in cattle movement data associated with the introduction of PrMT for bTB in England and Wales. A key finding was that, overall, two-thirds of respondents to the postal questionnaire reported no changes in themselves or others, although only around half of farmers in those areas directly affected by PrMT reported no change. This implies that many farmers were able to incorporate the effects of PrMT into their existing management strategies with little or no (noticeable) change. However, other farms did report changes since introduction of PrMT and there was evidence of an effect of PrMT in the cattle movement data.

Farmers' behavioural response to PrMT might be affected by factors including their beliefs about bTB, the actual and perceived financial (and other) costs associated with testing, their trust in the test and in government control of bTB more generally and their ability to undertake alternative behaviours. Trust in government to control bTB varied between areas, but not between PTI, suggesting that trust may be related to regional or cultural differences rather than a consequence of the presence of bTB *per se*.

Pre-movement testing imposes a financial cost on farmers but this varies, particularly with the number of animals being tested (Anon, 2006a; Bennett, 2009). Such additional costs may encourage farmers to limit the number of movements requiring PrMT and to avoid testing single or few animals at a time, in favour of larger groups of animals in order to reduce the unit cost of testing. There was evidence of a reduction of movement of cattle from farms that must comply with PrMT. The interviews and questionnaires reveal behavioural changes by farmers in PTI 1 and 2 that might reduce the number of off movements, including; finishing cattle for sale direct to slaughter, reducing cattle numbers, undertaking less cattle movement, purchase of bulls rather than hiring, and selling in larger batches. In contrast, farmers in PTI 4 areas reported very few

relevant changes due to PrMT. Farmer reported changes were supported by the movement data, which suggested that at or around the time of introduction of Phase I of PrMT, there was a sudden, but generally small, reduction in the relative rate of movements originating on farms in PTI 1 and 2 and increased rate of movements originating on farms in PTI 4 (with exceptions). Following the initial impact of introduction of PrMT, the rate of movements between farms subsequently decreased over time in most areas, and irrespective of PTI (but greatest in PTIs 1 and 2, again with exceptions). However, as this latter finding occurred over a wide area and in regions not directly affected by PrMT it is likely that other factors may have caused this effect.

The interviewed farmers tended to believe that PrMT was effective and noted that either they, or other farmers, believed that purchasing cattle from high risk areas would be safer following introduction of PrMT. This was corroborated in the questionnaire with around 40% of farmers agreeing that moving cattle will not spread bTB since they are tested prior to moving. In contrast, some interviewed farmers expressed doubts about the efficacy of the tuberculin skin test and this was supported in the questionnaire with a low confidence in the skin test evident in all regions. Farmers with trust in the efficacy of skin testing were more likely to have a favourable attitude to bring cattle onto their farms and it is therefore likely that trust in the skin test might lead toward more risky behaviour, as compared to farmers with low confidence in the skin test. However, the time series analysis found a limited effect of introduction of PrMT on the number of movements from high to low risk areas; if anything, there was a tendency toward a reduction of such movements (except for PTI 2 to 4). It is important to note that most movements occur locally (Christley *et al.*, 2005; Mitchell *et al.*, 2005; Robinson & Christley, 2007) and that PTI categories also cluster in space. Hence the majority of movements occur within a given PTI category and this is likely to reflect the preference for local trade which might offset farmers' willingness to undertake movement from a higher risk areas.

In both the interviews and the questionnaire many farmers suggested that introduction of PrMT would result in movement of larger batches of animals, whereas only few suggested that cattle would be more likely to be traded in smaller batches. In contrast, the time series analysis identified increased movement of single animals and reduced movement of large batches ($\geq$10 animals) directly between farms and little change in movements through markets. The apparent discrepancy between farmer opinion and the time series may be due to a bifurcation in behaviour, with some famers decreasing sale of large batches and (a smaller number) increasing this behaviour. However, other explanations are also possible. The time series results may suggest that many farmers are willing to endure the increased unit cost of testing small groups of animals in order to reduce the risk of detection of a positive animal. This is in keeping with considerable evidence that economic factors are often not the main drivers of farmer behaviour (Gasson, 1973). Such risk-averse behaviour is also suggested by anecdotal reports that some farmers are undertaking PrMT of small numbers of animals prior to routine annual herd tests in order to sell some animals before being exposed to the greater risk of detection of a positive animal (and hence enforced movement restriction) during the herd test. One caveat to these conclusions is that the movement data records the size of batches moved, not the size of the group tested. Hence, farmers may still be testing large numbers of animals (to reduce the unit cost), but moving these in smaller groups (e.g. selling to several, rather than a single, farm), although the rationale for such behaviour change is unclear. Furthermore, testing and moving single animals instead of groups could have a marked effect on the number of single animal movements; a group of 2 becomes 2 distinct movements, a group of 5 becomes 5 distinct movements and so on. Hence the observed results may result from the actions of relatively few farmers. In contrast, inclusion of extra animals in an already large group does not increase the number of large batches and amalgamation of large batches would decrease their number.

There are several limitations to the approaches used here. First, the time series analyses used data aggregated across space and time and this might have led to failure to identify some changes. For example, if PrMT caused divergent responses, with some farmers increasing and others decreasing a particular activity, the composite effect may appear as if there has been no change. Secondly, our analyses have tested the impact of PrMT by assessing the significance of the date of introduction in the model. However, other unmeasured events might have occurred approximately concurrently. We have explored this by considering the impact of PrMT in different PTIs +/- different regions. The finding that several changes are evident in PTI 1 and 2, but not PTI 4, is suggestive of PrMT being responsible for these changes. However the possibility of extraneous, unmeasured influences remains. Thirdly, the analyses consider 2 possible effects of policies on the time series; a step change and a change in linear trend. It is likely that the true nature of the impact of policy changes would be more subtle than this simple assumption (e.g. perhaps causing short term effects only or an initial decrease in a behaviour followed by an increase etc). However, in the absence of strong behaviour-specific hypotheses regarding the nature of the impacts, investigation of more complex effects is difficult and exploration of many possible patterns of effect increases the chance of type I error. Fourthly, we have assumed that all farms are free to move cattle on a given date. This ignores the issue of farms under restriction, e.g. due to bTB. Therefore a reduction in numbers of farms moving animals (particularly in areas of PTI 1 and 2) may change at certain times of the year due to the seasonality of bTB testing. Finally, the time series analyses considered the impact of phase I PrMT only for approximately 1 year following introduction. It is possible that further behavioural adaptation has occurred in response to Phase II and as farmers become more familiar with the effects of PrMT requirements. There was evidence following the 2001 Foot and Mouth outbreak in the UK and the introduction of the 6-day standstill ruling, that adaptation occurred over at least 3 years (Robinson *et al.,* 2007). It is also worth noting that the interview and questionnaire data were collected after the introduction of Phase II PrMT and hence some apparent discrepancies between these data and the time series results may reflect this difference. The response rate to the postal questionnaire, as in many similar studies involving UK farmers (Garforth *et al.,* 2006), was less than ideal and raises the possibility of non-response bias (Dohoo *et al.,* 2003). However, the respondents did not differ greatly from study population in several demographic and farm management variables, although small hobby-farms were under-represented in the respondents. Hence, whilst some caution is needed when interpreting the results of the postal questionnaire, the results are likely to represent the views of a substantial subset of the population.

In conclusion, despite a majority of farmers reporting little or no change in behaviour since the introduction of phase I PrMT the time series analysis provides evidence of changes to cattle movement at the national level. Therefore, the changes in the movement data might reflect behavioural modification by a minority of farmers. Alternatively, the behavioural modifications made by farmers might have been readily incorporated into existing management leading to failure to recall these changes. Taken together the results suggest reduced cattle movement in the areas required to undertake PrMT (i.e. PTI 1 and 2). A potentially important effect of introduction of PrMT might have been increased movement from high risk to low risk areas. Despite evidence that some farmers are now more likely to consider such movements due to a belief that tested animals (with a negative result) will not transmit disease, there is little evidence in the movement data to suggest movements from high to low risk areas are now more common. There is evidence that farmers are now more likely to move single animals and less likely to move large batches, particularly when moving animals directly between farms (rather than via a

market). This behaviour might be an attempt to mitigate the risk of detection of a single animal, but might increase the unit cost of testing.

ACKNOWLEDGEMENTS

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REFERENCES

Anon (2004). Preparing for a new GB strategy on bovine tuberculosis, Defra. p 82.

Anon (2005a). Agriculture in the United Kingdom 2004, Defra, SEERAD, DARDNI and WAG, London.

Anon (2005b). The Tuberculosis (Scotland) Order 2005, Queen's Printer for Scotland.

Anon (2006a). Regulatory Impact Assessment: Pre-Movement Testing in England.

Anon (2006b). The Tuberculosis (England) Order 2006, Queen's Printer of Acts of Parliament

Anon (2006c). The Tuberculosis (Wales) Order 2006, Queen's Printer of Acts of Parliament

Anon (2009). Bovine TB: Pre-movement testing monitoring data, Defra.

Bennett, R.M. (2009). Farm costs associated with premovement tetsting for bovine tuberculosis. Vet. Rec. 164, 77-79.

Biglan, A., Ary, D. and Wagenaar, A.C. (2000). The value of interupted time-series experiments for community intervention research. Prev. Sci. 1, 31-49.

Christley, R.M., Robinson, S.E., Lysons, R. and French, N.P. (2005). Network analysis of cattle movement in Great Britain. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, 234-244.

Dohoo, I., Martin, W. and Stryhn., H. (2003). Veterinary Epidemiologic Research, AVC Inc, Charlottetown.

Garforth, C., McKemey, K., Rehman, T., Tranter, R.B., Cooke, R., Park, J., P., D. and Yates, C.M. (2006) Farmers' attitudes towards techniques for improving oestrus detection in dairy herds in South West England. Livest. Sci. 103, 158-168.

Gasson, R. (1973). Goals and values of farmers. J. Agri. Econ. 24, 521.

Mitchell, A., Bourne, D., Mawdsley, J., Wint, W., Clifton-Hadley, R. and Gilbert, M. (2005). Characteristics of cattle movement in Britain - an analysis of records from the Cattle Tracing System. Anim. Sci. 80, 265-273.

Robinson, S.E. and Christley, R.M. (2007). The contact network resulting from cattle moving through auction markets in Great Britain. Prev. Vet. Med. 80, 21-37.

Robinson, S.E., Everett, M.G. and Christley, R.M. (2007). Recent network evolution increases the potential for large epidemics in the British cattle population. J. R. Soc. Interface 4, 669-674.

AVIAN INFLUENZA

AN ASSESSMENT OF BIOSECURITY PRACTICES AND CONTACT STRUCTURES ON PROFESSIONAL AND HOBBY POULTRY SITES

S. VAN STEENWINKEL*, S. RIBBENS, E. DUCHEYNE, E. GOOSSENS AND J. DEWULF

SUMMARY

This study investigated Belgian commercial and non-commercial poultry sites based on their biosecurity levels and farm movements. Questionnaire data was analyzed using a combination of a linear scoring system, a categorical principal component analysis and a two-step cluster analysis. An extrapolation exercise resulted in an estimate of 230,556 hobby poultry premises in Belgium. Most commercial farms and hatcheries had an acceptable level of standard biosecurity practices, however further enhancements are still possible. In general, the level of biosecurity was lower in hobby poultry flocks. Considerable variation in the movements and in the structure of the networks arising from these movements was found. Commercial and hobby poultry sites were connected, but movements of poultry (products) were found only to occur from commercial to non-commercial sites. Six clusters of poultry sites were differentiated, which were interpreted as very low to very high risk groups, based on the potential of disease introduction and spread.

INTRODUCTION

The probability of disease introduction and spread is determined by a complex combination of determinants, such as the number and density of animals, the type of species or breeds present, the number and type of contacts between flocks, and the sanitary measures that are put in place. To avoid the introduction of diseases into farms and to contain the spread of infections already present, implementation of preventive measures are required. Biosecurity refers to the implementation of such measures. Enhancement of biosecurity is generally agreed to be the best way to minimize the risk of introduction (Boklund et al., 2004; Niemi et al., 2009).

Poultry production is characterized by a huge diversity of production systems, with different scales of production, bird species, measures of biosecurity, production inputs and outputs. Both intensive professional production systems coexist with smallholder hobby poultry sites, with very different characteristics. Yet, both harbour animals that are susceptible to the same diseases. While the scope and impact of biosecurity measures may be obvious for large-scale poultry production, its significance for small poultry-keeping holdings must not be overlooked; either in their own right or as sources of infection for large commercial flocks.

* Sarah Van Steenwinkel, Department of Reproduction, Obstetrics and Herd Health, Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium. E-mail: sarah.vansteenwinkel@ugent.be

Classification of poultry sites, based on the risk for disease introduction and spread is an important step in the development of risk based surveillance strategies, policies and recommendations for farmers, as well as for modeling purposes (Lyytikäinen and Kallio, 2008; Ortiz-Pelaez & Pfeiffer, 2008; Niemi et al., 2009). For instance, the development of generic risk profiles could help policy makers to direct surveillance and early warning systems towards high-risk holdings and promote measures which reduce the farm's risk to disease introduction. In addition, examining characteristics of poultry sites from the point of view of risk for disease introduction and spread provides useful information for stochastic spatial simulation models, which simulate disease outbreaks.

In spite of the importance of biosecurity and contact structures in disease transmission, there is little information available on the biosecurity status of poultry farms (Nespeca et al., 1997; East, 2007). There are several papers which use multivariate analyses to classify livestock farms (Calavas et al., 1998; Solano et al., 2000; Rose & Madec, 2002; Köbrich et al., 2003; Kristensen, 2003; Boklund et al., 2004; Milán et al., 2006; Ribbens et al., 2008; Costard et al., 2009), however, to our knowledge no paper classified all poultry systems.

The objective of this study was to describe the presently applied biosecurity measures and onto/off farm movements of Belgian poultry sites for different flock types, as well as to search for possible links between the commercial poultry sector and hobby poultry sites. Secondly, the aim was to use the output of this biosecurity survey to investigate whether poultry sites could be categorized into risk groups for disease introduction and spread.

This study served as a supportive tool for the development of a stochastic spatial model to simulate highly pathogenic avian influenza (HPAI) outbreaks in Belgium. Therefore, an epidemiological approach towards the investigation of the level of biosecurity for HPAI has been pursued. However, the majority of poultry pathogens are transmitted via the faeco-oral route, and thus the outcome of this study can be interpreted as a generic risk-classification of disease introduction and spread.

MATERIALS AND METHODS

Selection of poultry premises

Our target population comprised both commercial and hobby poultry sites in Belgium. Commercial poultry holdings were defined as premises were more than 200 birds are kept at the same location.

A list of all Belgian commercial poultry operations was available from the identification and registration database of animals (SANITEL-Poultry). This database provides information on the type of operation, the maximum capacity for each bird species kept and the geographical coordinates. Eighty commercial poultry operations were randomly selected from the SANITEL database, corresponding to a sampling fraction of 4.4%. Selection was done by a stratified proportional allocation, accounting for: (1) animal species (chicken, duck, pigeon, pheasant, turkey, quail, guinea fowl and partridge), (2) type of operation (rearing, multiplier hens, layer hens, broilers, selection and show) and (3) geographical spread. In addition, all existing recognized hatcheries, 32 in total, were selected.

For smallholder poultry sites (<200 birds) no official database exist. Yet, in early 2006 all communities of Belgium were asked – as a precautionary measure in light of the HPAI threat –

to identify all inhabitants that were having hobby birds. Two hundred and eight different communities which surrounded the selected commercial poultry operations and hatcheries were contacted and asked whether they still had this hobby poultry inventory at their disposal and were willing to give access to this database. Seventy communities (34%) responded positively, which provided a database of 28,288 hobby poultry keepers. Consequently, all addresses were geocoded and 2000 hobby poultry holdings, lying within a 3 km radius of a commercial poultry operation, were randomly selected.

Both random selections (commercial and hobby flocks) were performed using a computer-generated list (Survey Toolbox, Cameron, 1999).

Mapping hobby poultry premises

Based on the database of 28,288 hobby poultry keepers of 70 communities, an extrapolation of the total number of hobby poultry premises for the whole of Belgium was made. Therefore, the relationship between the human population density and the number of hobby poultry premises was determined, using linear regression analysis (SPSS, 15.0). In addition, the mean distance between hobby poultry premises and the nearest neighbour was calculated using R-2.2.1. Based on the former regression formula, the estimated amount of hobby poultry premises for each community could be randomly allocated, taking into account the mean distance to the nearest neighbour. These point locations were plotted using a geographical information system and were converted into a continuous raster using the quadratic kernel density estimation function (ArcMap 9.3, ESRI, Redlands, CA, USA). This way, the density plots expressed the number of hobby poultry premises per square kilometre. Bandwidth selection for the kernel smoothing was 7.5 kilometres. Output cell size was 900 metres. We defined 3 density classes based on Jenks Natural Breaks Classification Method (ArcMap 9.3, ESRI FAQ).

Administration of the questionnaire

The 80 selected commercial poultry farms were subjected to an interview-based questionnaire by telephone. Additionally, a questionnaire was administered to the 32 hatcheries by regular mail. The 2000 hobby poultry holdings were contacted by regular mail and asked to fill out an online questionnaire. The surveys were conducted between September and December 2008. All telephone interviews, including the coding and typing of the information, were conducted by the first author.

To increase the response rate, an incentive was given: each participant of the hobby poultry holdings received a voucher worth €2 when buying a bag of 25kg poultry feed. In addition, 50 gift vouchers worth €10 were put up for raffle. All commercial poultry farms that participated were offered a free yearly subscription to a professional poultry magazine.

Questionnaire design

For each type of poultry premise an adapted questionnaire was designed, both in Dutch and French. A preliminary draft was pre-tested on 8 hobby poultry farmers, 1 professional poultry farm and 3 experts. The questionnaire was a standardised closed and semi-closed questionnaire, consisting of 9 pages, divided into four parts:

1. *General data:* Identification, type of farm, capacity, bird species, presence of other animals.
2. *Infrastructure:* presence of farm fences, boot dips, sanitary transition zone, paved place of (dis-)charge, free-range and housing secure against wild birds.

3. *Hygiene:* cleaning and disinfection, all in all out, pest control, accessibility of wild birds to fresh litter and manure, accessibility of rodents and wild birds to feed storage, feeding outside, type of drinking and cleaning water, allocation of waste water, dead bird disposal, employees and visitors.
4. *Contacts/movements:* this part provided a table of all kinds of external contacts that could be applicable to the poultry premise (poultry supply and discharge, suppliers of food and litter, veterinarians, pest control, disinfection, manure and dead bird removal, advisors and controlees, hobby poultry keepers, local vendors, employees). The participant was asked to select those external contacts that were applicable to his situation and to complete the table with the frequency for each type of contact, how many persons were involved, whether they were entering the bird compartments and whether they wear company clothing.

The questionnaire (in Dutch or French) can be obtained upon request.

Data processing

All information was coded numerically to assist analysis, entered into a database worksheet program (Microsoft Excel, 2007) and recoded into categorical data (nominal and ordinal level) for further analysis. The data was subsequently exported for analysis into SPSS 15.0 (SPSS Inc., Chicago, IL).

Data analysis

Creating a biosecurity scoring system:

First, variables which were assumed to have a similar influence on the potential risk of introduction of contagious disease on the farm were combined into a single variable, thereby creating an index of poultry farm biosecurity. To this end, all variables were first coded into 1 (biosecurity measure present) or 0 (absent). Then, for each biosecurity category (made up of several measures) these points were summed up, creating a score. Finally, each score was converted to a scale from 1 to 10, except for one category ('sensitivity of birds', see further). Thus, a higher score implied a 'better' biosecurity level for the category concerned.

The following categories were considered:

1. *Susceptibility of birds:* according to their susceptibility to HPAI, bird species were grouped into two groups (Alexander, 2007; Sharkey et al., 2008): highly susceptible and low susceptible species. Chickens, turkeys, pheasants, partridges and mixed bird species were categorised in the susceptible group. Ducks, geese, pigeons and ostriches were categorised in the low susceptible group. The hatching eggs of hatcheries were also categorized in the low susceptible group (Ligon, 2005; Alexander, 2007, expert opinion).

2. *Other animals:* this category groups variables concerning the presence of other animals such as pets, production animals, hobby poultry and rodents.

3. *Wild birds:* included all measures that prevent (in)direct contacts with wild birds, such as housing secure against wild birds, wild birds having no access to fresh litter/manure/food storage and no surface water used.

4. *Hygiene of infrastructure:* This referred to the elements that are fixed within the farm or belong to the daily management (presence of boot dips, sanitary transition zone, cleaning and disinfection, dead bird disposal, etc.).

5. *Hygiene of persons:* this included measures reducing the number and intensity of direct contacts between the birds and external persons (no visitors allowed in poultry houses/free-range, company clothing provided to all kinds of (professional) visitors).

6. *Hygiene of transport:* this class grouped variables giving information on the safe transportation of poultry or poultry products (truck no contact with other poultry farms in one day, single purpose of transportation truck, company transport material when selling to local traders or hobby poultry keepers).

The information on the external contact types and frequencies, were split into 'onto-farm' and 'off-farm' movements, followed by a subdivision into 'living poultry', 'poultry products' (eggs and manure) and 'fomites' (inanimate objects which are able of transmitting infectious organisms). The monthly frequencies were coded into 'no', 'low', 'medium' and 'high' movement frequencies. In this way, a scoring was created on a scale of 1 to 4, with a higher score implying lower movement frequencies (more secure against infectious agents).

Categorical principle component analysis (CATPCA):

To analyze the categorical data, the CATPCA was used (SPSS 15.0). The optimal scaling process transforms the original, categorical variables into metric variables, by means of monotonic optimal least squares transformations. The results of a CATPCA can be represented in a graphical display. The component loadings are correlations between the variables and the components (dimensions), and they give coordinates to represent the variables as vectors in the component space. The squared distance of the vector tip to the origin corresponds to the percentage of variance accounted for (PVAF). If the VAF for two variables is adequate, a small angle between the two vectors in the space indicates a large correlation between the two variables. The theory of CATPCA is described, among others, in Meuleman et al. (2004) and Linting at al. (2007).

All scored variables were given an ordinal scaling level. Three supplementary variables were included and analyzed multiple nominally (i.e. as grouping variables) (Linting et al., 2007): type of farm, capacity of the farm and commercial poultry-farm density of the region. The quantifications of a supplementary variable have no influence on the actual analysis but are computed afterwards to establish its relationship with the solution obtained. Type of farm was presented by seven categories: broiler, layer, breeder, multiplier, multiple-category farms, hatcheries and hobby farms. The capacity of the farm was divided into tree sizes: small, medium and large. As cut-off points, the 33.3 and 66.6 percentile of the maximum capacity of all commercial poultry operations from the SANITEL database, were used. All hobby poultry sites were considered small. The poultry-farm density of the region was measured using three categories: sparsely populated poultry are (SPPA), medium populated poultry area (MDPA) and densely populated poultry area (DPPA). To this end, the point locations were converted into a continuous raster using the quadratic kernel density estimation function (Spatial Analyst, ArcMap 9.3, ESRI, Redlands, CA, USA). This way, the density plots expressed the number of commercial poultry sites per square kilometre. Bandwidth selection for the kernel smoothing was 7.5 kilometres. Output cell size was 900 metres. We defined the 3 density classes based on the Jenks Natural Breaks Classification Method (ArcMap 9.3, ESRI FAQ).

In our analyses, the number of dimensions was set at a default value of 2, which consequently allowed for two-dimensional graphic representation. The reduction to two dimensions was allowed for since the sum of VAF, which is a measure of model fit, was largely sufficient. Because 44% of the respondents had one or more missing values on these 12 scored

variables, a missing data treatment strategy other than deleting all cases with missing data was required. It was decided to use the straightforward CATPCA option of imputing the modal category for each of the variables (Ferrari and Anonni, 2005). Finally, the variable principal normalization option was used, which optimizes the association between variables.

Two-step cluster analysis (TSCA):

The object scores obtained from the CATPCA solutions were then included in a two-step cluster analysis (TSCA, SPSS 15.0), to find clusters of poultry systems with a similar biosecurity level and onto/off farm movement frequencies (Ribbens et al., 2008).

RESULTS

Mapping hobby poultry premises:

The relationship between the population density (inhabitants/km²) and the number of hobby poultry premises per km² is described in the following regression function (Eq. 1):

$$\text{No. hobby poultry premises}_i \text{ per km}^2 = 495.75 - 0.21 * \text{population density}_i \quad (1)$$

with $_i$ = community i

Indicating a decreasing number of hobby poultry keepers with an increasing population density. The mean distance between a hobby poultry premise and the nearest neighbour was 91.64m. An extrapolation for the whole of Belgium resulted in an estimate of 230,556 hobby poultry premises, with an estimated mean density of 3.75 per km² (min. 0, max. 21.55 and st.dev. 4.27). Figure 1 shows the estimated kernel density of the number of hobby premises per km², together with the point location of all commercial poultry farms and hatcheries.

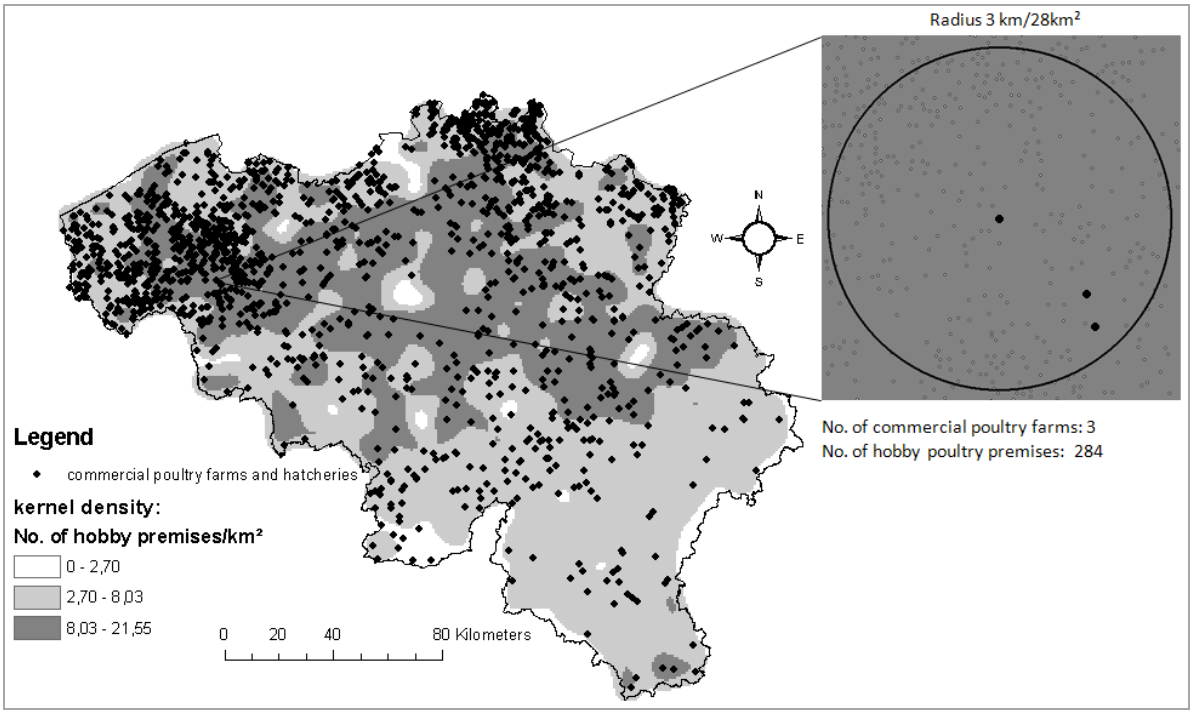

Fig. 1 Estimated kernel density plot of the number of hobby poultry premises per km², together with the point location of all commercial poultry farms and hatcheries, Belgium (2008)

General hobby poultry flock characteristics are shown in Table 1. Poultry species that were kept most frequently are chickens (85%), pigeons (12%), geese (10%) and ducks (9%). Eighty-six percent of the hobby flocks with chickens had fewer than 10 birds. Pigeon flocks were generally larger, with a median flock size of 25 birds.

Table 1. Flock characteristics of Belgian hobby poultry premises.

	% that keep specified species	Median [min.- max.]	Percentage keeping this number of birds					
			[1-10]	[10-20]	[20-30]	[30-50]	[50-100]	>100
Chicken	85.8	5 [1-100]	86.2	10.8	1.9	0.8	0.2	0.0
Pigeon	12.1	25 [1-200]	34.3	12.8	7.2	6.4	23.1	9.2
Goose	10.3	3 [1-80]	95.5	3.5	0.7	0.2	0.1	0.0
Duck	9.2	3 [1-100]	90.2	7.1	1.6	0.5	0.3	0.0
Other[a]	8.7	6 [1-260]	63.3	18.4	7.0	3.4	4.3	1.3
Pheasant	2.4	3 [1-110]	90.6	7.0	1.3	0.6	0.3	0.1
Peacock	2.0	2 [1-100]	95.6	3.2	0.7	0.4	0.2	0.0
Quail	1.5	3 [1-182]	90.5	4.7	1.9	0.5	0.5	0.2
Guinea fowl	1.1	3 [1-200]	70.5	4.2	3.6	4.5	10.4	1.3
Turkey	0.9	2 [1-48]	97.1	1.2	1.2	0.0	0.0	0.0
Running bird	0.5	2 [1-54]	94.6	3.4	0.7	0.0	0.7	0.0
Partridge	0.2	3 [1-62]	86.5	7.7	0.0	0.0	1.9	0.0
Swan	0.2	2 [1-48]	95.3	3.1	0.0	0.0	0.0	0.0
Total		6 [1-500]	72.7	13.4	4.3	2.4	4.0	1.7

[a] Such as birds of prey and other aviary birds kept outside the house

Survey response: Of the 80 selected commercial poultry farms, 78 registered addresses and telephone numbers were valid; responses were received from 48 (61.5%) of them, but only 37 (47.4%) surveys were completed because 11 farms had ceased production. For the hatcheries a response rate of 59.4% was obtained (19/32). The questionnaires for hobby poultry holdings were mailed to 2000 addresses, of which 1905 were valid. Of these 373 responded. Eighty seven no longer raised poultry, resulting in 286 (15.0%) complete questionnaires. In fig. 2 all participants are geographically represented.

Of the total population of commercial poultry farms today, 56% consist of broilers, 19% of layers, 8% of multipliers, 10% of rearing farms, 1% of show and 5% of poultry farms with multiple category activities. Similar proportions were obtained within the respondents: 51% broilers, 11% layers, 11% multipliers, 8% rearing farms and 19% of poultry farms with multiple category activities. Most poultry farms are situated in the Northern part of the country (79%), with the highest proportion in the provinces West-Flanders (30%) and Antwerp (21%). The responding farms showed approximately the same distribution throughout the country. General characteristics of the studied population sample, regarding the bird species kept or hatched, size of the farm and commercial poultry population density of the area, are summarized in table 2.

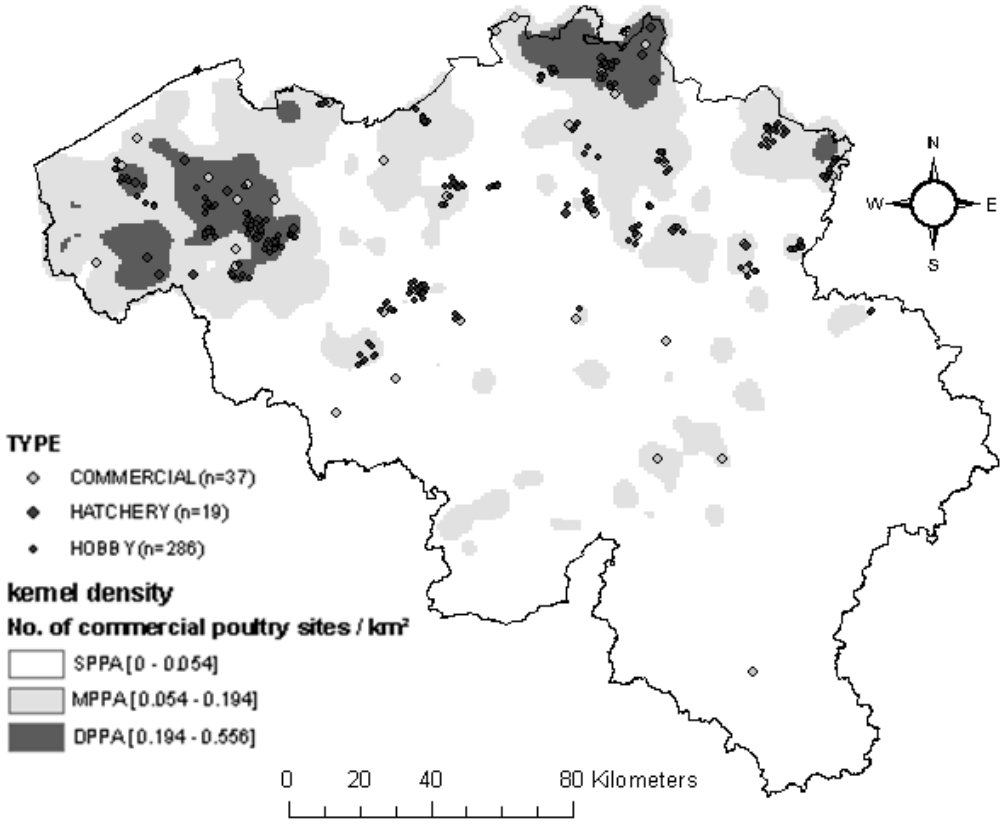

Fig. 2 Geographical presentation of participants in the 2008 biosecurity survey, Belgium. In the background, the kernel density plot (bandwidth 7.5 km; raster size 0.9 km) of all commercial poultry sites is given.

Table 2. General characteristics of the studied population sample, Belgium 2008.

		Commercial farms (n=37)	Hatcheries (n=19)	Hobby poultry premises (n=286)
Bird species	pigeon			5%
	duck			1%
	pheasant	3%	11%	
	goose			1%
	turkey	3%		
	chicken	68%	68%	62%
	ostrich		11%	
	partridge		5%	
	multiple species	27%	5%	31%
Size[a]	Small	38%	32%	100%
	Medium	43%	26%	
	Large	19%	42%	
Density[b]	SPPA	38%	5%	26%
	MPPA	41%	21%	39%
	DPPA	22%	74%	35%

[a] 33.3 and 66.6 percentile of the maximum capacity of all commercial poultry operations from the SANITEL database.
[b] based on a spatial Kernel density estimate of the number of commercial poultry sites per square kilometre (bandwidth 7.5 km; raster size 0.9 km, density classes based on Jenks natural breaks)

Biosecurity:

All commercial poultry farms in the sample had highly susceptible birds for HPAI present at the farm. Hatcheries did not, as hatching eggs are not expected to be able to infect one day old chickens. Ninety-four percent of the hobby poultry premises also had highly susceptible bird species for HPAI. Table 3 shows the percentage of farms executing several biosecurity measures, for the three types of poultry operations, together with the mean biosecurity score for each category.

Table 3. Percentage of farms executing several biosecurity measures, together with the mean biosecurity-score per category (scoring index on 10 points; the higher the score, the better).

Cat.	Biosecurity components	COMM (n=37)	HAT (n=19)	HOB (n=286)
Other Animals	no other farm animals present	32	84	78
	no pets present	46	63	40
	no hobby poultry present (hatchery= live poultry)	95	32	/[a]
	no contacts between poultry & other animals	91	100	47
	permanent rodent control	84	95	72
	stored feed is not accessible to rodents	89	/	90
	Score (mean; st.dev.)	**(7.29;1.60)**	**(7.95;1.43)**	**(7.07;1.56)**
Wild Birds	free range/compartments not accessible to wild birds	76	100	19
	used cleaning water is not drained outside (open)	70	83	/
	wild birds have no access to stored fresh litter	84	/	76
	permanent wild bird control (chasing, shooting)	0	16	6
	wild birds have no access to stored manure	57	/	49
	wild birds have no access to stored food	97	/	92
	No feeding outside & no access to it by wild birds	92	/	32
	surface water is not used for drinking	100	/	83
	surface water is not used for cleaning	100	89	100
	Score (mean; st.dev.)	**(7.88;1.26)**	**(8.89;0.758)**	**(6.34;1.73)**
Hygiene infrastructure	fence present around the farm yard perimeter	68	68	0
	boot dips present	86	84	0
	sanitary transition zone(s) present	92	74	0
	presence of paved place of (dis)charge	97	100	0
	no multiple ages are kept together	84	58	0
	no partial depopulation	62		/
	regular cleaning & disinfection	95	100	50
	proper cleaning & disinfection of egg containers	89	89	/
	proper disposal of dead birds	57	68	27
	staff no contact with other poultry farms/poultry at home	92	63	/
	Score (mean; st.dev.)	**(8.28;1.05)**	**(8.00;1.63)**	**(3.75;0.74)**
Hygiene persons	visitors no access to poultry compartments	81	63	0
	good hygiene[b] of supply teams	65	95	/
	good hygiene of discharge teams	65	100	/
	good hygiene of professionals	76	63	/
	good hygiene of control agencies	62	58	/
	Score (mean; st.dev.)	**(8.64;0.10)**	**(8.81;0.98)**	**(9.00.0.00)**
Hygiene Transport	transp. vehicles do not visit more than1 farm/day	56	47	/
	transp. vehicles are not used for double purpose (poultry& products)	90	50	/
	poultry(products) not sold to several companies	71	0	/
	hygienic trade[c] of poultry/products to traders	81	100	92[d]
	hygienic trade of poultry/products to persons	78	100	100
	Score (mean; st.dev.)	**(8.72;1.27)**	**(7.21;1.31)**	**(9.92;0.27)**

COMM = commercial poultry farms; HAT = hatcheries; HOB = hobby poultry premises)

[a] not applicable

[b] wearing company clothing when entering poultry

[c] trader/private person does not use own transport material

[d] no selling/buying at bird shows

Several contacts between commercial farms and both poultry traders and private persons were identified: 46% traded live poultry (products) with local traders and 38% traded live poultry (products) with private persons. Also 16% of the hatcheries traded with local traders and even 58% traded with private persons (hatching eggs or one day old chickens). Eleven and nineteen percent of respondents from hatcheries and commercial poultry farms respectively stated that they visited bird shows on average once a year, but without selling or purchasing birds. Twenty-one percent of the hobby poultry keepers visited bird shows more often, on average 6.28 times/year and 8% also sold or purchased birds at these bird shows.

Hobby poultry keepers ranked egg production and processing kitchen waste highest as the reason for keeping birds. The majority of the hobby poultry keepers (77%) intended to apply preventive measures (creating a covered and fenced outdoor scavenging area or to put all poultry indoors) when asked by the government.

Movements:

Figure 3 shows the contact structure between commercial poultry farms, hatcheries, traders and private persons, arising from the trade of live poultry or (hatching) eggs. For each contact, the movement direction and the average monthly frequency is shown. In addition, extra movements occurred resulting from farm visits by veterinarians, feed and litter suppliers, vaccinators, rodent control teams, manure removal trucks, cleaning and disinfection teams, dead bird/rotten eggs disposal trucks, control agencies, repairers and advisors. Of these, a summary is provided in table 4. Multiple category farms showed on average the highest total movement frequencies (24.90/mo.), mainly resulting from the high frequency of off farm trade of live poultry. Hatcheries had on average 20.16 onto and off farm movements per month, and showed on average the largest frequencies of monthly professional visitors (14.38/mo.). The majority of hobby poultry sites did not have off farm movements, however exceptions did occur.

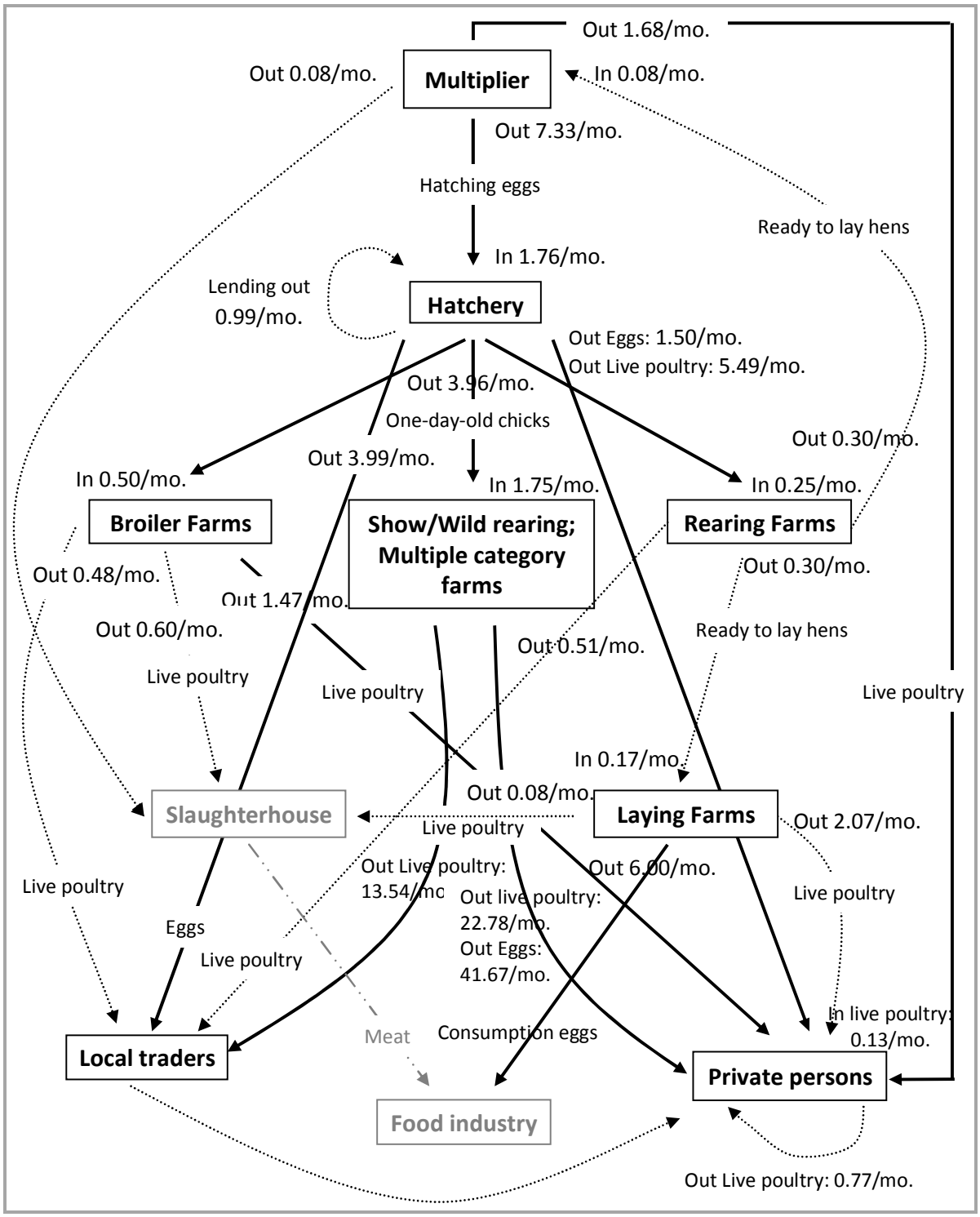

Fig. 3 Contact structure between commercial poultry farms, hatcheries, local traders and private persons, arising from the trade of live poultry or (hatching) eggs. For each contact, the movement direction and the average monthly frequency is shown. Survey response, 2008.

Table 4. Mean number of monthly contacts on a poultry site, attributable to onto and off farm movements of live poultry, (hatching) eggs and professional visitors, 2008.

	Onto poultry/ hatching eggs	Off poultry	Off eggs	Professionals	TOTAL (onto&off)
	Mean freq./mo. [min., median, max.]				
Multiplier	0.08 [0.08, 0.08, 0.08]	0.48 [0.08, 0.08, 1.67]	7.33 [6.00, 8.00, 8.00]	6.29 [1.75, 7.63, 8.17]	12.33 [3.41, 14.79, 16.33]
Hatchery	1.76 [0.08, 1.00, 4.33]	5.98 [0.42, 2.92, 20.83]	3.2 [0.17, 1.50, 13.33]	14.38 [0.13, 10.96, 50.71]	20.16 [1.46, 19.33, 54.54]
Rearing	0.25 [0.21, 0.21, 0.33]	0.47 [0.21, 0.21, 1.00]		5.31 [1.33, 6.29, 8.29]	6.03 [2.67, 6.71, 8.71]
Broiler	0.5 [0.25, 0.54, 0.58]	1.02 [0.25, 0.58, 2.75]		7.54 [4.00, 6.18, 13.92]	9.03 [5.50, 8.75, 15.42]
Layer	0.17 [0.08, 0.08, 0.33]	0.78 [0.08, 0.08, 2.17]	6.00 [4.00, 6.00, 8.00]	10.84 [4.62, 9.46, 19.83]	17.55 [9.08, 15.52, 30.08]
Multiple category	1.75 [0.17, 1.75, 3.33]	23.85 [1.67, 12.50, 68.75]	41.67 /	4.81 [1.41, 3.96, 9.08]	24.9 [2.17, 6.58, 121.33]
Hobby poultry	0.13 [0.01, 0.03, 4.33]	0.77 [0.02, 0.25, 4.33]		1.56 [0.00, 0.08, 45.51]	1.96 [0.01, 0.17, 45.56]

The survey also examined the distances travelled by hobby poultry keepers to purchase live poultry or (hatching) eggs from commercial poultry farms and hatcheries. The percentage of hobby poultry keepers travelling certain distances were the following: 6.38% [<1km], 40.43% [2-5km], 10.64% [6-10km], 27.66% [11-20km], 8.51% [21-30km], 2.13% [31-50] and 4.26% [> 50km].

Categorical principle component analysis and two-step cluster analysis:

The result of a two-dimensional solution of the CATPCA explained 56.63% of the variance of the scores of the 342 respondents on the 12 variables. The percentage of variance accounted for (PVAF) in the first dimension (40.30%) was more than two times the PVAF in the second dimension (16.32%). Figure 4 shows the plot of component loadings, together with the centroid coordinates of the multiple nominal category points. The vectors (lines) are relatively long (always between -1 and 1), indicating that the first two dimensions account for most of the variance of all of the quantified variables.

The vector of a variable, points in the direction of the highest category of the variable, in this case indicating a higher level of biosecurity or lower onto/off farm movement frequencies. The active variables approximately formed 4 groups (Fig. 4). The orientation of the vectors for the variables in the first and the third groups was approximately the same, but the vectors pointed in opposite directions, indicating a strong negative relationship between these groups of variables. The same conclusions could be made for the variables in the second and the fourth group. Examining category points for the variable size, we saw that the first dimension revealed the contrast between small and large poultry farms, whereby the later showed high frequencies

of onto and off farms movements and a high level of infrastructural hygiene relative to the first. The distinction between medium and large farms was made by dimension 2.

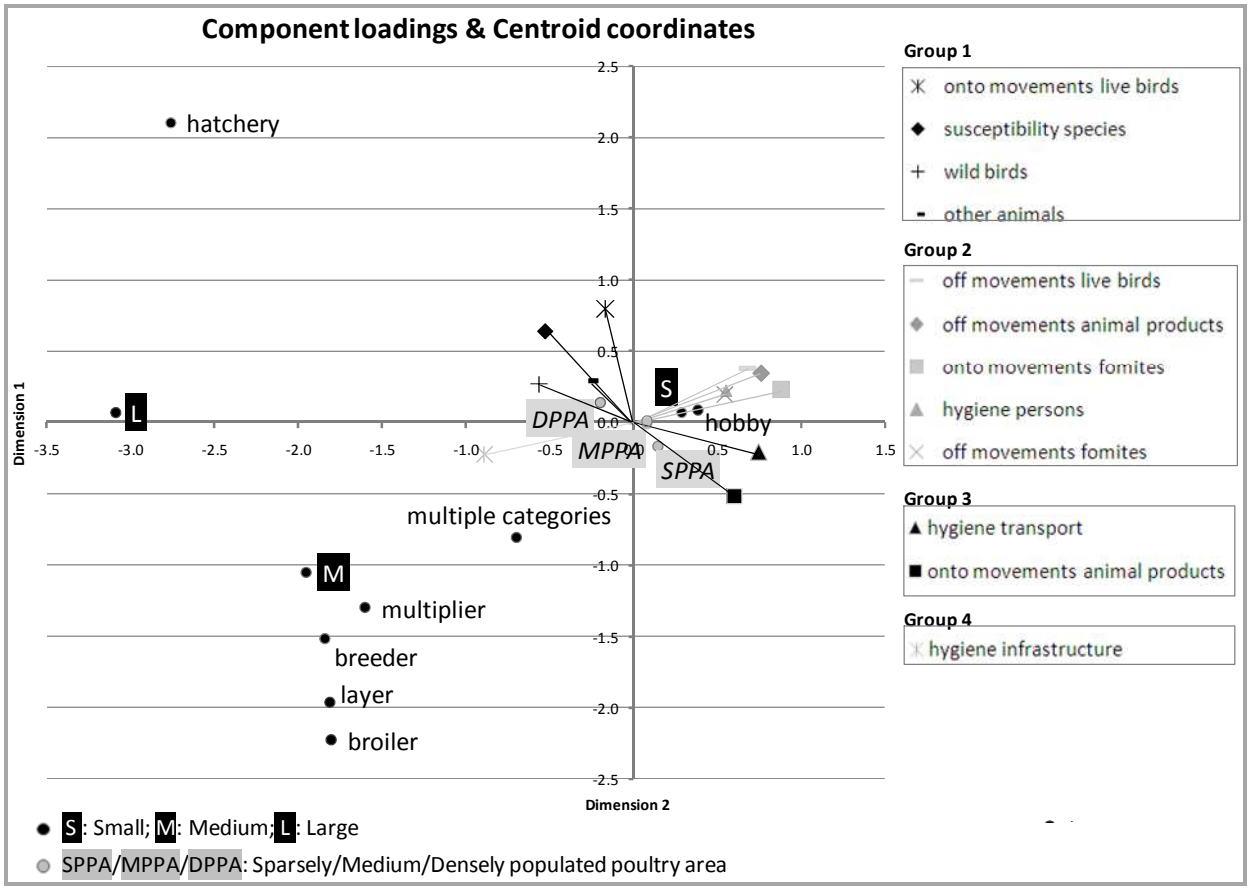

Fig. 4 Biplot of component loadings for the active variables and multiple nominal category points, CATPCA analysis, survey 2008

The object scores obtained from the CATPCA solution, together with the solutions of two consecutive Two-Step cluster Analysis (TSCA), are presented in fig. 5. A first TSCA revealed 3 clusters in the whole population. Because there was a larger variation within the sample of the commercial poultry farms and hatcheries than in the sample of the hobby poultry premises, one TSCA was not able to reveal subgroups within these 3 systems. Therefore, the object scores of the 3 different clusters were subjected to a second TSCA. This extra analysis reveals 2 sub-clusters within the former clusters.

1. *Very low risk group – Cluster 1A (n=14):* This cluster consisted of hobby poultry premises (71%) and small to medium sized hatcheries (29%). These sites were characterized by having no susceptible birds for the HPAI virus, a high level of confinement of birds against other animals, birds and visitors, but rather a low level of infrastructural hygiene. They were also characterized by very little onto and off farm movements.

2. *Low risk group – Cluster 1B (n=12):* All sites in cluster 1B were hatcheries, predominantly being medium to large sized and mainly situated in densely populated poultry areas. They did not have susceptible birds at the site and had a high level of biosecurity both on confinement as infrastructural hygiene. However, they generally had a relative low score on

hygienic transportation and were characterized by relative high frequencies of onto farm movements of animal products and off farm movements of living birds.

3. *Rather low risk group – Cluster 3B (n=203):* This cluster was totally made of small hobby poultry premises that had susceptible birds, with a very poor confinement and low infrastructural hygiene. However, they had almost no onto and off farm movements.

4. *Rather high risk group – Cluster 3A (n=78):* This cluster was predominantly made of small hobby poultry premises (94%) and small commercial poultry farms with multiple categories (6%). The majority of these sites had susceptible birds, with poor confinement, a low infrastructural hygiene and low onto and off farm movements of living birds and fomites.

5. *High risk group – Cluster 2A (n=19):* This cluster was predominantly made of small to medium commercial poultry farms (84%) and hatcheries (16%), with mainly susceptible birds, medium confinement and good infrastructural hygiene. These sites had high frequencies of onto farm movements of fomites and medium high frequencies of off farm movements of living birds and animal products.

6. *Very high risk group – Cluster 2B (n=16):* These sites were all commercial poultry farms, mainly broiler farms (75%). All had susceptible birds, with medium confinement, but scored a bit lower on the biosecurity level towards infrastructure and visiting persons in comparison with cluster 2A. They also exerted high frequencies of onto and off farm movements of living birds and fomites.

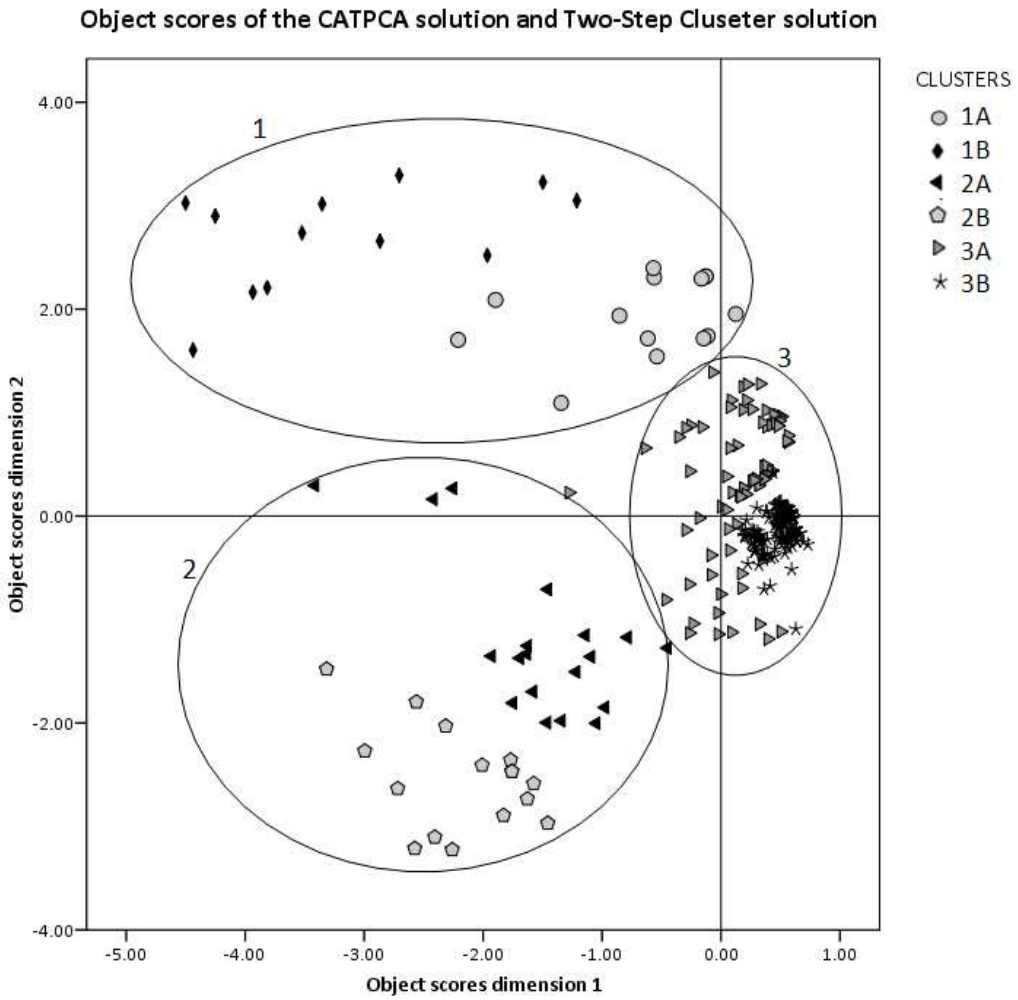

Fig. 5 Object scores of the CATPCA solution and two-step cluster solution

DISCUSSION

Designing a study from which conclusions could be drawn on the interactions between commercial and non-commercial poultry populations, posed a unique challenge, since registers of hobby poultry premises in Belgium, as in most countries, are non-existent. The results of this study made it possible to gain a first insight into the population density, characteristics and practices of hobby poultry sites. Following the described procedure, a total of 230,556 hobby poultry premises in Belgium was estimated. This means that around 5% of the Belgian households (4,569,519 anno 2008) keep hobby poultry at home. The density of hobby poultry premises around commercial operations was high, with the most dense areas going from 8 premises, up to 22 premises per km². However, it has to be stressed that for the extrapolation procedure assumptions had to be made and therefore the outcome should only be interpreted as an indication. These results showed a geographical connection between commercial and non-commercial poultry farms. A study of contacts between commercial and non-commercial poultry farms in Switzerland found similar results, with high density areas with more than 8 poultry sites per km² (Fiebig et al., 2009).

The stratified selection of the commercial farms led to a good representativeness of the farms within each category, bird species and region. The response rates of 61.5% for

commercial poultry farms and 59.4% for hatcheries exceed other similar surveys (Hurnik et al., 1994; Fiebig et al., 2009). The response rate of 19.6% of hobby poultry keepers was rather low.

This study used two complementary approaches to deal with the problem of large numbers of independent variables (35 variables were used to evaluate biosecurity in this study) (Dohoo et al., 1997). First, variables linked to the same kind of risk were combined. In this, it was decided to put 'hatching eggs' in the 'low susceptible species' group. Although HPAI virus may contaminate the surfaces of hatching eggs, it was assumed that the length of the hatching process and strict hygiene measures ensure that the virus does not survive the hatching process and therefore cannot act as a source of infection for the one-day-old chick (expert opinion).

The assumption that all potential biosecurity measures are equally weighted in the scoring system (1/0 each variable) could be challenged, as some measures might play a more prominent role in reducing the risk for disease introduction and spread than other measures. However, for the vast majority of the different biosecurity measures suggested at present no evidence-based quantitative estimate is available of how much they contribute to reducing transmission risks (Hagenaars, 2008). Therefore this study aimed at creating a linear scoring system whereby poultry sites can be compared relative to each other, rather than creating a quantitative scoring system.

The level of biosecurity was, not surprisingly, associated with being a commercial poultry site or not. The extensive scale of hobby poultry facilities does not require the implementation of e.g. boot dips, sanitary transition zone and paved place of discharge. But their low confinement against the outdoor environment facilitates vector's access to domestic poultry and thus the risk of disease dissemination. It is sometimes stated that smaller conventional sites do not have a great motivation to implement preventive measures, because the costs would be relatively small if a new pathogen were introduced (Boklund et al., 2004). However, this study explored the behavioural intention of hobby poultry sites to mandatory measures (creating a covered and fenced outdoor scavenging area or to put all poultry indoors) and showed that a positive attitude towards preventive measures did exist.

Larger facilities are often assumed to implement more advanced biosecurity measures, but the intensity of their operations also poses higher risks for infection and pathogen propagation. Although Belgian commercial poultry farms and hatcheries had in general an acceptable level of adoption of standard biosecurity practices, further enhancement of their preventive measures is still possible. For example, a high proportion of the hatcheries (68%) also had live poultry production activities at the hatchery site. In addition, 53% of them visited two or more farms per day with one transportation vehicle and 50% even used one transportation vehicle for double purpose. The small amount of hatcheries purchasing hatching eggs from multiplier farms and selling one-day old chicks to broiler and rearing farms, can act as a bridge between otherwise separate sectors of the industry. When hatcheries also have live poultry production activities (susceptible species) on site, this source of pathogen transmission (through transportation) might become even more important. On top of this: 37% of the hatcheries had staff that had contact with other poultry farms or had poultry at home, which is something that should be avoided. In the same perspective, multi-species sites or multiple-categories sites are also at higher risk. One potentially underutilized practice in commercial poultry farms appeared to be the provision of company clothing when supply or discharge teams and control agencies enter their facilities. Since common service providers routinely contact different classes of farms over wide areas and many pathogens, including AI virus, may survive for a moderate time at ambient temperatures in

organic material, it is possible that infection could be disseminated over large distances by the movement of service providers.

This study identified considerable variation in the movements and in the structure of the networks arising from these movements. Movement frequencies were higher at commercial farms compared to non-commercial farms. Results showed that multiple category farming systems had the highest total movement frequencies. Hatcheries also had high total movement frequencies. Monthly frequencies of professional visits often exceeded those of poultry and egg movements. Such contacts may be a commonly overlooked means of disease transmission among facilities and provide evidence that increased biosecurity awareness is essential.

Although hobby poultry sites practiced less biosecurity measures in general, they moved birds very infrequently. Commercial and non-commercial poultry sites were connected, but movements of poultry and eggs were found only to occur from commercial to non-commercial farms and not in the other direction. Yet, hobby poultry keepers were personally purchasing the poultry and eggs on the commercial poultry site and by doing so they might pose a risk of indirect disease transmission. Further connections were found through visiting the same bird shows. These results were comparable to the findings of the Swiss study done by Fiebig et al. (2009). Thus, the common assumption of a closed circuit of the commercial poultry production without any connections to hobby farms does not entirely hold true (Bavinck et al., 2009).

An important result of this study was the identification and characterisation of the different clusters of farms existing in Belgium. The practices investigated were assumed to have a potential influence on the introduction and spread of contagious diseases. Therefore the resulting different clusters exhibit differences which may impact on disease spread and control. The results of this study may be useful for mathematical models of pathogen transmission between farms and aid the development of surveillance programs and tailored recommendations towards farmers.

CONCLUSIONS

In conclusion, this study provided a description of biosecurity practices and onto/off farm movements in commercial and non-commercial poultry sites of Belgium. It has also been shown that high densities of hobby poultry sites coexist with commercial poultry farms and that links between the commercial poultry sector and hobby poultry sites do exist. This might pose a risk for the spread of infectious agents between these two compartments. Therefore all kinds of poultry sites, irrespective of being commercial or not, should be counselled with tailored recommendations and used in models to simulate poultry disease spread. Six groups of poultry sites were differentiated and risk-classified according to their risk of disease introduction and spread.

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REFERENCES

Alexander, D.J. (2007). An overview of the epidemiology of avian influenza. Vaccine 25, 5637-5644.

Bavinck, V., Bouma, A., Van Boven, M., Bos, M.E.H., Stassen, E. and Stegeman, J.A. (2009). The role of backyard poultry flocks in the epidemic of highly pathogenic avian influenza virus (H7N7) in the Netherland in 2003. Prev. Vet. Med. 88, 247-254.

Boklund, A., Alban, L., Mortensen, S. and Houe, H. (2004). Biosecurity in 116 Danish fattening swineherds: descriptive results and factor analysis. Prev. Vet. Med. 66, 49-62.

Calavas, D., Bugnard, F., Ducrot, C and Sulpice, P. (1998). Classification of the clinical types of udder disease affecting nursing ewes. Small Ruminant Res. 29, 21-31.

Costard, S., Porphyre, V., Messad, S., Rakotondrahanta, S., Vidon, H., Roger, F. and Pfeiffer, D.U. (2009). Multivariate analysis of management and biosecurity practices in smallholder pig farms in Madagascar. Prev. Vet. Med. In Press, Corrected Proof.

Dohoo, I.R., Ducrot, C., Fourichon, C., Donald, A. and Hurnik, D. (1997). An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Prev. Vet. Med. 29, 221-239.

East, I.J. (2007). Adoption of biosecurity practices in the Australian poultry industries. Aust. Vet. J. 85, 107-112.

Ferrari, P. and Anonni, P. (2005). Missing data in Optimal Scaling. Lavoro presentato al CLADAG 2005. Universita di Milano, p. 9.

Fiebig, L., Smieszek, T., Saurina, J., Hattendorf, J. and Zinsstag, J. (2009). Contacts between poultry farms, their spatial dimension and their relevance for avian influenza preparedness. Geospatial Health 4, 79-95.

Hagenaars, T.J. (2008). between-farm transmission routes of highly transmissible diseases in livestock: a literature study on the quantitative knowns and unknowns. Central Veterinary Institute of Wageningen UR, Lelystad, p. 13.

Hurnik, D., Dohoo, I.R., Donald, A. and Robinson, N.P. (1994). Factor analysis of swine farm management practices on Prince Edward Island. Prev. Vet. Med. 20, 135-146.

Köbrich, C., Rehman, T. and Khan, M. (2003). Typification of farming systems for constructing representative farm models: two illustrations of the application of multi-variate analyses in Chile and Pakistan. Agri. Sys. 76, 141-157.

Kristensen, S.P. (2003). Multivariate analysis of landscape changes and farm characteristics in a study area in central Jutland, Denmark. Ecol. Model. 168, 303-318.

Ligon, B.L. (2005). Avian Influenza Virus H5N1: A Review of Its History and Information Regarding Its Potential to Cause the Next Pandemic. Sem. Pediat. Infect. Dis. 16, 326-335.

Linting, M., Meulman, J.J., and Groenen, P.J.F. (2007). Nonlinear Principal Components Analysis: Introduction and Application. Psychol. Meth. 12, 336-358.

Lyytikäinen, T. and Kallio, E.R. (2008). Risk-classification of finnish pig farms by simulated FMD spread. In E.J. Peelr, L. Alban, A. Russell, and the SVEPM Executive Committee, Eds. Society for Veterinary Epidemiology and preventive medicine, proceedings of a meeting held at Liverpook, UK, 285-300.

Meulman, J.J., van der Kooij, A.J.and Heiser, W.J. (2004). Principal Components Analysis With Nonlinear Optimal Scaling Transformations for Ordinal and Nominal Data. In: D. Kaplan (ed.), Handbook of quantitative Methods in the social Sciences. CA: Sage Publications, Newbury Park, pp. 49-70.

Milán, M.J., Bartolomé, J., Quintanilla, R., Garcia-Cachán, M.D., Espejo, M., Herráiz, P.L., Sánchez-Recio, J.M. and Piedrafita, J. (2006). Structural characterisation and typology of beef cattle farms of Spanish wooded rangelands (dehesas). Livest. Sci. 99, 197-209.

Nespeca, R., Vaillancourt, J.P. and Morgan Morrow, W.E. (1997). Validation of a poultry biosecurity survey. Prev. Vet. Med. 31, 73-86.

Niemi, J.K., Lyytikäinen, T., sahlström, L., Virtanen, T. and Lehtonen, H. (2009). Risk Classification in Animal disease Prevention: Who Benefits from Differentiated Policy? Selected Paper prepared for presentation at the Agricultural & Applied Economics Association 2009 AAE & ACCI Join Annual Meeting, Milwaukee, Wisconsin, 28.

Ortiz-Pelaez, Á. and Pfeiffer, D. (2008). Use of data mining techniques to investigate disease risk classification as a proxy for compromised biosecurity of cattle herds in Wales. BMC Vet. Res. 4, 24.

Ribbens, S., Dewulf, J., Koenen, F., Mintiens, K., De Sadeleer, L., de Kruif, A. and Maes, D. (2008). A survey on biosecurity and management practices in Belgian pig herds. Prev. Vet. Med. 83, 228-241.

Rose, N. and Madec, F. (2002). Occurrence of respiratory disease outbreaks in fattening pigs: relation witht he features of a densely and a sparsely populated pig area in France. Vet. Res. 33, 179-190.

Sharkey, K.J., Bowers, R.G., Morgan, K.L., Robinson, S.E. and Christley, R.M. (2008). Epidemiological consequences of an incursion of highly pathogenic H5N1 avian influenza into the British poultry flock. Proc. R. Soc. B: Biolog. Sci. 275, 19-28.

Solano, C., Bernués, A., Rojas, F., Joaquín, N., Fernandez, W. and Herrero, M. (2000). Relationships between management intensity and structural and social variables in dairy and dual-purpose systems in Santa Cruz, Bolivia. Agri. Sys. 65, 159-177.

FARMING PRACTICES AND THE RISK OF HIGHLY PATHOGENIC AVIAN INLUENZA H5N1 IN BACKYARD POULTRY: A CASE-CONTROL STUDY IN THAILAND

M. PAUL*, S. WONGNARKPET, C. POOLKHET, S. THONGRATSAKUL, S. BORD, P.GASQUI, F. ROGER, C. DUCROT

SUMMARY

Highly pathogenic avian influenza (HPAI) H5N1 is continuing to devastate poultry flocks and posing an ongoing threat to human health (Briand & Fukuda, 2009). Little is known about the association between farming practices and the introduction of HPAI in a poultry farm. A case-control study was carried out in Thailand to investigate the risk factors for HPAI introduction in small-scale poultry farms. Six-hundred and thirty houses with backyard chickens or fighting cocks were investigated using a questionnaire. A random effects logistic regression model was developed with explanatory variables, such as farming practices and environmental factors included. The presence of a pond around the house was found to be the main risk factor regarding HPAI H5N1. The risk of HPAI was low for houses located at an altitude of >100 m. Also, the role of backyard chicken trade in HPAI H5N1 spread was highlighted, while the role of fighting cocks remains debatable.

INTRODUCTION

Since its emergence in China in 1996-97, the Highly Pathogenic Avian Influenza (HPAI) H5N1 virus has spread widely in more than 60 countries across Asia, Europe, Africa and Middle-East (Tiensin et al., 2009). HPAI H5N1 is continuing to devastate poultry flocks and posing an ongoing threat to human health, to a degree significantly beyond that of any previously known influenza virus (Briand & Fukuda, 2009). Controlling the spread of H5N1 disease in poultry is a major issue regarding the reduction of risk for humans. Determining the pathways by which H5N1 virus is spread has critical implications for targeting of control measures (Kilpatrick et al., 2006). Research to date suggests that the spread of HPAI H5N1 is influenced primarily by human activities related to poultry production and poultry trade (Normile, 2008) The persistence of HPAI H5N1 virus in Southeast Asia has been linked to a specific agro-ecosystem that combines free-grazing ducks with rice cultivation (Gilbert et al., 2008). In addition, some studies mentioned the potential role in HPAI spread of fighting cocks or backyard poultry farms with low biosecurity systems (Webster et al., 2006).

Thailand was severely affected by the HPAI H5N1 epizootics with 1717 outbreaks reported during the first and second 'wave' of influenza epizootics, from January 2004 to July 2005. Backyard poultry flocks represented over 50% of the outbreaks recorded. Backyard flocks

* Mathilde Paul, CIRAD UR 22-AGIRs / INRA UR 346 - Epidémiologie Animale, INRA Clermont-Ferrand Theix, 63122 Saint-Genes-Champanelle, FRANCE.
Email: mpaul@clermont.inra.fr

account for a small part of the overall poultry population in Thailand, but constitute approximately three quarters of the flocks (Otte et al., 2006). Small-scale poultry farming systems deserve special attention as backyard chickens constitute an important source of protein and cash income for rural families. In addition, diffusion of HPAI H5N1 virus among backyard chickens is a public health concern because of the frequent and close contacts between poultry and humans. Analytic epidemiologic reports about risk factors for backyard poultry are rare, apart recent studies in Bangladesh (Biswas et al., 2009) and in Vietnam (Henning et al., 2009). Identifying the role of human practices and the pathways of H5N1 spread between backyard farms remains challenging.

In order to identify the factors which played a role in HPAI H5N1 spreading from one house to another, a case-control study was carried out on backyard chicken farms in Thailand.

MATERIALS AND METHODS

Study design

The study was conducted in Phitsanulok province, which is located in the upper part of the Central plain of Thailand. Among all Thai provinces, this area is the one which recorded the highest number of outbreaks in chickens during the first and second wave of HPAI H5N1.

Small-scale chicken farms were the units of interest for the case-control study. The study was restricted to the farms which were recorded as 'native chickens' or 'fighting cocks' in databases of the Department of Livestock Development. To be included in our study, farms had to rear at least five backyard chickens or fighting cocks in 2005.

Selection of case and control farms:

Epidemiological data relevant to HPAI H5N1 outbreaks in poultry were provided by the Avian Influenza Control Center, Department of Livestock Development (DLD, Bangkok, Thailand), a unit in charge of surveillance and monitoring of avian influenza (AI) in poultry. Since January 2004, DLD has been recording information on all poultry outbreaks confirmed by a diagnostic test. Tests were carried out by diagnostic laboratories on sick or dead poultry or cloacal samples using reverse-transcriptase polymerase chain reaction and virus isolation. From 1 January 2004 to 31 July 2005, 216 outbreaks were recorded in the Phitsanulok province, among which 173 have been reported in backyard or fighting cock farms. All of them were recruited as case farms for the study. 'Cases' were farms where a positive laboratory result had been found on backyard chickens or fighting cocks, during the study period.

In order to have at least two useable controls per case, 400 control farms were randomly selected in the 2005 DLD poultry census database, from the 52,232 houses which had backyard chickens or fighting cocks at this time. Inclusion criteria were checked as part of the information collected in the questionnaire. Farms with either poultry mortality or HPAI clinical signs and without any result of diagnostic test were excluded from the analysis. 'Controls' were farms which raised backyard chickens or fighting cocks, which had no poultry mortality and no HPAI clinical sign, or a negative laboratory result in case of suspicion, during the study period.

Data collection and data management

Study period:

A 12-months study period was set for each farm. For case flocks, poultry owners were interviewed about their farming practices during the year just before the occurrence of HPAI outbreak on their poultry. As most of the outbreaks were reported between the start and middle of 2004, owners of control farms were interviewed about the whole 2003. The aim was to ensure that both cases and controls were questioned about farming practices over similar time periods.

Questionnaire:

A questionnaire which consisted mostly of closed questions was designed in English and translated into Thai. The questionnaire included general questions on the household and farm characteristics, detailed questions on poultry disease status and HPAI, poultry farming and poultry trade practices, activities related to fighting cocks, and environmental variables such as altitude and agro-ecological characteristics in a 100 m radius around the house. Sixteen researchers were trained over a 5 day period to conduct interviews with farmers. Interviews were carried out during 7 weeks, from March to April 2009, with 3 field coordinators. Interviews were conducted in Thai and answers were recorded on printed copies of the Thai-English questionnaire. Interviews lasted an average of 45 minutes. Geographical coordinates and elevation of the farms were recorded on the field using Global Positioning System (GPS).

Statistical analysis

Data were entered into a Microsoft (Redmond, WA, USA) Access 2003 database. All statistical analyses were performed using R 2.9.2 for Windows. All risk factors extracted from the questionnaire were categorical variables. Elevation was the only continuous variable and was categorised into three levels to account for non-linearity of effects. Screening of all variables was performed using univariate logistic regression, to assess the relationship between each biologically plausible explanatory variable and the dependant dichotomous variable, *i.e.* status of the house regarding HPAI H5N1 infection. Variables with $p \leq 0.25$ in the univariate analysis (likelihood ratio test) were considered for inclusion in multivariate logistic regression models (Hosmer and Lemeshow, 2000). Multicollinearity in the starting model was investigated by checking the variance inflation factors (VIF) (Dohoo et al., 2003). Multivariate analysis was based on random effects logistic regression. District was introduced as random effect variable to take spatial autocorrelation between houses into account (Pfeiffer et al., 2007).Variable selection for the final model was carried out with a backward elimination process based on the LR test, according to the recommendations of (Hosmer and Lemeshow, 2000). Odds-ratios and their 95% confidence intervals were derived from the coefficient estimates and variance parameters of the models. *P*-values of the different variables were computed based on the Wald test.

RESULTS

Study population

A total of 630 farmers were interviewed. Of these 144 were removed from statistical analysis as they did not fully meet the inclusion criteria. Statistical analyses were run on 486 farms, of which 104 were cases and 382 were controls. The 486 houses were located throughout the nine districts of Phitsanulok province.

Univariate analysis

Univariate analysis identified several variables that were significantly ($p \leq 0.25$, LR test) associated with the occurrence of HPAI H5N1 outbreak on chickens. Out of the 17 potential risk factors, 13 were significantly associated to HPAI. The remaining 5 variables were: visit of a fighting cock owner, giving backyard chickens to friends, confinement of fighting cocks, selling of fighting cocks, bringing a cock to the fighting arena.
Several environmental factors were significantly associated with the risk of HPAI in backyard farms (Table 1). Elevation > 50m was found to be a protective factor. Presence of a pond in a 100 meter radius around the house, high rice cropping intensity (> 2 crops / year) and presence of free-grazing ducks around the house were associated to high odds-ratios.

Table 1. Results of the univariate regression model for the environmental factors

		OR	CI 95%	Number of farms	*p* (LR test)
Elevation	≤ 50 m	1		245	< 0.001
	50 m - 100 m	0.56	0.34 - 0.94	128	
	> 100 m	0.11	0.04 - 0.27	113	
Pond in a 100 m radius	No	1		156	< 0.001
	Yes	3.47	1.93 - 6.23	330	
Number of rice crops in a 500 m radius	No rice field	1		113	< 0.001
	1 crop / year	0.76	0.38 - 1.51	159	
	2 crops / year	2.17	1.13 - 4.15	110	
	3 crops / year	2.56	1.34 - 4.91	104	
Free-grazing ducks around the house	No	1		297	< 0.001
	Yes	2.3	1.48 - 3.57	189	

Also, five variables related to poultry farming practices were found to be significantly associated with the risk of HPAI H5N1 (Table 2). Houses with large flocks of backyard chickens or houses raising both chickens and ducks were associated with high HPAI risk. The daily time spent with fighting cocks was significantly associated to a low risk of HPAI.

Among the four significant variables related to backyard poultry trading activities (Table 3), the purchase of live backyard chickens from a trader was found to be the most relevant risk factor (p=0.002). Houses visited by two traders or more during the study period were found to be associated to high risk of HPAI H5N1.

Table 2. Results of the univariate regression model for the poultry farming practices

		OR	CI 95%	Number of farms	*p* (LR test)
Number of backyard chickens	≤ 50 chickens	1		318	< 0.001
	51- 100 chickens	2.74	1.66 - 4.54	108	
	> 100 chickens	2.54	1.36 - 4.75	60	
Ducks in the farm	No	1		432	0.01
	Yes	2.22	1.21 - 4.07	54	
Confinement of backyard chickens	No	1		73	0.19
	During the day or night only	1.02	0.55 - 1.88	399	
	All the time	2.9	0.87 - 9.64	14	
Time spent with backyard chickens	Less than 1 h / day	1		469	0.18
	1 h / day or more	2.06	0.75 - 5.72	17	
Time spent with fighting cocks	Less than 1 h / day	1		400	0.25
	1 - 3 h / day	0.86	0.46 - 1.61	71	
	3 h / day or more	0.25	0.03 - 1.92	15	

Table 3. Results of the univariate regression model for backyard poultry trading activities

		OR	CI 95%	Number of farms	*p* (LR test)
Number of traders who visited the house	No trader	1		286	0.05
	1 trader	1.49	0.9 - 2.47	127	
	2 traders or more	1.99	1.11 - 3.57	73	
Selling of backyard chickens to the trader	Never	1		285	0.098
	1 time	1.31	0.61 - 2.82	45	
	2 to 4 times	1.66	1.03 - 2.68	143	
	5 times or more	2.87	0.9 - 9.13	13	
Purchase of backyard chickens from a trader	No	1		418	0.002
	Yes	2.49	1.44 - 4.33	68	
Purchase of fighting cocks	No	1		424	0.228
	Yes	1.46	0.8 - 2.68	62	

Multivariate analysis

Two variables were found positively correlated: the number of traders who visited the houses and the selling of backyard chickens to the trader. Thus, only the former was entered in the multivariate model. Multicollinearity was finally assumed not to cause any serious problem in the model, with all VIF values < 2 (Dohoo et al., 2003). The set of candidate variables included 12 categorical variables. The final model included five explanatory variables (Table 4), three related to poultry farming and poultry trading activities, two related to the characteristics of environment around the house.

Three variables were positively associated with the risk of having a positive laboratory test for HPAI H5N1: presence of a canal or pond in a 100 m-radius around the house, number of backyard chickens in the farm, purchase of live backyard chickens from a trader. Two variables were associated to lower risk of HPAI: elevation > 100 m and time spent with fighting cocks.

Table 4. Results of the random effects logistic regression analysis for risk factors associated with HPAI H5N1 occurrence in small-scale farms (n=486 houses, 9 districts). Variance of random coefficient was 0.50

		OR	CI 95%	*p* (Wald test)
Elevation	≤ 50 m	1		
	50 m - 100 m	0.62	0.29 - 1.27	0.190
	> 100 m	0.21	0.06 - 0.79	0.020
Pond in a 100 m radius	No	1		
	Yes	3.11	1.61 - 5.99	< 0.001
Number of backyard chickens	≤ 50 chickens	1		
	51- 100 chickens	2.90	1.61 - 5.24	< 0.001
	> 100 chickens	3.52	1.69 - 7.27	< 0.001
Time spent with fighting cocks	Less than 1 h / day	1		
	1 - 3 h / day	0.38	0.18 - 0.80	0.011
	3 h / day or more	0.05	0.01 - 0.48	0.009
Purchase of backyard chickens from a trader	No	1		
	Yes	2.89	1.46 - 5.67	0.002

DISCUSSION

To clarify the source and modes of transmission of influenza H51N from farm to farm, a case-control study was carried out in the province of Phitsanulok (Thailand). This targeted all outbreaks of avian influenza H5N1 recorded in backyard chickens and fighting cocks during the first and second wave of HPAI H5N1.

First, findings from the multivariate analysis evidenced the role at the farm level of several agro-environmental factors. Altitude was found to be a protective factor. Farms located at an altitude of >100 m were associated with a low risk of HPAI. This finding is consistent with results of a previous agro-ecological study (Gilbert et al., 2006). Compared with the plain, these areas are characterized by a sparse irrigation network, moderate land use intensity, absence of free-grazing ducks and a lower human population density. Such an environment may have not been as favourable for the HPAI HN51 virus as the agro-ecosystem found in the plains. In addition, contact between farms is more difficult in upland areas. Besides, the farms located in areas with a high rice cropping intensity (more than 2 rice cycles / year) as well as farms where free-grazing ducks were observed in the surroundings were found to be positively associated with the risk of HPAI in the univariate analysis only. Thus, while previous studies showed that free-grazing ducks may contribute to the spread of the virus when they are moved among rice fields (Gilbert et al., 2006; Gilbert et al., 2008), the role of the ‘free grazing ducks - rice paddy

fields' agro-ecosystem in HPAI infection was not evidenced in this study, at the farm level. In addition, the presence of a canal or a pond in a 100 m-radius around the house was a relevant risk factor in the multivariate analysis. These results are consistent with findings of a recent study in Bangladesh (Biswas et al., 2009). Small ponds or irrigation canals usually serve as a water source for backyard animals and gardening. Ducks from surrounding houses or wild birds have access to these ponds and may deposit large amounts of faeces. It was also found that, in the univariate analysis only, the presence of ducks within the farm was a factor associated with the risk of HPAI. Ducks are known to play the role of 'Trojan horse' for HPAI H5N1 virus, and can carry a high level of viral particles without showing any clinical signs (Hulse-Post et al., 2005).

Results of the analysis did not show any evidence of the role of fighting cocks in HPAI H5N1 risk at the farm level. Previous ecological studies highlighted a slight association between the risk of HPAI and the number of fighting cocks in the area (Gilbert et al., 2006), indicating that fighting cocks may not have been the major factor involved in HPAI spread in Thailand. In this analysis, neither the farms where the owner brought one of their cocks to the fighting arenas nor those where the owner trained their cocks at friend's houses were found to be significantly associated with the risk of HPAI. Thus, fighting cocks activities could not be evidenced as a risk factor in this study. The results also showed a protective effect of the daily time spent to take care of fighting cocks. The houses where the owner spent more than one hour per day with the cocks were found to be significantly associated to a low risk of HPAI H5N1. Fighting cocks have a very high monetary and cultural value, and thus receive very special attention from their owners. Moreover, in Thailand, fighting cocks were targeted when control measures were implemented in 2004, with a prohibition on cockfighting, compulsory registration, and disease monitoring (Tiensin et al., 2007). Fighting cock owners may have changed their practices early to protect their poultry from the disease.

Several findings indicate that besides the agro-ecological pattern, poultry trade activities played a key role in HPAI spread between farms. In the multivariate analysis, farms with more than 50 backyard chickens were significantly associated to a higher risk of HPAI H5N1 than smaller farms. The number of backyard chickens in the house was the most relevant risk factor of HPAI H5N1 evidenced in this study. Large backyard farms - with more than 50 backyard chickens - commercialized more poultry than the small ones and as a consequence may have been exposed to more contacts. Several variables related to the role of the live poultry traders were positively associated with the risk of HPAI H5N1 in the univariate analysis. In Thailand, live poultry markets have always been rare (Amonsin et al., 2008). Backyard chickens are collected by traders in several farms, before being slaughtered at the middleman's house and brought to the food market. Such chains may constitute an efficient pathway for HPAI H5N1 spread from house to house. However, among all poultry trading activities, only the purchase of live chickens from a trader was evidenced as a risk factor in the multivariate analysis. Backyard farms may have been contaminated by HPAI H5N1 through introduction of infected live chickens that they got from the trader.

These findings provide new perspectives on risk factors of HPAI H5N1 at the farm level. Altitude of > 100m was found to be a protective factor. The agro-ecosystem based on flocks of ducks grazing in intensively cultivated rice paddy fields - which has been previously demonstrated by ecological approaches (Gilbert et al., 2006; Gilbert et al., 2008), was not found to be a major risk factor at the farm level. In addition, the results showed that the activities related to fighting cocks may have not played a key role in disease spread from house to house. However, because this activity involves close and frequent contacts between human and poultry,

prevention measures should keep targeting fighting cock owners. Findings from this study highlighted several risk factors related to backyard poultry trading and small-scale poultry trading chains may constitute an efficient pathway for the spread of HPAI H5N1.

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REFERENCES

Amonsin, A., Choatrakol, C., Lapkuntod, J., Tantilertcharoen, R., Thanawongnuwech, R., Suradhat, S., Suwannakarn, K., Theamboonlers, A. and Poovorawan, Y. (2008). Influenza Virus (H5N1) in Live Bird Markets and Food Markets, Thailand. Emerg. Infect. Dis 14, 1739 - 1742.

Biswas, P.K., Christensen, J.P., Ahmed, S.S.U., Das, A., Rahman, M.H., Barua, H., Giasuddin, M., Hannan, A.S.M.A., Habib, M.A. and Debnath, N.C. (2009). Risk for Infection with Highly Pathogenic Avian Influenza Virus (H5N1) in Backyard Chickens, Bangladesh. Emerg. Infect. Dis 15, 1931 - 1936.

Briand, S. and Fukuda, K. (2009). Avian Influenza A (H5N1) Virus and 2 Fundamental Questions. J. Infect. Dis., 1717-1719.

Dohoo, I., Martin, W. and Stryhn, H. (2003). Veterinary Epidemiologic Research. AVC Inc., Charlottetown, Prince Edward Island, Canada.

Gilbert, M., Chaitaweesub, P., Parakamawongsa, T., Premashthira, S., Tiensin, T., Kalpravidh, W., Wagner, H. and Slingenbergh, J. (2006). Free-grazing ducks and Highly Pathogenic Avian Influenza, Thailand. Emerg. Infect. Dis 12, 227-234.

Gilbert, M., Xiao, X., Pfeiffer, D.U., Epprecht, M., Boles, S., Czarnecki, C., Chaitaweesub, P., Kalpravidh, W., Minh, P.Q., Otte, M.J., Martin, V. and Slingenbergh, J. (2008). Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. Proc. Natl. Acad. Sci. U. S. A. 105, 4769-4774.

Henning, K.A., Henning, J., Morton, J., Long, N.T., Ha, N.T. and Meers, J. (2009). Farm- and flock-level risk factors associated with Highly Pathogenic Avian Influenza outbreaks on small holder duck and chicken farms in the Mekong Delta of Viet Nam. Prev. Vet. Med. 91, 179-188.

Hosmer, D.W. and Lemeshow, S. (2000). Applied logistic regression. Wiley New York.

Hulse-Post, D.J., Sturm-Ramirez, K.M., Humberd, J., Seiler, P., Govorkova, E.A., Krauss, S., Scholtissek, C., Puthavathana, P., Buranathai, C., Nguyen, T.D., Long, H.T., Naipospos, T.S.P., Chen, H., Ellis, T.M., Guan, Y., Peiris, J.S.M. and Webster, R.G. (2005). Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proc. Natl. Acad. Sci. U. S. A. 102, 10682-10687.

Kilpatrick, A.M., Chmura, A.A., Gibbons, D.W., Fleischer, R.C., Marra, P.P. and Daszak, P. (2006). Predicting the global spread of H5N1 avian influenza. Proc. Natl. Acad. Sci. U. S. A. 103, 19368-19373.

Normile, D. (2008). Avian Influenza: Flu Virus Research Yields Results but No Magic Bullet for Pandemic. Science 319, 1178-1179.

Otte, J., Pfeiffer, D., Tiensin, T., Price, L. and Silbergeld, E. (2006). Evidence-based Policy for Controlling HPAI in Poultry: Bio-security Revisited. Pro-Poor Livestock Policy Initiative Research Report. p. 13.

Pfeiffer, D.U., Minh, P.Q., Martin, V., Epprecht, M. and Otte, M.J. (2007). An analysis of the spatial and temporal patterns of highly pathogenic avian influenza occurrence in Vietnam using national surveillance data. Vet. J. 174, 302-309.

Tiensin, T., Ahmed, S. S. U., Rojanasthien, S., Songserm, T., Ratanakorn, P., Chaichoun, K., Kalpravidh, W., Wongkasemjit, S., Patchimasiri, T., Chanachai, K., Thanapongtham, W., Chotinan, S., Stegeman, A. and Nielen, M. (2009). Ecologic Risk Factor Investigation of Clusters of Avian Influenza A (H5N1) Virus Infection in Thailand. J. Infect. Dis. 199, 1735-1743.

Tiensin, T., Nielen, M., Songserm, T., Kalpravidh, W., Chaitaweesub, P., Amonsin, A., Chotiprasatintara, A., Damrongwatanapokin, S., Wongkasemjit, S., Antarasena, C., Songkitti, V., Chanachai, K., Thanapongtham, W. and Stegeman, A. (2007). Geographic and temporal distribution of Highly Pathogenic Avian Influenza A virus (H5N1) in Thailand, 2004-2005: an overview. Avian Dis. 51, 182-188.

Webster, R.G., Peiris, J.S.M., Chen, H. and Guan, Y. (2006). H5N1 outbreaks and enzootic influenza. Emerg. Infect. Dis 12, 3-8.

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North Mymms
Hatfield
Herts, AL9 7TA
UK

TEL +44 (0) 1707 666 625
FAX +44 (0) 1707 666 574
Email: kverheyen@rvc.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