SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

Proceedings of a meeting held in

Elsinore, Denmark

16th – 18th March 2016

Edited by A. Lindberg and H.-H. Thulke

and the SVEPM Executive Committee

Cite as: Lindberg, A. and Thulke, H.-H. (eds). Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine annual meeting held in Elsinore, SVEPM

ISBN 978-0-948073-35-9

ACKNOWLEDGEMENTS

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

University of Copenhagen
Technical University of Denmark (DTU)
Danish Agriculture & Food Council
Scottish Rural University College (SRUC)
Royal Veterinary College (RVC)
University of Liverpool
Carlsberg Foundation
Peter Christian Abildgaards fond
Overdyrlæge Hans A. Madsen og hustrus fond
NOSOVE
DIANOVA
Zoetis
SAFOSO
Avia-GIS
MSD Animal Health
FOSS
Elanco
King Thermofisher
Biomed Central
Frontiers

CONTENTS

ENHANCED DETECTION

TRANSBOUNDARY DISEASES

PREDICTIVE MODELLING

SHEEP EPIDEMIOLOGY

PERCEPTIONS AND PRACTICES

ANTIMICROBIAL USAGE AND RESISTANCE

EMERGING INFECTIOUS DISEASES

SURVEILLANCE FRAMEWORKS

***

Society for Veterinary Epidemiology and Preventive Medicine

ENHANCED DETECTION

HOW FEW POOLED TESTS ARE NEEDED TO DETECT A SINGLE POSITIVE SAMPLE?

K. GRÆSBØLL*, L.O. ANDRESEN, T. HALASA, N. TOFT

SUMMARY

Testing large quantities of samples to detect one or more positive sample(s) is expensive and time consuming. Pooling of samples can optimize this process. Several different pooling schemes were simulated to compare the efficiency as a function of prevalence and number of pooled samples.

The sensitivity of ELISAs on pooled samples for antibodies in bovine milk to *Salmonella Dublin* (SD), *Mycobacterium avium* spp. *paratuberculosis* (PTB), and bovine virus diarrhea was tested; alongside ELISAs for antibodies in serum to SD, PTB and infectious bovine rhinotracheitis. For milk assays the sensitivity decreased rapidly with increased pool sizes. However, for serum the detection limits were between 25 and 100 individual samples.

The best pooling scheme depended mainly on the prevalence and the sensitivity of the test in a pooled sample. The combinatorial scheme named Shifted Transversal Design proved to be the best framework for determining the most efficient pooling scheme.

INTRODUCTION

Within the veterinary field, pooling is used extensively to detect farm status and/or as an indicator for further investigation. This can be in form of testing the bulk tank milk of dairy cows for, for instance *Salmonella Dublin* (Nielsen & Ersbøll, 2005), or pooling of ticks, midges or mosquitoes - often in geographical strata (Rasmussen et al., 2013; Lorraine et al., 2014). However, little pooling seems to be have been done when the objective is to detect disease in individual animals.

How few pooled tests are needed to detect a single positive sample? The answer firstly depends on how much a positive sample can be diluted and still be detected; in other words: How does sensitivity change with the pool size? Secondly, how many negative samples does this positive sample hide between; in other words: What is the prevalence?

When determining whether pools are positive or negative, it can be desired to determine alternative lower cut-offs for the ELISAs compared to the defaults determined by the manufacturer. A lower cut-off is needed because pooling dilutes positive samples causing a

---

* Kaare Græsbøll, National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C. Email: kagr@dtu.dk

lower signal which lowers the sensitivity of the test. Therefore, alternative cut-offs were determined to as low as possible to increase sensitivity, while maintaining specificity.

Pooling of samples can be done in structures of different dimensionality. The 1D is the traditional pooling method: pool from a line. The 2D is arranging samples in a matrix and pool on the edges. The 3D is to arrange samples in a cube and pool on the edges. Beyond the traditional 123D methods this paper investigates a combinatorial scheme: the Shifted Transversal design (STD), which can also go to even higher dimensions. The advantage of going to high dimensional and combinatorial pooling is that the need to retest samples to identify the individual positive sample may be significantly reduced or even eliminated. The reduction of retests potentially saves time and money. Thus, the objective of this paper is to investigate when testing to determine the individual positive animal; what are the possible savings using pooling; and what is needed to achieve those savings.

MATERIALS AND METHODS

Firstly, pooling experiments with ELISA testing will be presented, followed by the pooling schemes and the approach to simulate them.

ELISA tests

The sensitivity of commercially available ELISAs on pooled samples for detection of antibodies in bovine milk to *Salmonella Dublin* (SD) (PrioCHECK Salmonella AB bovine Dublin, Prionics, Swizerland), *Mycobacterium avium spp. paratuberculosis* (PTB) (ID Screen Paratuberculosis Indirect, IDVet, Grabels, France), and bovine virus diarrhea (BVD) virus (Svanovir BVDV-Ab, Svanova, Uppsala, Sweden) were evaluated using the following approach: The commercially available ELISA tests were performed according to the manufacturer’s instructions. The numbers of positive milk samples included in the study were 9 for SD, 8 for PTB and 10 for BVD. Positive milk samples were pooled with known negative milk samples resulting in one positive sample being pooled with 4, 9, 24, 49, 99, 149 and 199 negative samples, respectively. An equal volume from each sample was used for pooling. The optical density (OD) was measured at 450 nm. Results were calculated as percent positivity (PP) by Eq. (1).

$$PP = 100 \cdot (OD_{\text{sample}} - OD_{\text{negative control}}) / (OD_{\text{positive control}} - OD_{\text{negative control}}) \qquad (1)$$

The sensitivity of ELISAs on pooled samples for detection of bovine antibodies in serum to SD, PTB and bovine herpesvirus-1 (BHV-1) causing bovine rhinotracheitis (IBR) (Nylin et al., 2000) was also investigated in a similar manner. The numbers of positive bovine serum samples included in the study were 7 for SD, 5 for PTB and 4 for IBR. Positive serum samples were diluted in negative bovine serum and tested as undiluted and in dilutions of 1:5, 1:10, 1:25, 1:50, 1:100, 1:150 and 1:200.

Estimation of the specificity of the ELISAs and alternative cut-offs for milk samples was performed by testing 460 known negative milk samples in each of the three ELISAs. The alternative cut-off was calculated as the mean percent positivity relative to the positive control of the assay plus 3 times the standard deviation.

Pooling schemes

The three traditional 123D pooling schemes and the combinatorial STD scheme were tested. The first three schemes have easily identifiable physical representations, while the STD combinatorial scheme requires more complicated pooling and decoding algorithms. For simplicity it is assumed that the test here used has 100% sensitivity and specificity. The pooling schemes are:

1D: This is the traditional pooling scheme in use as far back as 1915 (Hughes-Oliver, 2006). Each sample is pooled once with a number of other samples. If a pool is positive, all samples belonging to this pool must be retested to identify a single positive sample. Notice that in the 1D pooling scheme, there will always be a retest when there are positive samples. This means that a 1D pooling scheme can be expensive if retesting carries a large cost. A 1D pooling scheme is equivalent to testing pools P1-P8 in Fig. 1.

2D: This pooling scheme is also known as the matrix or row/column pooling scheme (Barillot et al., 1991; Hughes-Oliver, 2006). The physical representation of this scheme is all samples arranged in a matrix and then pools are created by sampling all rows and columns. Positive samples can be identified by intersection of positive rows and columns (Fig. 1). Notice that the 2D pooling scheme have situations were retesting is not necessary, most commonly when there is only one positive sample. Therefore, if the pool size is reduced then the probability of retesting will also be reduced.

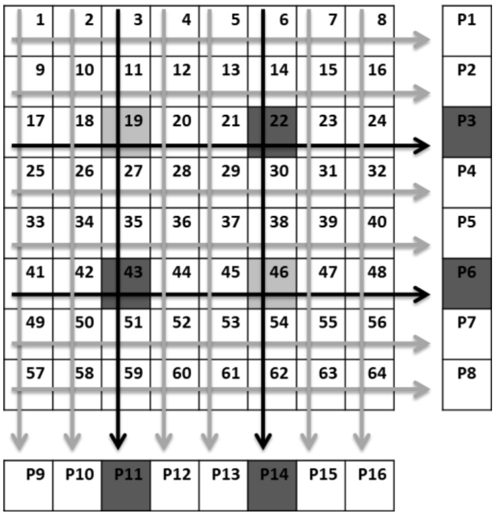


Fig. 1 An example of a 2D pooling scheme with 64 samples pooled into 16 pools of pool size 8. Here samples 22 and 43 are positive, causing pools 3, 6, 11, and 14 to be positive, thus prompting a retest of the samples on the intersections; namely samples 19, 22, 43, and 46

3D: This pooling scheme is also known as the cube scheme (Barillot et al., 1991). The physical representation is that samples are arranged in a cube (stacked matrices), planes in the xy, xz an yz direction are pooled. Positive samples can be identified by intersection of positive planes (Fig. 2). Because the pools in the 3D are planes of a cube the pool sizes are all square numbers. Therefore, the 3D pooling schemes with pool size 4, 9, 16, 25, and 36 were the only ones examined. Contour plots in the results section of 3D are based on interpolation.

STD: The Shifted Transversal Design was introduced by Thierry-Mieg (2006). It is a combinatorial pooling scheme, i.e. it formalises how to pool samples with a minimal co-occurrence of samples in the pools – the STD minimizes the amount of times that a given sample is in the same pool as another given sample. Minimizing co-occurrence has the effect, that the STD is a method that can detect multiple positive samples in multiple dimensional pooling schemes - often without retesting. The STD is defined by multiple parameters. However, in this paper results are presented as a function of pool size. There will at most times be more than a hundred different pooling combinations with a similar pool size proposed by the STD, of these the optimal pooling scheme is selected for presentation. For the complete mathematical description of the STD, see Thierry-Mieg (2006).

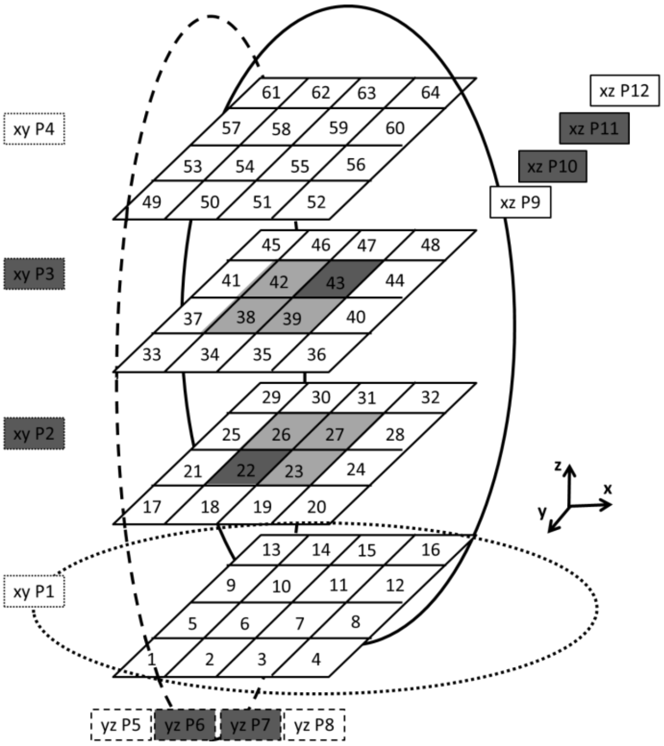


Fig. 2 An example of a 3D pooling scheme where 64 samples are pooled into 12 pools of pool size 16. Pooling is done 4 times along each of the xy, yz, and xz planes. Two positive samples (samples 22 and 43 indicated by dark grey) give rise to 8 possible positives (light grey)

Simulation: For some of the simpler schemes the number of retests can be deduced with relative ease. However, with increasing number of positive samples the combinations of locations of positives in the pooling structures give rise to a very complicated probabilistic structure. For the STD, the number of possible pooling schemes made an analytical solution unfeasible. Therefore, the pooling schemes were simulated to use the average number of retests found in the simulation. The simulation method was: All possible pooling schemes with a pool size smaller than or equal to 36, were tested in combination with prevalence from 0.1% to 90%. For each combination of pool size and prevalence, the number of individual samples that were needed in the scheme was drawn from a binomial distribution. This was repeated a thousand times for each of the 1D, 2D, and 3D schemes and one hundred times for the STD. The number of times to retest and the average number to be retested were saved for each combination of pool size and prevalence. The limit on pool size of 36 was imposed because the time to simulate the STD scheme increases exponentially with pool size. All simulations were done using R: A

Language and Environment for Statistical Computing ver. 3.1.1 (R development Core Team, 2014) in RStudio ver. 0.99.447 (RStudio Team, 2015).

Comparison of pooling schemes: When comparing pooling schemes to the testing of individual samples the trivial comparison is to count the total number of tests needed to detect the positive samples. However, there may be costs associated with the pooling itself and/or the storage and preparation for retesting individual samples identified as possible positives by the pooling schemes. In this paper, the cost of pooling is assumed to be negligible, and only a fixed cost for the retrieval of possible positive samples to be retested is included. The cost of testing a single sample or pool is set to index 1, and the cost of retrieving is given as a relative cost to this index. The total cost of a single pooling scheme is number of test used for pooled samples + number of samples to be retested + cost of retrieval. Thus, when the cost of retrieving is set to zero, the costs and the number of tests are equivalent. In the results section, the savings of the pooling scheme are presented relative to the cost of individual testing of all samples. To get savings in Euros, multiply the total number of samples to be tested and the cost of testing an individual sample/pool in Euros onto the values in the savings plots.

RESULTS

Table 1 summarizes the results of the testing of negative milk samples in the three ELISAs. Results from the pooling of the nine SD positive milk samples with negative milk samples are presented in Fig. 3. Similar results were obtained when testing milk samples in assays for antibodies to PTB and BVD virus. Results show that the percent positivity (PP) decreases drastically when positive samples are pooled with increased number of negative samples. Pool sizes higher than 25 showed results at the level of the negative samples. From Fig. 3 it can be seen that a maximum pool size of five would give positive results with the alternative cut-off of 21 PP. Pool sizes higher than five could result in false negative measurements for milk.

Figure 4 shows the results from diluting serum samples positive for PTB in known negative bovine serum. In the case of the ELISA for PTB the positive serum samples could be diluted up to 100 times, corresponding to a pool size of 100, and still have a positive result in the ELISA using the cut-off of 70% positivity. For SD and IBR positive serum samples, the maximum pool size was 50 and 10, respectively, when using the default cut-off values recommended by the manufacturer.

Table 1. Results from test of negative milk samples in ELISA

ELISA for antibodies to*	Negative samples tested	Mean Percent Positivity	Standard deviation	Alternative cut-off	Default cut-off	Specificity using alternative cut-off
Salmonella Dublin	460	6.65	4.62	21	35	0.99
Mycobacterium avium spp. paratuberculosis	460	2.00	2.18	9	15	0.995
Bovine Diarrhea Virus	460	3.50	0.93	7	12	0.99

*ELISAs used are presented in materials and methods

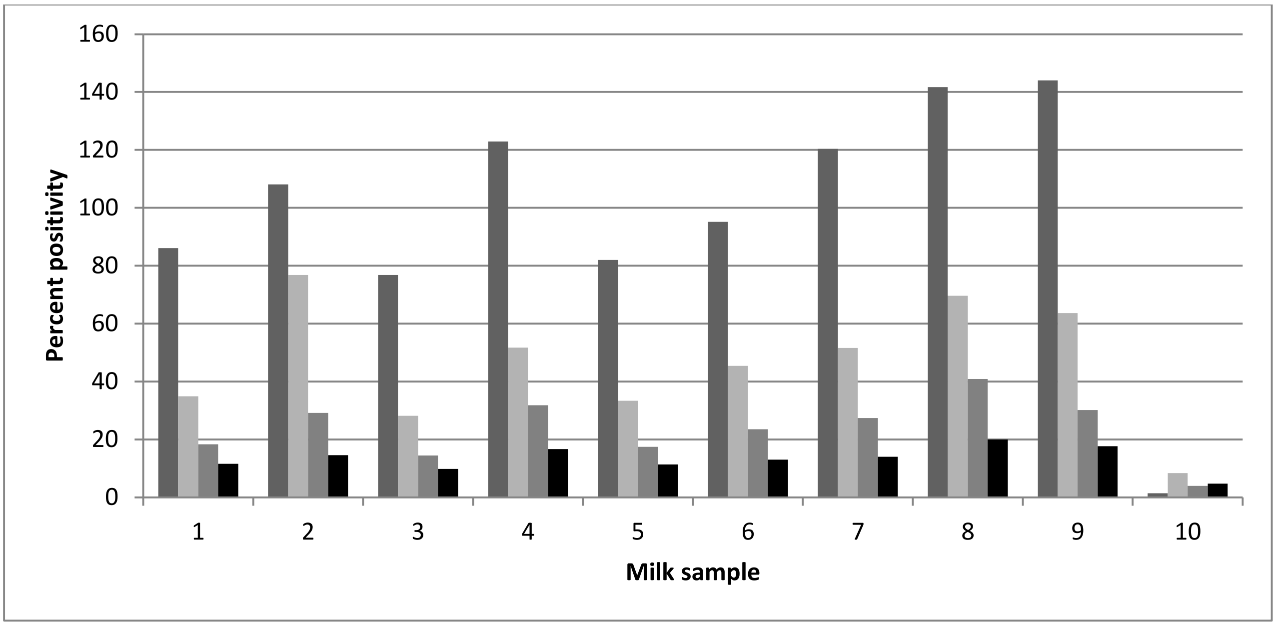


Fig. 3 Salmonella Dublin ELISA: Percent positivity in pooled milk samples. Nine antibody positive samples and one antibody negative sample were pooled with known negative milk samples. Results of testing the milk samples undiluted (dark grey), diluted 1:5 (light grey), 1:10 (grey) and 1:25 (black) are presented as mean values of three tests performed on separate days

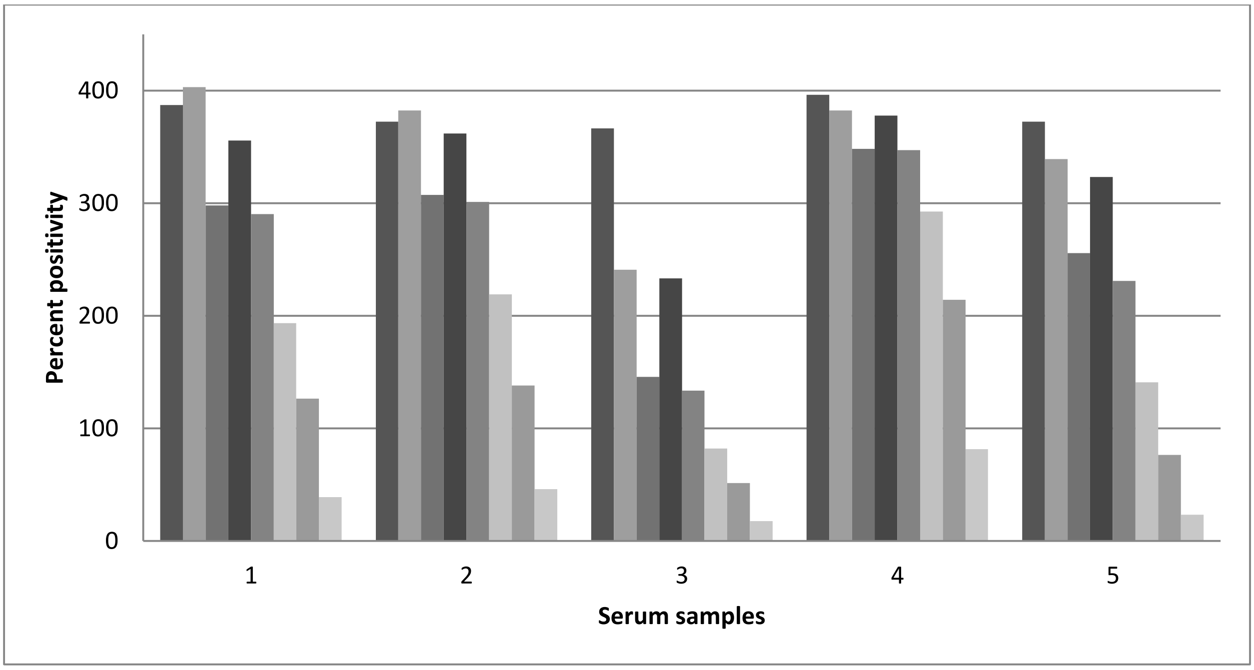


Fig. 4 PTB ELISA: Percent positivity in pooled serum samples. Five antibody positive samples were diluted in known negative bovine serum. Bars from left to right represent results for undiluted serum, and serum dilutions 1:5, 1:10, 1:25, 1:50, 1:100, 1:150 and 1:200, respectively

The results of the simulations show that STD can provide the most efficient pooling schemes regardless of pooling size or prevalence (Figures 5, 6, and 7). It can be difficult to interpret what goes on in the STD, for that reason 123D are compared, because the mechanisms that govern the optimal schemes within those schemes generally also apply to the optimal scheme selected by the STD.

Figures 5 and 6 show that a 1D scheme is more efficient than 2D and 3D when the maximum pool size, the retrieval cost and the prevalence are low. However, as soon as the retrieval cost increases, 1D is less often the optimal scheme. Generally, for higher retrieval cost the pooling schemes of higher dimensions becomes optimal.

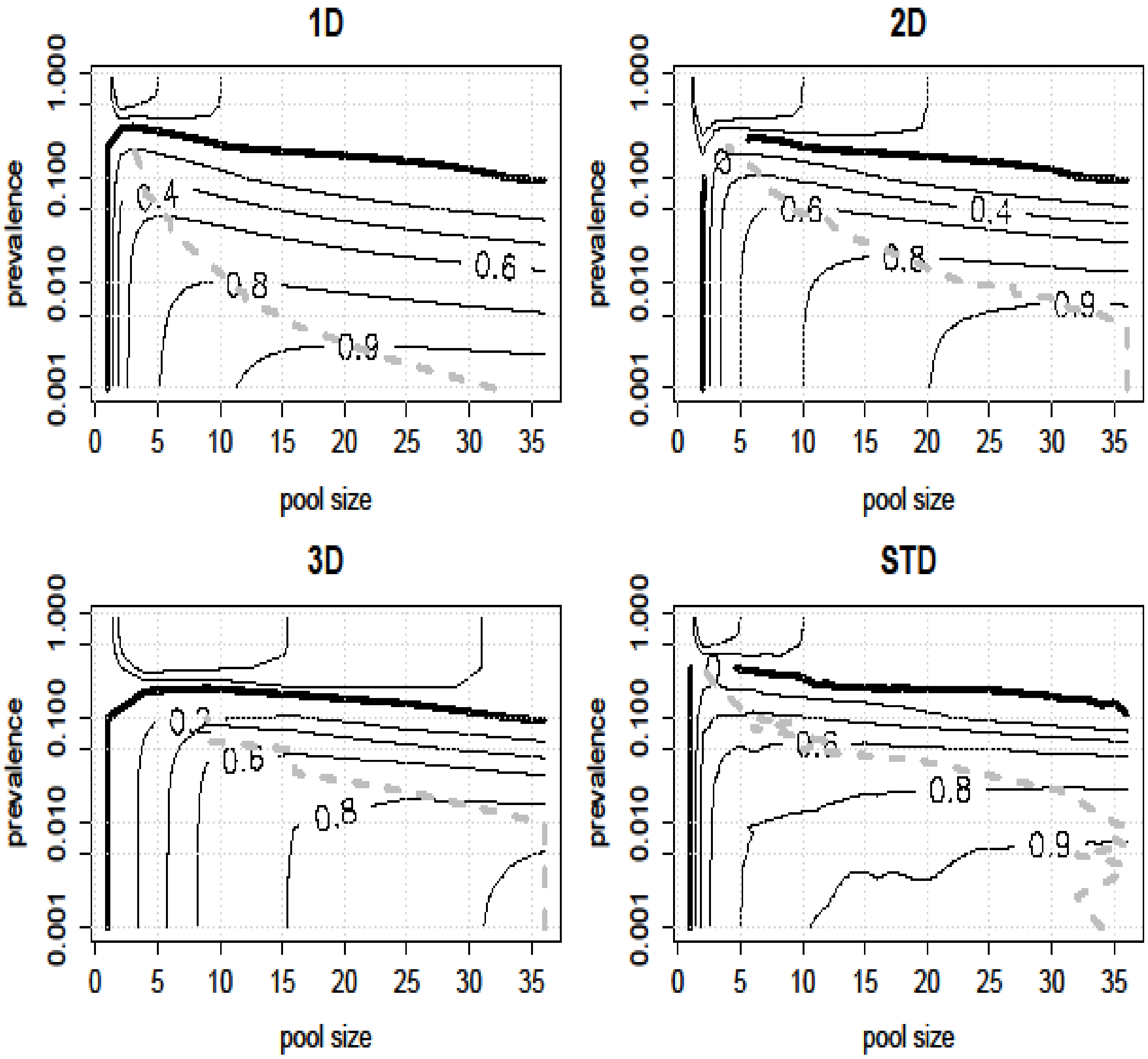


Fig. 5 Contour plot of the fraction of tests saved compared to individual testing as a function of pool size and prevalence. The thick line indicates where the pooling scheme requires the same number of tests as individual testing, and above this line pooling cannot be cost effective. However, there are large areas in the bottom right corners where the number of tests saved is more than 50%. The grey line indicates the pool size that gives the maximal saving of tests for a given prevalence. This plot is equivalent to a savings plot where the cost of retrieval is 0

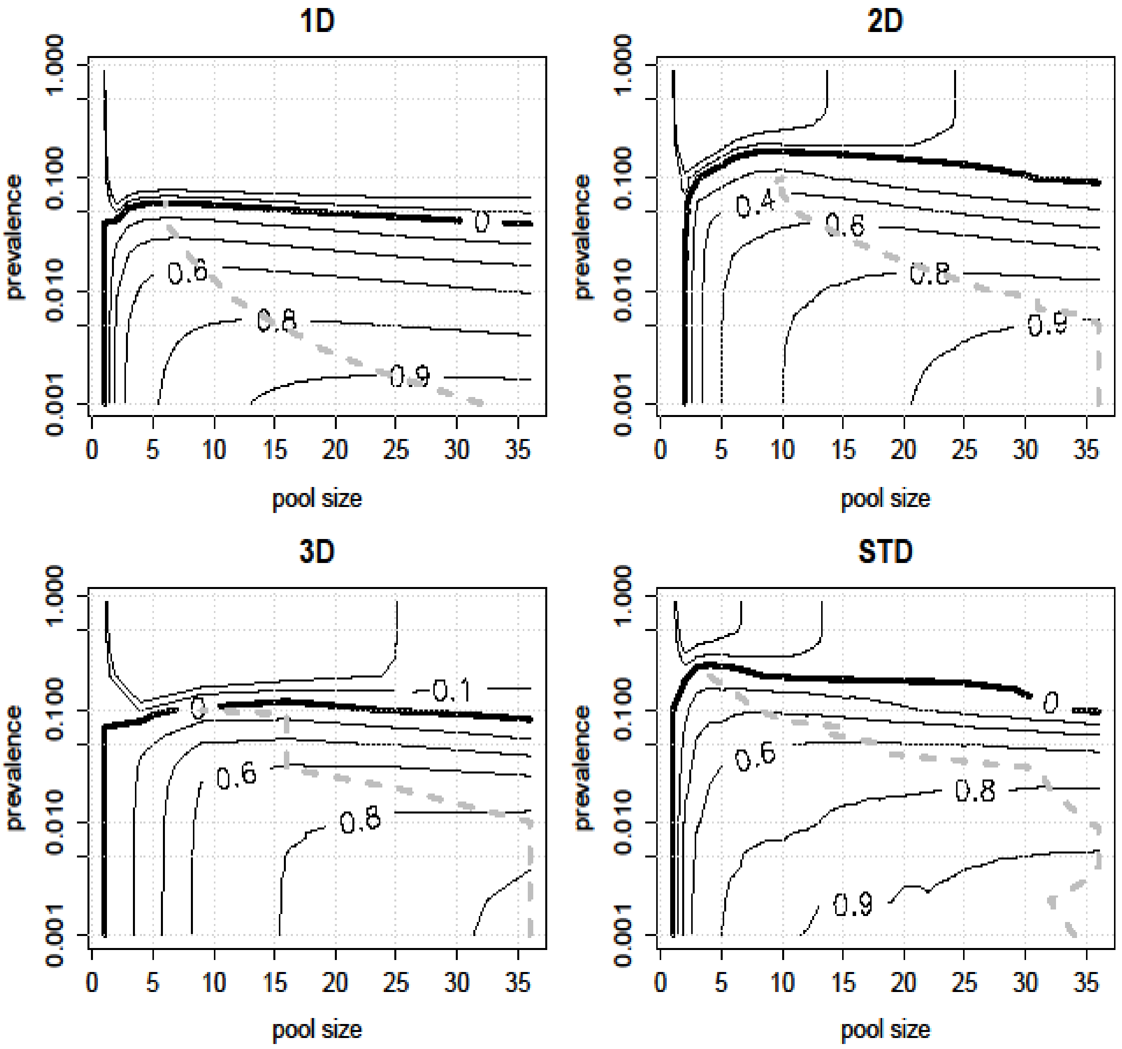


Fig. 6 Contour plot of the fraction of savings compared to the price of individual testing as a function of pool size and prevalence, where each retrieval for retesting caries the cost of 10 individual tests. The thick line indicates where the pooling scheme has the same cost as individual testing, and above this line pooling is not cost effective. However, there are large areas in the bottom right corners where the savings are more than 50% compared to the price of individual testing. The grey line indicates the pool size that gives the maximal saving for a given prevalence

There exists for all pooling schemes an optimal pool size for a given prevalence (dashed grey lines in Figures 5-7). It can also be observed that pooling is not cost-efficient when the prevalence is higher than 30% (Fig. 5). If only the maximum prevalence is known (Fig. 7), there is a large difference between the 1D and the higher dimensional pooling schemes. The 1D scheme is only cost-efficient if the maximum prevalence is below 10%, while the 2D can be cost-efficient for a maximum prevalence as high as 50%.

DISCUSSION

The physical representations of some of the pooling schemes are not necessarily connected to the ideal way of implementing them in practise. The 1D and 2D schemes can be done by conventional pipetting, e.g. ‘by hand’, but higher dimensional pooling schemes and the STD are more easily done by a robot. The robot should not arrange samples in the physical structures, but rather from software receive a list of pools that each sample is assigned to. In this way, each individual sample is only visited once, and directly distributed to the relevant number of pools determined by the pooling scheme. In this paper the additional cost of pooling was assumed to be zero, because large scale laboratories often have a robot that distribute samples from test tubes to test assay (personal communication with Eurofins Steins Laboratory). For 1D pooling this robot could deliver the sample to a pooled well on the assay instead of individual wells without increasing time spend. For higher dimensional pooling schemes each sample need be delivered to multiple wells, this cost in time may be negligible if e.g. the cleaning process between samples or the time to test is comparatively longer.

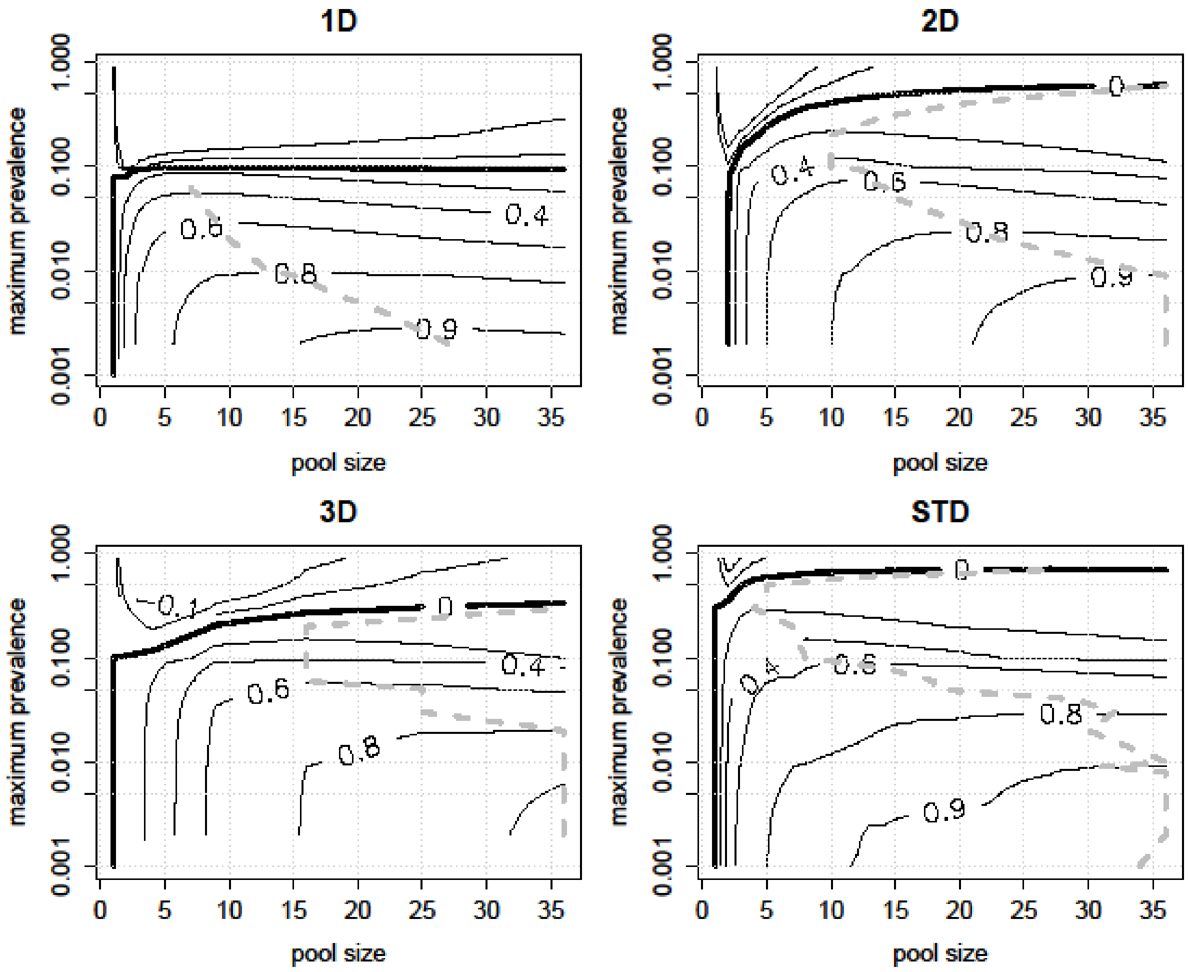


Fig. 7 Contour plot of the fraction of savings compared to the price of individual testing as a function of pool size, but only the maximum prevalence is known. Prevalence is assumed to be uniformly probable between 0.001 and the maximum prevalence. Every retrieval for retesting caries the cost of 10 individual tests. The thick line indicates where the pooling scheme has the same cost as individual testing. The grey line indicates the pool size that gives the maximum saving for a given maximum prevalence

The sensitivity and specificity were not specifically handled in the simulations. For a specific disease, dilution series to determine Se/Sp for different pool sizes should be performed. The results in this paper are reported in terms of the pool size, which may allow a user to impose his or her cut-off in OD to the desired Se/Sp. To use the results in such a way, it must be assumed that Se/Sp is sample specific: A sample that test negative in an individual test will also test negative in all pooled test, and vice versa. Furthermore, it has been assumed that samples do not give rise to added unspecific reactivity when pooled.

The maximum pool size for a given disease using a specific test kit can be determined by dilution trials as presented in this paper. In this work, this initial step was further used to determine an alternative cut-off in order to maximize the possible maximum pool size. The experiments presented here are examples. To achieve a good measurement of the change in sensitivity when pooling the number of positive samples tested should be larger. Specifically, weakly positive samples must be included in the test series, to correctly estimate changes in sensitivity due to pooling. It may also be possible to adjust the procedure of the ELISA if there are steps of pre-dilution before the OD measurements to further increase sensitivity (Brinkhof et al., 2007).

In this paper only positive/negative test results following the use of a cut-off are reported from the pooling schemes. However, using cut-offs can remove information from the test, and it could potentially be better and/or easier to make algorithms to identify positive samples based on the distribution of the continuous outcomes in a pooling scenario.

Hierarchical group testing is a class of pooling schemes that require additional number of retests as samples are pooled, and re-pooled depending of the results of the first pools (Black et al., 2015). Preliminary results show that hierarchical schemes can be cost optimal if price of retesting is low.

The results indicate that a higher retrieval cost leads to pooling schemes of higher dimensions becoming optimal. This is due to higher dimension schemes contain more samples per scheme, and given retrieval is a onetime expense, this will lower total cost.

The STD is always the most cost effective scheme, because it implicitly also includes the 123D schemes and many more. The difference between the STD and the other schemes does, however, depend on the price of retesting and the combination of pool size and prevalence. Within the explored parameters in this paper, the STD was seldom more than 10% points better than any of the other schemes. However, in Thierry-Mieg (2006) it was shown that for very low prevalence combined with opportunity to go to high pool sizes the STD becomes very efficient compared to simpler schemes.

CONCLUSION

The results of the simulations show that for a wide range of prevalence and pool sizes, there are large potential savings with pooling. However, certain restrictions apply before those savings can be achieved: Firstly, initial tests must be performed on the specific test kit intended for use, to determine how large a pool size can be used, ideally in combination with defining an alternative cut-off to maximize Se/Sp. Secondly, samples should not display an increase in any unspecific reactivity in the test when pooled, otherwise results may be invalid.

The code that produced the savings figures in this paper has been integrated into a Shiny WebApp, which allows the user to specify the cost of retrieval and the cost of pooling. The WebApp is freely available at https://kagr.shinyapps.io/SMARTPOOL.

ACKNOWLEDGEMENTS

All milk samples and the serum samples positive for IBR were kindly supplied by Eurofins Steins Laboratorium, Vejen, Denmark. This work was carried out as a part of the SMARTPOOL project which is funded by GUDP grant no. 34009-13-0760. The funder had no influence on the design, execution, results, or conclusions of the experiments or simulations in this study.

REFERENCES

Barillot, E., Lacroix, B., and Cohen, D. (1991). Theoretical analysis of library screening using a N-dimensional pooling strategy. Nucleic Acids Res. 19, 6241-6247

Black, M.S., Bilder, C.R., and Tebbs, J.M. (2015). Optimal retesting configurations for hierarchical group testing. J. R. Stat. Soc.: Series C (Applied Statistics)

Brinkhof, J.M.A., Houwers, D.J., and Van Maanen, C. (2007). Development of a sample pooling strategy for the serodiagnosis of small ruminant lentiviral infections using the ELITEST-MVV ELISA. Small Ruminant Res. 70, 194-199

Hughes-Oliver, J. (2006). Pooling experiments for blood screening and drug discovery. Screening: Methods for Experimentation in Industry, Drug Discovery, and Genetics. Springer, N.Y. 48-68p

Lorraine, M., Delannoy, S., Devillers, E., Umhang. G., Aspan, A., Juremalm, M., Chirico, J., Wal, F.J., Pihl, T.P.B., Schou, K.K., Bødker, R., Fach, P., and Moutailler, S. (2014). High-throughput screening of tick-borne pathogens in Europe. Front. Cell. Infect MI. 4, 103

Nielsen, L.R., and Ersbøll, A.K. (2005). Factors associated with variation in bulk-tank-milk Salmonella Dublin ELISA ODC% in dairy herds. Prev. Vet. Med. 68, 165-179

Nylin, B., Strøger, U. and Rønsholt, L. (2000). A retrospective evaluation of a Bovine Herpesvirus-1 (BHV-1) antibody ELISA on bulk-tank milk samples for classification of the BHV-1 status of Danish dairy herds. Prev. Vet. Med. 47, 91-105

R Development Core Team. (2014). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. www.R-project.org

Rasmussen, L.D., Kirkeby, C., Bødker, R., Kristensen, B., Rasmussen, T.B., Belsham, G.J., and Bøtner, A. (2013). Rapid Spread of Schmallenberg Virus-infected Biting Midges (Culicoides spp.) across Denmark in 2012. Transbound. Emerg. Dis. 61, 12-16

RStudio Team. (2015). RStudio: Integrated Development Environment for R. RStudio, Inc. Boston, MA. www.rstudio.com

Thierry-Mieg, N. (2006). A new pooling strategy for high-throughput screening: the Shifted Transversal Design. BMC bioinf. 7, 28

DESIGNING A TARGETED MEAT INSPECTION SYSTEM FOR *CYSTICERCUS BOVIS*

B.CHENGAT*, L.R.MARSHALL, J.GUITIAN

SUMMARY

Cysticercus bovis, is the larval stage of the zoonotic parasite *Taenia saginata* with a life cycle involving both cattle and humans respectively. Current meat inspection system has low sensitivity and requires all cattle to be inspected leading to huge economic burden. Therefore, in the interests of public health and food production efficiency, more targeted and cost effective meat inspection activities for the detection of *C.bovis* have been recommended. This study utilised cattle movement history data and meat inspection results from Great Britain (GB) to assess farm characteristics, cattle movement history and individual cattle characteristics, as potential predictors of *C.bovis* infection in cattle at slaughter. The results of these analyses were integrated into a simulation model, which showed significant potential for the utilisation of such information in a cost-effective methodology, to target meat inspection activities at cattle with a higher risk of *C.bovis* infection.

INTRODUCTION

Bovine cysticercosis is caused by the larval stage of the human tapeworm *T.saginata*. Humans are the definitive host and hold the adult tapeworm (taeniasis), while cattle act as the intermediate host and harbour the larvae (cysticercosis). Humans become infected after ingestion of raw or undercooked beef containing infective cysticerci. The disease does not typically cause major health problems in humans, being characterised by mild symptoms, if any (Dorny et al., 2010). Cattle become infected through accidental ingestion of food or water which is contaminated with human faeces containing viable *T.saginata* eggs. After 8-10 weeks the eggs have developed into larvae which establish in bovine skeletal and cardiac muscle, and less commonly in fat and visceral organs. They develop into cysticerci (viable cysts), remaining infective for approximately nine months before they eventually die and calcify, becoming non-infective, non-viable cysts (Hiepe, 2006).

A collection of estimates for the apparent prevalence of *C.bovis* in cattle within member states of the European Union were compiled within the European Food Safety Authority (EFSA) Scientific Report, published in 2010, on "*monitoring and reporting of Cysticercus in animals and foodstuffs in the European Union"* (Dorny et al., 2010). Based upon these estimates, in addition to more recent literature sources (reporting figures relating to 2011), the apparent prevalence of *C.bovis* infection in cattle (at individual animal level, based on post mortem inspection) appears to range between 0.01% and 0.06% in European countries (Dorny et al., 2010; Calvo-Artavia et al., 2013). However, as described below, this range may be an

---

* Bhagyalakshmi Chengat, Veterinary Epidemiology, Economics and Public Health Group, Royal Veterinary College, Hawkshead Lane, Hertfordshire, AL9 7TA, UK. Email: bchengat2@rvc.ac.uk

underestimate of the prevalence of *C.bovis* in the EU, due to the insensitivity of current meat inspection methods (Dorny et al., 2010).

The presence of *T.sanginata* cysts in cattle in the UK is determined during post mortem meat inspection and enforced through Regulation (EC) No 854/2004. This current slaughterhouse regulation requires that meat inspectors evaluate all susceptible bovine carcasses above six weeks of age, using the following palpation and incision techniques:

- visual examination and palpation of the tongue;
- two deep incisions in the external- and one in the internal- cheek muscles parallel to the mandible;
- visual examination of the heart (incised lengthwise to open ventricles and to cut through the interventricular septum;
- visual examination of the diaphragm and oesophagus

Carcasses with visible cysts are either downgraded (requiring extra handling and freezing to inactivate cysticerci) or condemned, depending upon the amount and type of visible cysts, (i.e. whether they are infective or non-infective). Whilst this process is necessary to detect carcases harbouring cysts and posing a public health risk, it does lead to significant economic losses.

The sensitivity of this visual post mortem meat inspection method is low (<30%) and positively correlated with the amount and size of the cysts present in a carcass (Dorny et al., 2010; Dorny & Praet, 2007; Allepuz, 2012). In recognition of this, the 2010 European Food Safety Authority (EFSA) Scientific Report on "*monitoring and reporting of Cysticercus in animals and foodstuffs in the European Union"* (Dorny et al., 2010) recommended the development of more sensitive tests for *C.bovis* detection at slaughter. However, such developments will take considerable time, and even if a more sensitive test is developed, it is likely that its use in all slaughtered cattle would not be practical or cost effective under the current meat inspection system as a whole.

One plausible solution to this complex portfolio of requirements, would be the development of a framework for more targeted meat inspection, with regard to the detection of *C.bovis*. Such targeted surveillance should be both economically and logistically feasible, without compromising public health. This system, instigating targeted meat inspection for the detection of *C.bovis,* could involve classifying animals based on their *a priori* risk of *C.bovis* infection, and conducting an 'enhanced' meat inspection on those identified as at high risk of being infected by point of slaughter. Such an 'enhanced' meat inspection would be engineered to be more sensitive than those undergone by animals classified as at lower risk of *C.bovis* infection.

It has been hypothesised that enlisting such a tiered system of meat inspection methodology, based upon classification of animals in this manner, would lead to a more efficient and effective system for the detection of *C.bovis* infected carcasses overall. The 2010 EFSA Scientific Report (Dorny et al., 2010) also supports this conclusion, emphasising the need for more targeted, risk-based and cost effective methods of *C.bovis* surveillance in cattle at slaughter.

Based on these recommendations, our study aimed to identify risk factors for *C.bovis* infection in cattle at slaughter in the UK. Both the individual characteristics of such cattle (movement history, age and sex), and the characteristics of the farm(s) of origin of these cattle, were assessed with regard to their potential as risk factors for *C.bovis* infection in cattle at slaughter. It was hypothesised that the identification of such risk factors could enable the classification of cattle arriving for slaughter into risk groups for *C.bovis* infection, based upon their individual characteristics and the profile of characteristics pertaining to the farms within their movement histories. As previously described, such a classification system has been identified as a potential route to enabling targeted meat inspection efforts for the detection of *C.bovis* in cattle at slaughter.

Data on the individual characteristics and movement history of cattle resident in the UK is comprehensively recorded. In GB, Collection and Communication of Inspection Results (CCIR), such as the diagnostic details of those *C.bovis* positive cattle detected during post mortem inspection, is facilitated through record storage within the Food Standards Agency (FSA) national meat inspection database. Food Chain Information (FCI) such as the identification details of the animal and farm of immediate origin, are also recorded within this database. The British Cattle Movement Service (BCMS) of the Rural Payments Agency (RPA) holds details of the individual characteristics and movement history (county parish holding (CPH) number and address of all farms) of any bovine slaughtered in GB. It is mandatory that a farmer should inform BCMS within three days of the movement of any individual bovine animal into, or within, GB (e.g. movements into and out of farms and into slaughter houses). This system enables the maintenance of a centralised and regulated identification system for cattle in GB, aiding with traceability “from farm to fork”, which is key in the investigation of disease outbreaks.

A systematic review of the literature, carried out as part of this study, discovered that several other studies have already explored both individual animal and farm characteristics as risk factors for *C.bovis* infection in cattle. For example, extensive studies carried out in Denmark and Switzerland identified evidence of farm-level risk factors, these included: cattle having (drinking) access to streams contaminated with effluent from sewage treatment plants; the presence of a railway line or car park close to cattle pasture; leisure activities close to cattle pasture; feeding purchased roughage to cattle; and holding public activities on cattle farms, such as open farm events or visits (Kyvsgaard et al., 1990; Flutsch et al., 2008).

We present the results of a study carried out to assess the relationship between i) farm-level risk factors and ii) individual animal level sex, age and movement history; and the likelihood of an animal being found as *C.bovis*–infected at slaughter.

MATERIALS AND METHODS

The overall design of this study, and analyses conducted, is represented in Fig. 1.

Data collection

Abattoirs across GB were contacted to enable the collection of CCIR records for all cattle detected as infected with *C.bovis* at slaughter (cases), over a two year period. In parallel, for every CCIR record pertaining to a *C.bovis* infected animal, the CCIR records of three uninfected animals (controls), which underwent slaughter on the same day, within the same abattoir, were also appended to this dataset. This process yielded a dataset comprising a large

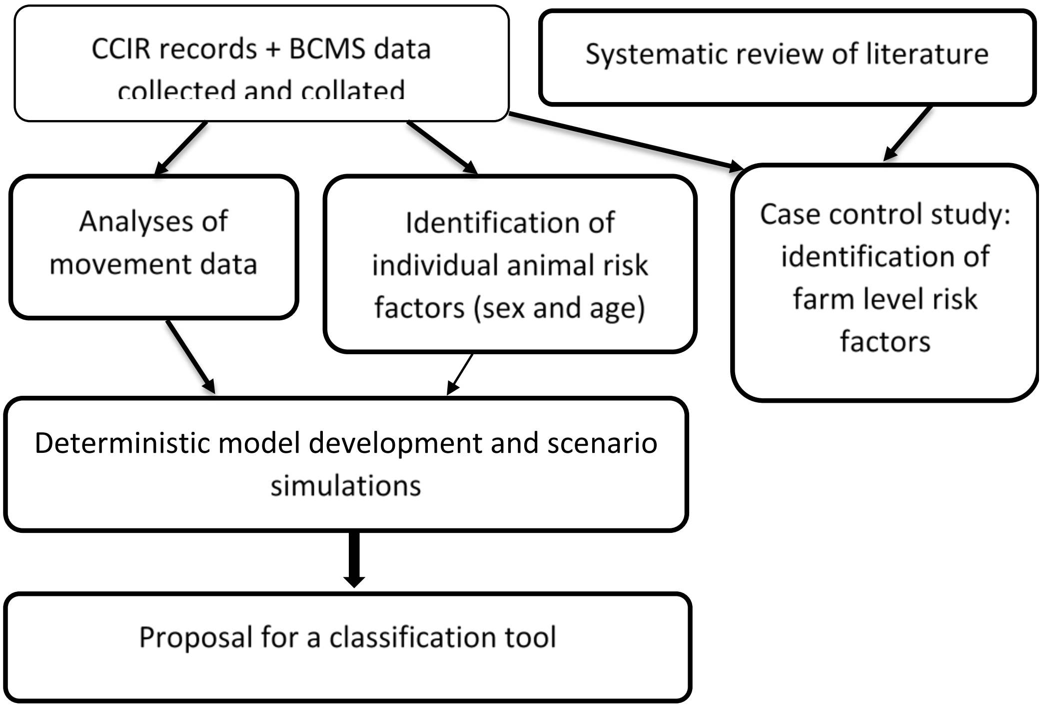


Fig. 1 Schematic representation of the study

number records for cattle infected with *C.bovis*, in combination with affiliated records for uninfected cattle, in a 3:1 ratio. BCMS then provided data on the movement history (for at least the previous three years), together with the sex and age at slaughter, for all cattle within the dataset. Only 1.5% of these animals had movement histories unaccounted for. Slaughterhouses and markets were excluded from the movement history data, after its receipt from BCMS. The final study sample of cattle and associated individual and movement history data, formulated on completion of this refinement process, was then utilised for further analyses. ‘Case’ and ‘control’ farms were selected from the collective data on the movement histories of the infected and uninfected cattle respectively, within the final study sample. A panel of experts from the field of C.bovis research were elicited in the development of separate selection criteria for case and control farms. Case farms were derived solely from the collective movement histories of infected cattle, and were defined as those farms cited more than once in the movement history of one or more infected bovines. Control farms were derived solely from the collective movement histories of uninfected cattle, i.e. no control farm existed in the movement history of an infected bovine.

All selected case and control farms received a postal questionnaire designed to collect information on putative risk factors, previously identified through the systematic review of published literature.

Data analysis

Identification of farm level risk factors: A case control study was carried out, to identify evidence for farm characteristics which may act as risk factors for *C.bovis* in cattle slaughtered in GB. Twenty-one farm characteristics, extracted from the completed questionnaires returned by case and control farms, were analysed as predictor variables, through univariable logistic regression methods. Within this analysis, the response variable was the assigned farm status; case or control. Those predictor variables with a P value of <0.02 within univariable analyses were then selected for involvement in multivariable logistic regression analyses utilising a stepwise backward elimination method; after consideration of their biological plausibility and potential for collinearity with other predictor variables. Logistic regression was carried out using epicalc package in R-software version 3.1.0. The elicitation of policy and industry advice lead to the conclusion not to incorporate the results of this analysis into the final deterministic model and scenario simulations for generation of a proposal for a classification tool at meat inspection.

Identification of individual animal risk factors: Logistic regression was carried out to assess the relationship between age at slaughter, sex, and the infection status of an animal. Age at slaughter was divided into two groups for each sex, namely 0-20 months, and greater than 20 months. This grouping held biological significance regards the structure of the cattle production industry. Logistic regression was carried out using STATAIC software version 14.

Analysis of movement data: Using the movement data and infection status of the animals within the dataset, a new variable, defined as: "whether the animal had been on a farm previously occupied by an infected animal" was created. Any change in the odds of *C.bovis* infection in cattle, associated with having resided on a farm in which a bovine previously diagnosed as infected at slaughter had resided, was assessed for the animals slaughtered within the second year of study.

Subsequently, network analysis was utilised (using the igraph package in R software) to visualise and describe the movement networks of case and control animals within the final study sample. The movement history data from only one control animal per case animal was used for the purpose of the network analysis. Furthermore, those animals that had made no movements between farms in their lifetime, and those animals that were imported, were excluded from the network analysis; animal movements to markets and slaughterhouses were also omitted. Farms acted as nodes (connection points) within the networks produced, with cattle movements expressed as the edges (the relationship between nodes) (Fig. 2).

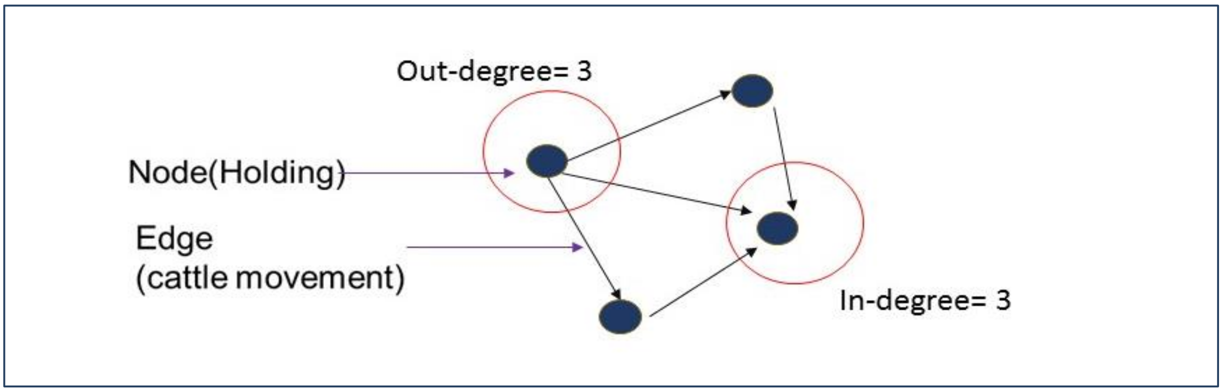


Fig. 2 Diagrammatic representation of a directed network: an 'out degree' of 3 indicates that there were 3 cattle movements off a farm, an 'in degree' of 3 indicates that there were 3 cattle movements onto a farm, within the network

Animals were divided into high risk and low risk groups for *C.bovis* infection, based on their individual profile of characteristics, derived from the results of analyses explained above. A deterministic model was created in @Risk software version 7.0 to simulate various scenarios of meat inspection. The sensitivity of current meat inspection was assumed to be 15%; whilst the sensitivity of an enhanced meat inspection methodology (hypothesised as one engineered to be more sensitive than those undergone by animals classified as at lower risk of *C.bovis* infection, as previously explained) was assumed to be 30%. Different scenarios included different proportions of animals subjected to either enhanced or current meat inspection, based upon the risk group they belonged to. The prevalence of *C.bovis* in the UK cattle population was calculated based on the apparent prevalence derived from the data collected for this study, and the assumed sensitivity of current routine meat inspection (15%) where the apparent prevalence is calculated as shown by Eq. (1).

Calculation of the apparent prevalence demonstrated in Eq. (1).

$$AP = T_P / T_N \tag{1}$$

Where AP is the apparent prevalence, T_p is the total number of positive carcasses detected in one year and T_n is the total number of carcasses inspected in that year.

The total number of animals inspected in one year was assumed to be 2.5 million, hence the model was iterated once with 2.5 million simulations. The outputs from the model gave the total number of infected animals per year, and the total number of infected animals detected through meat inspection per year.

RESULTS

The case control study revealed that farms close to a permanent source of human faecal contamination; and farms which utilised manure from animals other than cattle are positively associated with the occurrence of *C.bovis* in cattle derived from them. In addition, the analysis of individual animal characteristics (sex and age) produced evidence that male animals of less than 20 months of age at slaughter have a lower odds of *C.bovis* infection, by comparison to males of greater than 20 months of age at slaughter, and females of any age at slaughter, the latter two sex-age groups were thus classified as the ‘high risk’ sex-age group.

Analysis of the movement data revealed that 40% of the case animals and 14% of the control animals had inhabited a ‘high risk’ farm in which an animal, previously diagnosed as infected at slaughter, had formerly resided. Those animals with a history of being on a farm which is exhibited in the movement history of a previously infected animal have a four times higher odds of been found as positive for *C.bovis* at meat inspection.

Analysis of the separate movement networks of infected animals and uninfected animals respectively revealed a similar number of farms in the infected animal network or ‘case network’ to the number represented in the uninfected animal network or ‘control network’. The range of in-degree in the case network was wider than that within the control network, suggesting that some farms in the case network received many more ‘on-movements’ of cattle, than did farms within the control network. In the case network, several ‘hub’ farms received cattle from a large number of ‘supplier’ farms, however, in the control network there was only one such ‘hub’ farm, with a large number of suppliers.

The outputs of the simulations showed that a classification tool which utilised the following criteria could lead to a reduction in the total number of meat inspections required annually, while at the same time increasing the proportion of infected carcases detected at inspection, by comparison to the current situation:

- an enhanced inspection methodology (assumed sensitivity 30%) in animals with a movement history including one or more ‘high risk’ farms;

- current inspection methods enforced through Regulation (EC) No 854/2004 (estimated sensitivity 15%) in animals in the ‘high risk’ sex-age group;

- no inspection with regard to *C.bovis* in all remaining animals not falling into these categories.

DISCUSSION

The yields from the data collection and collation phases of this research indicate that cattle movement history data and inspection results are very comprehensively recorded in GB. This supports the notion of a classification tool, based on such data, whereby inspection activities can be targeted at cattle with a higher risk of *C.bovis* infection at slaughter. The analysis of data collected through the case control study suggest that it may not be plausible to design a tool for targeted meat inspection based on farm level risk factors. This was because the risk factors found to be significantly associated with a farm being a case farm are difficult to measure, and hence of low practical value.

The results of the movement data analyses were indicative of heterogeneities and linkages in the movement histories of cattle found to be infected with *C.bovis* at meat inspection, by comparison to those found to be uninfected. There was found to be a high degree of connectivity between animals diagnosed as infected with *C.bovis* at slaughter, through farms common to their movement histories. Such incites are suggestive of a key role, played by certain farms, as a source of *C.bovis* infection. Targeting meat inspection on animals arriving from such farms is likely to increase the chances of identifying positive animals, which is particularly valuable given the low sensitivity of procedures currently used. An increase in the proportion of infected carcasses detected when enhanced and current meat inspection methods are combined in high risk animals, with no inspection in the remaining animals, suggests a potential application of the model for targeted inspection.

The results of this study are suggestive of a strong potential for the use of previous *C.bovis* diagnoses at slaughter (CCIR) and cattle movement history records (stored within the RPA BCMS database), to cost effectively target meat inspection for *C.bovis* detection at slaughter, within GB abattoirs. Further work is therefore ongoing to validate the model by exploring more stochastic scenarios and through incorporation of an economic analysis.

ACKNOWLEDGEMENTS

The authors would like to thank the Food Standards Agency (FSA) for funding this research as part of a broader and ongoing project; and for assisting with the coordination of data collection. Additional thanks must go to our collaborators within Safe Food Solutions (SAFOSO). The authors would also like to thank the relevant FSA-approved abattoir personnel, RPA BCMS personnel and Svetlana Buzdugan from the RVC for their central roles in the initial data collection phase. Additionally, we would like to thank those experts in the field of *C.bovis* research and who provided much appreciated insights to this project.

REFERENCES

Allepuz, A., Gabriël, S., Dorny, P., Napp, S., Jansen, F., Vilar, M.J., Vives, L., Picart, L., Ortuno, A., Gutiérrez, J., and Casal, J. (2012). Comparison of bovine cysticercosis prevalence detected by antigen ELISA and visual inspection in the North East of Spain. Res. Vet. Sci. 92, 393–395

Calvo-Artavia, F.F., Nielsen, L.R., Dahl, J., Clausen, D.M. and Alban, L. (2013). Occurence and factors associated with bovine cysticercosis recorded in cattle at meat inspection in Denmark in 2004-2011. Prev. Vet. Med. 110**,** 177-182

Dorny, P. and Praet, N. (2007). Taenia saginata in Europe. Vet. Parasitol. 149, 22–24

Dorny, P., Vallée, I., Alban, L., Boes, J., Boireau, P., Boué, F., Claes, M., Cook, A.J.C., Enemark, H., van der Giessen, J., Hunt, K.R., Howell, M., Kirjusina, M., Nöckler, K., Pozio, E., Rossi, P., Snow, L., Taylor, M.A., Theodoropoulos, G., Vieira-Pinto, M.M. and Zimmer, I.A. (2010). Development of harmonised schemes for the monitoring and reporting of Cysticercus in animals and foodstuffs in the European Union. 1–23

Flutsch, F., Heinzmann, D., Mathis, A., Hertzberg, H., Stephan, R. and Deplazes, P. (2008). Case-control study to identify risk factors for bovine cysticercosis on farms in Switzerland. Parasitology. 135**,** 641-646

Murrell, K.D. and Dorny, P. (2005). WHO/FAO/OIE Guidelines for the surveillance, prevention and control of taeniosis/cysticercosis. OIE (World Organisation for Animal Health), 32

Kyvsgaard, N. C., Ilsoe, B., Henriksen, S. A. and Nansen, P. (1990). Distribution of Taenia saginata cysts in carcases of experimentally infected calves and its significance for routine meat inspection. Res Vet Sci. 49**,** 29-33

TRANSBOUNDARY DISEASES

INFERRING WITHIN-HERD TRANSMISSION PARAMETERS FOR AFRICAN SWINE FEVER OUTBREAKS IN THE RUSSIAN FEDERATION USING MORTALITY DATA

C. GUINAT[*], S. GUBBINS, A. GOGIN, L. DIXON AND D.U. PFEIFFER

SUMMARY

This study quantifies the transmission of African Swine Fever Virus (ASFV) within pig herds for outbreaks in the Russian Federation (RF) between 2010 and 2014. A stochastic compartmental model was fitted to pig mortality data to estimate epidemiological parameters using Approximate Bayesian Computation (ABC). Infected herds were characterised by intensive indoor pig production with stringent biosecurity measures, pig health and welfare management. Estimates of the median of the basic reproduction number (R_0) varied from 7.4 (95% credible interval (CI): 2.0-35.9) to 14.7 (95% CI: 3.0-55.0). Results also show that approximately 10-20 days can elapse between a single ASFV introduction into a herd and the first observation of increased pig mortality. These findings will inform future between-farm transmission models and allow the assessment of alternative control strategies in simulation studies to other Eastern Europe countries, given similar pig production systems.

INTRODUCTION

Since 2007, the pig industry in the Russian Federation (RF) has experienced severe African Swine Fever (ASF) outbreaks throughout the western part of the country, having a serious impact on pig production and trade, as well as on farmers' livelihoods (Gogin et al., 2013). The epidemic has resulted in the death of around 12,000 infected domestic pigs as well as approximately 550,000 domestic pigs which have been slaughtered in order to control the outbreaks (OIE, 2007-2015). According to the Russian Ministry of Agriculture, the total cost of ASF control was estimated at over $10 million in 2014. Pig farming is the second largest meat market in the RF, producing over 2 million tonnes of pig meat per year (2008-2015) valued at around £4 million (FAO, 2014). ASF is considered to be a major threat to the Russian pig industry as a result of various factors, such as the lack of farmers and local authorities' compliance with the reporting process, limited biosecurity in rural households and some industrial farms, and interactions between domestic pigs and wild boar populations (Gogin et al., 2013).

Within-herd transmission dynamics can be quantified using the transmission rate β, defined as the number of new infections per infectious individual per time unit and the basic reproduction number R_0, defined as the number of new infections that result from a single

---

[*] Claire Guinat, Royal Veterinary College, Veterinary Epidemiology, Economics & Public Health Group, Hawkshead Lane, Hatfield, AL9 7TA, UK / The Pirbright Institute, Ash Road, Pirbright, GU24 0NF, UK. Email: cguinat@rvc.ac.uk

infectious individual (Keeling and Rohani, 2008). These parameters provide insight into how rapidly and extensively a virus may spread, and estimates are needed for simulation models to assess the effectiveness of intervention strategies. Detailed epidemiological data on ASF outbreaks within herds are generally not collected, but mortality data are often available. Simulation-based approaches using approximate algorithms for Bayesian inference (e.g. Approximate Bayesian Computation: ABC) can be used to infer the underlying dynamics of infection from mortality data and, hence, permit estimation of epidemiological parameters (Tanaka et al., 2006; Conlan et al., 2012; Brooks-Pollock et al., 2014; Gubbins et al., 2014). In addition, ABC methods allow the uncertainty associated with the unobserved times of infection to be incorporated in analysis and also accommodate existing information from previous studies (Jewell et al., 2009). These methods offer the advantage of allowing inferences to be drawn, without the need for computing complicated likelihood functions, by simulating data similar to the actual observations (Lopes and Beaumont, 2010; Sunnåker et al., 2013).

The objective of this study was to estimate within-herd transmission parameters for African Swine Fever Virus (ASFV) using mortality data from outbreaks that occurred in large commercial pig herds in the RF from 2010 to 2014. A stochastic compartmental model was fitted to the data and posterior distributions of the model parameters were generated by repeated stochastic simulations of the model using an ABC approach.

MATERIALS AND METHODS

Data collection

Data on daily pig mortality were collected from nine fattening pig herds during the 2010-2014 ASF epidemic in the RF (Table 1). Herds were selected based on the availability and quality of the data collected on pig mortality. They were located in four western regions of the RF (Tver, Rostov, Stavropol and Oryol) where pig farming is mostly concentrated (FAO, 2014). Herd size ranged from 600 to 2145 pigs, with a median of 1614. During the observation period, higher numbers of dead pigs and clinical signs suggestive of ASF (depression, loss of appetite, redness of the skin and fever) were reported by farmers. ASFV infection was confirmed by virus isolation on randomly collected blood and tissue samples taken from dead pigs.

Model formulation

The within-herd transmission dynamics of ASFV were expressed using a stochastic SEIR epidemic model (Fig. 1), in which animals are susceptible (S), latent (E; i.e. infected, but not yet infectious), infectious (I) and removed (R; i.e. dead) (Keeling and Rohani, 2008). The durations of the latent and infectious periods were assumed to be gamma-distributed with means μ_E and μ_I and shape parameters k_E and k_I, respectively (i.e. with variances μ_E^2/k_E and μ_I^2/k_I), so the latent and infectious classes were subdivided into k_E and k_I stages each of mean duration μ_E/k_E and μ_I/k_I, respectively (Anderson and Watson, 1980). The force of infection was given by $\lambda(t) = \beta\, I(t)/N(t)$ where β is the within-herd transmission rate parameter, $I(t)$ is the number of infectious pigs and $N(t)$ is the total number of pigs at time t. This formulation assumes homogeneous mixing (i.e. individuals uniformly and randomly contact each other) and frequency-dependent transmission (i.e. the number of contacts is independent of the population size) (de Jong, 1995; Keeling and Rohani, 2008). A natural mortality rate (r_M) was included in the model.

Table 1. Daily mortality data from nine ASF-infected pig herds in the RF (2010-2014)

	Herd number								
	1	2	3	4	5	6	7	8	9
Initial number of pigs	1614	1949	1753	1833	1320	600	600	600	2145
Day	Number of dead pigs								
1	1	0	0	1	0	0	2	0	0
2	0	1	0	2	0	0	0	0	7
3	0	1	0	5	4	0	1	0	7
4	1	1	0	7	6	0	0	0	3
5	0	2	1	5	4	1	2	0	2
6	0	3	1	7	4	1	1	2	0
7	1	1	1	7	6	2	4	0	5
8	1	9	2	8[a]	8[a]	2	2	3	1
9	0	6	2	4	6	1[a]	8[a]	3[a]	0
10	0	4	5	5	1	0	12	4	6
11	0	4[a]	4[a]	4	3	0	8	3	0
12	1	11	9	10	5	1	6	0	1
13	1	3	1	2	9	1	5	1	4
14	0	5	3	14	8	8	0	0	0
15	4	7	3	18	19	b	b	b	10
16	6	2	5	3	b				36
17	5	3	6	b					24[a]
18	6	b	b						17
19	1								18
20	8								7
21	0								40
22	1[a]								42
23	11[a]								31
24	13								12
25	B								b

a: day of ASFV positive diagnosis

b: day of culling of all animals as part of disease control

ABC rejection sampling

Prior distributions for model parameters (Table 2) were determined in such a way that they were concentrated around estimates from previous studies of ASFV strains currently circulating in the region (Gulenkin et al., 2011; Guinat et al., 2015). Gamma prior distributions were used for the within-herd transmission rate β (mean 2, shape 2), the mean duration of the latent period μ_E (mean 6.25, shape 10), the shape parameter of the latent period k_E (mean 19.39, shape 5), the mean duration of the infectious period μ_I (mean 9.12, shape 10) and the shape parameter of the infectious period k_I (mean 22.20, shape 5). An exponential prior distribution was used for the natural mortality rate r_M (0.0002), calculated from daily mortality data obtained from 34 surrounding ASF-free pig herds. All prior distributions were assumed to be independent of each other.

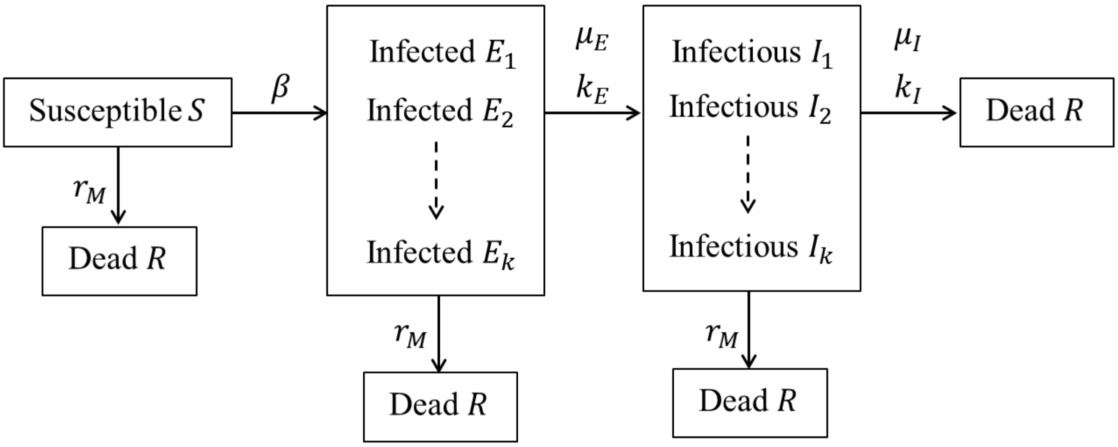


Fig. 1 Flow diagram of the within-herd transmission dynamics of ASFV infection including the number of susceptible pigs *S(t)*, the number of infected pigs *E(t)* (divided into k_E stages), the number of infectious pigs *I(t)* (divided into k_I stages) and the number of removed/dead pigs *R(t)*. Movement flows between compartments depend on the within-herd transmission rate (β), the mean duration of the latent period (μ_E), the shape parameter for the latent period (k_E), the mean duration of the infectious period (μ_I), the shape parameter for the infectious period (k_I) and the natural mortality rate (r_M)

Table 2. Prior distributions for model parameters for the within-herd transmission of ASFV

Parameter	Description	Prior distribution
β	Within-herd transmission rate (day^{-1})	Gamma (2, 2)
μ_E	Mean duration of latent period (day)	Gamma (6.25, 10)
k_E	Shape parameter for latent period	Gamma (19.39, 5)
μ_I	Mean duration of infectious period (day)	Gamma (9.12, 10)
k_I	Shape parameter for infectious period	Gamma (22.20, 5)
r_M	Natural mortality rate (day^{-1})	Exponential (0.0002)

Posterior distributions for model parameters were generated using an ABC rejection sampling algorithm (Pritchard et al., 1999; Sunnåker et al., 2013):

(1) A parameter set θ is sampled from the joint prior distribution $\pi(\theta)$;

(2) The time of infection for each herd *h* is sampled uniformly between 30 days prior to the first observation and the last observation. The daily pig mortality is simulated in each herd *h* during the observation period, using the model with the sampled parameter set θ;

(3) The simulated (*sim*) mortality is compared to the observed (*obs*) mortality independently for each herd *h* on day *t* (denoted by M_{ht}) and the parameter set θ (and introduction time) for the herd *h* is accepted if the distance measure D_h is below the acceptance criterion $\mathcal{E}_h$ Eq. (1), that is,

$$D_h = \sum_t |M_{ht}^{(obs)} - M_{ht}^{(sim)}| \leq \varepsilon_h \tag{1}$$

(4) Steps (1)-(3) are repeated until 1,000 parameter sets have been accepted so that posterior distributions can be estimated with reasonable accuracy (Gelman et al., 2014) with $\mathcal{E}_h$ selected based on visual assessment of model fit.

(5) The accepted parameter sets provide a sample from the joint posterior distribution approximated by the procedure.

The basic reproduction number (R_0) was calculated using the relationship $R_0 = \beta\mu_I$ (Keeling and Rohani, 2008). All statistical inferences were performed using R statistical software.

RESULTS

Posterior distributions for each model parameter are shown in Fig. 2. Informative posteriors were obtained for the transmission rate β and the mortality rate r_M. The inferred values for the transmission rate β were significantly different amongst herds. Among the herds, the posterior median for β ranged from 0.9 (95% CI: 0.3-3.9) (herd 2) to 1.7 (95% CI: 0.4-5.9) (herd 6) per day, while the posterior median for r_M ranged from 1.1×10^{-4} (95% CI: 0.0×10^{-4}-5.0×10^{-4}) (herd 1) to 3.1 (95% CI: 0.1×10^{-4}-12.6×10^{-4}) (herd 2) per day.

Among the herds, the posterior median for R_0 varied from 7.4 (95% CI: 2.0-35.9) to 14.7 (95% CI: 3.0-55.0) (Fig. 3).

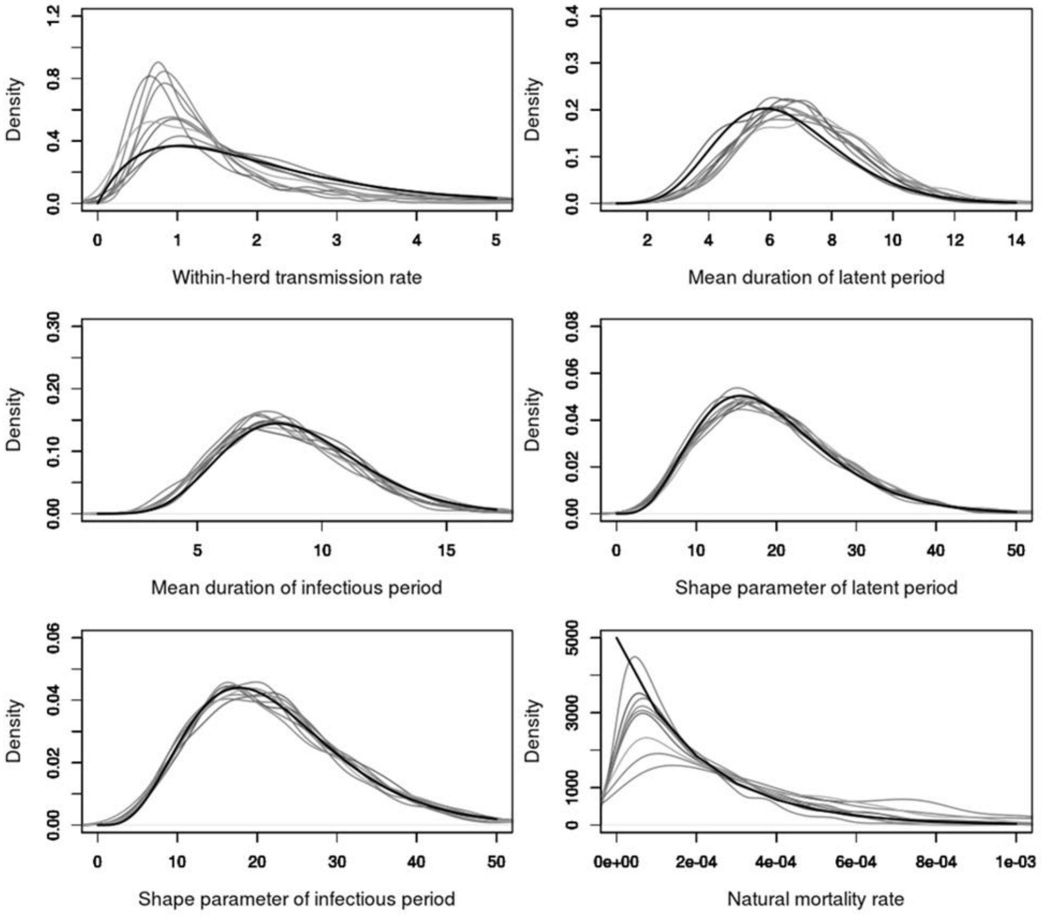


Fig. 2 Posterior and prior densities for model parameters describing within-herd transmission of ASFV. Prior distributions are displayed in black; posterior distributions are displayed in grey

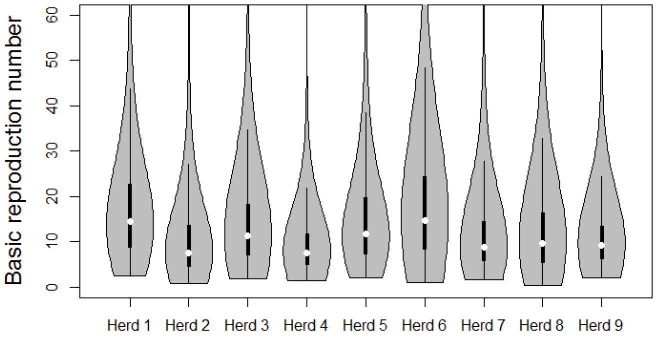


Fig. 3 Posterior densities of the R_0 for within-herd transmission of ASFV. The shape width indicates the probability density, the white dot is the median, the thick black bar represents the interquartile range and the thin black bar represents the 95% CI

Informative posteriors for the period of infection were obtained (Fig. 4), with ASFV most likely to have been introduced for most herds around 10-20 days before the observation period began. Later introductions could be observed for herds with lower mortalities in the first days of the observation period (such as herds 1, 2 and 8), which could be accounted for by natural mortality rather than ASFV infections. The model resulted in an acceptable fit to the mortality data from nine ASF-infected pig herds (Fig. 5).

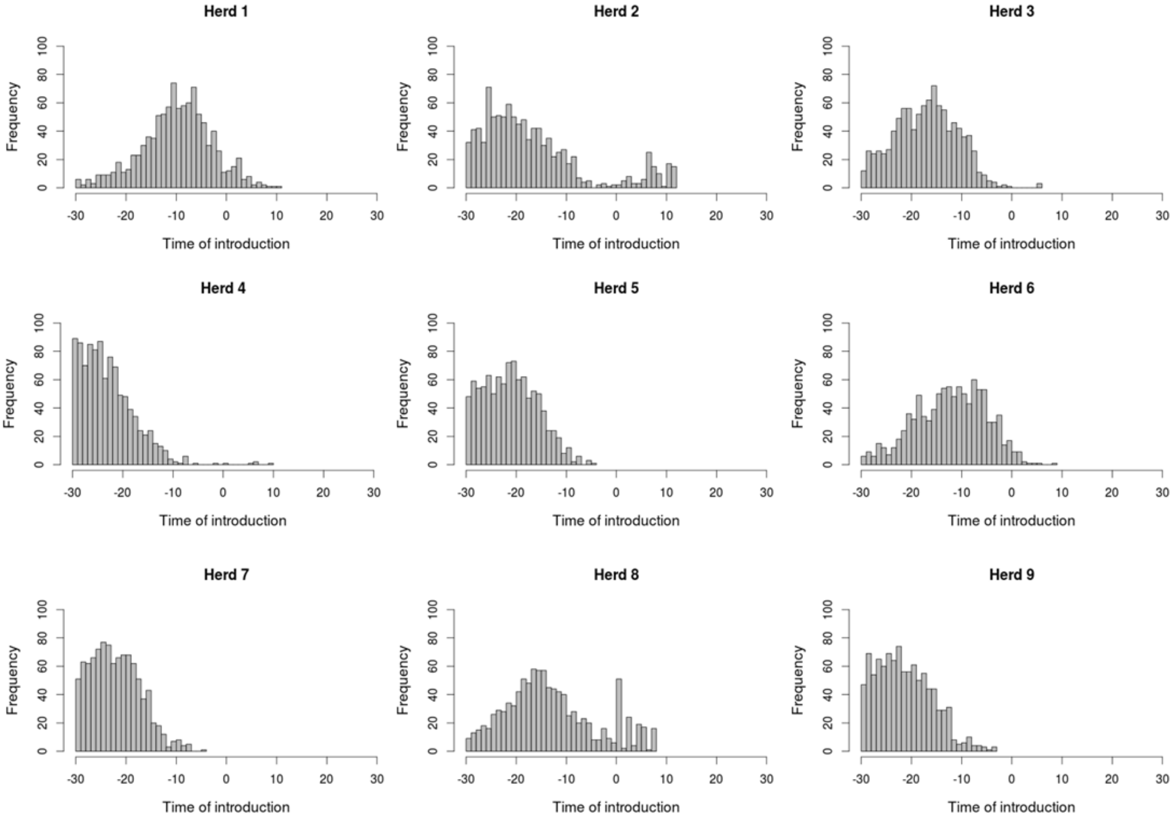


Fig. 4 Posterior distributions for the time of introduction (relative to the start of the observation period) for within-herd transmission of ASFV

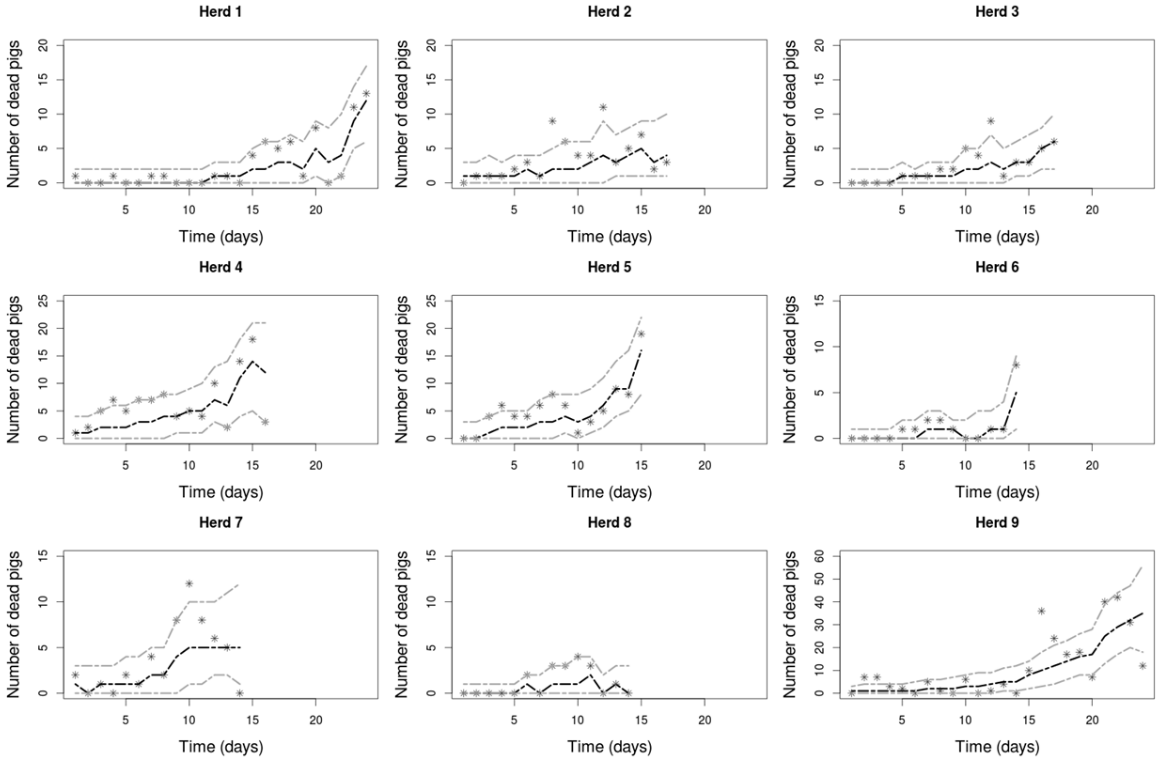


Fig. 5 Observed (asterisk dots) and simulated (median (black dashed line) and 95% CI (grey dashed lines)) daily number of dead pigs for the within-herd transmission of ASFV. Results are shown for 1,000 replicates of the model with parameters sampled from the joint posterior distribution

DISCUSSION

This study has demonstrated the usefulness of ABC methods for drawing inferences about disease outbreaks, even where there is only limited available field data. Estimates of within-herd transmission parameters for ASFV were based on mortality data, which was easily available since they are routinely recorded by pig farmers from large industrial pig farms in the RF. An increase in pig mortality was a sensitive signal for detecting ASF occurrence. Farmers should therefore be encouraged to monitor mortality, as this dataset has proven to be most useful for the estimation of key transmission parameters.

Transmission estimates were significantly different between herds. This was likely due to differences in the contact structure, such as intensity and frequency of contact, and in farm management practices (Hutchings and Harris, 1997; Lurette et al., 2008). The herds originated from industrial farms that were breeding indoor fattening pigs using high-level sanitary measures. In the RF, this farm type represents the majority of pig farms (65%); individual farms and rural households account for 5% and 30% of pig farms, respectively (FAO, 2014). As a result, ASFV transmission estimates may apply to other Eastern Europe countries with comparable pig production systems in which similar ASFV strains circulate. Findings also suggest that by the time pig farmers notice an increase in pig mortality, ASFV may have been spreading within the herd for 10-20 days. This is also important for disease control, as it allows the tracing back of contacts that occurred between infected and susceptible herds, between the time of virus introduction and the implementation of culling (Elbers et al., 2001).

Investigation reports suggest that the possible pathways for ASFV introduction into these herds were contact with potentially contaminated wild animals (birds, rodents), sharing of equipment and movement of animal workers between herds, ingestion of potentially contaminated feed or food brought in by animal workers. However, relevant data, such as on visitors and vehicle management, disinfection procedures and rodent control, were not available to better establish the causes and sources of ASFV introduction. Estimates for R_0 were similar to those reported by previous studies of ASFV (Gulenkin et al., 2011). These estimates were also based on daily pig mortality data collected in three farms from Stavropol and Orenburg regions in 2008-2009.

The advantage of ABC methods is that they can be used with complex models and large datasets, without being too computationally demanding (Lopes and Beaumont, 2010; Sunnåker et al., 2013). However, care needs to be taken when considering the credible intervals of the parameter estimates, as they could be increased due to the loss of information when summary statistics are used to approximate the likelihood. In addition, ABC rejection sampling can be computationally inefficient, requiring a very large number of simulations to generate sufficient accepted parameter sets (Toni et al., 2009). In this study, this has not been the case, but alternative, more efficient methods (e.g. Markov chain-Monte Carlo or sequential Monte Carlo methods) may be needed, especially when the posteriors are very different from the priors (Toni et al., 2009).

More data are needed to improve estimates of ASFV transmission. There were other potential routes of within-herd ASFV transmission, such as fomites (e.g. pig bedding and worker clothing) or aerosols, which may influence the extent of the outbreak (Costard et al., 2013). Pigs were assumed to be equally susceptible and able to mix randomly, although this may differ from actual housing conditions. Multiple virus introductions were not considered, although this is likely to result in a quicker increase in mortality and a shorter time period between ASFV infection and detection.

While there is scope for further improvement in data collection, the ABC methods used here could be applied in epidemiological analysis of animal disease outbreaks. The method could be also employed to model other infectious diseases that result in high mortality and to herds in which daily mortality data are available. ABC produced useful outputs that can make an important contribution in modelling of between-farm transmission dynamics of ASFV and may lead to more effective prevention and control measures.

ACKNOWLEDGEMENTS

The authors are grateful to the National Institute of Veterinary Virology and Microbiology (VNIIVViM, RF) for providing the data. They also thank Jan Kim (Pirbright Institute, UK) for compiling the original Fortran code so that it could be used in R, Timothée Vergne (RVC, UK) for providing valuable advice in relation to R coding, Milen Georgiev (RVC, UK) and Svetlana Buzdugan (RVC, UK) for translating the outbreak reports from Russian to English.

REFERENCES

Anderson, D. and Watson, R. (1980). On the spread of a disease with gamma distributed latent and infectious periods. Biometrika 67, 191-198

Brooks-Pollock, E., Roberts, G.O. and Keeling, M.J. (2014). A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature 511, 228-231

Conlan, A.J., McKinley, T.J., Karolemeas, K., Pollock, E.B., Goodchild, A.V., Mitchell, A.P., Birch, C.P., Clifton-Hadley, R.S. and Wood, J.L. (2012). Estimating the hidden burden of bovine tuberculosis in Great Britain. PLoS Comput. Biol. 8, e1002730. doi: 1002710.1001371/journal.pcbi.1002730

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. and Pfeiffer, D. (2013). Epidemiology of African swine fever virus. Virus Res. 173, 191-197

de Jong, M.C. (1995). How does transmission of infection depend on population size? In: Mollison, D. (Ed.), Epidemic models: their structure and relation to data. Cambridge University Press, Cambridge, 84

Elbers, A., Moser, H., Ekker, H., Crauwels, P., Stegeman, J., Smak, J. and Pluimers, F. (2001). Tracing systems used during the epidemic of classical swine fever in the Netherlands, 1997-1998. Rev. Sci. Tech. (International Office of Epizootics) 20, 614-629

FAO (2014). Russian Federation - Meat sector review [available at: http://www.fao.org/3/a-i3533e.pdf (accessed July 2015)]

Gelman, A., Carlin, J.B., Stern, H.S. and Rubin, D.B. (2014). Bayesian data analysis. Taylor and Francis London

Gogin, A., Gerasimov, V., Malogolovkin, A. and Kolbasov, D. (2013). African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012. Virus Res. 173, 198-203

Gubbins, S., Turner, J., Baylis, M., Van der Stede, Y., van Schaik, G., Abrahantes, J.C. and Wilson, A.J. (2014). Inferences about the transmission of Schmallenberg virus within and between farms. Prev. Vet. Med. 116, 380-390

Guinat, C., Gubbins, S., Vergne, T., Gonzales, J., Dixon, L. and Pfeiffer, D. (2015). Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. Epidemiol. Infect., 1-10

Gulenkin, V., Korennoy, F., Karaulov, A. and Dudnikov, S. (2011). Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. Prev. Vet. Med. 102, 167-174

Hutchings, M. and Harris, S. (1997). Effects of farm management practices on cattle grazing behaviour and the potential for transmission of bovine tuberculosis from badgers to cattle. Vet. J. 153, 149-162

Jewell, C.P., Kypraios, T., Neal, P. and Roberts, G.O. (2009). Bayesian analysis for emerging infectious diseases. Bayesian Analysis 4, 465-496

Keeling, M.J. and Rohani, P. (2008). Modeling infectious diseases in humans and animals. Princeton University Press

Lopes, J. and Beaumont, M. (2010). ABC: a useful Bayesian tool for the analysis of population data. Infection, Genet. Evol. 10, 825-832

Lurette, A., Belloc, C., Touzeau, S., Hoch, T., Seegers, H. and Fourichon, C. (2008). Modelling batch farrowing management within a farrow-to-finish pig herd: influence of management on contact structure and pig delivery to the slaughterhouse. Animal 2:1, 105-116

OIE (2007-2015). WAHID Interface: African swine fever - Summary of immediate notifications and follow-ups [available at: http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Immsummary (accessed in July 2015)]

Pritchard, J.K., Seielstad, M.T., Perez-Lezaun, A. and Feldman, M.W. (1999). Population growth of human Y chromosomes: a study of Y chromosome microsatellites. Mol. Biol. Evol. 16, 1791-1798

Sunnåker, M., Busetto, A.G., Numminen, E., Corander, J., Foll, M. and Dessimoz, C. (2013). Approximate bayesian computation. PLoS Comput. Biol. 9, e1002803

Tanaka, M.M., Francis, A.R., Luciani, F. and Sisson, S. (2006). Using approximate Bayesian computation to estimate tuberculosis transmission parameters from genotype data. Genetics 173, 1511-1520

Toni, T., Welch, D., Strelkowa, N., Ipsen, A. and Stumpf, M.P. (2009). Approximate Bayesian computation scheme for parameter inference and model selection in dynamical systems. Roy. Soc. Interface J 6, 187-202

RIFT VALLEY FEVER IN MADAGASCAR: ESTIMATION OF THE YEAR-SPECIFIC FORCE OF INFECTION IN CATTLE FROM MID-2007 TO MID-2013

M.M. OLIVE[*], V. GROBOIS, A. TRAN, L.A. NOMENJANAHARY, M. RAKOTOARINORO, J.M. HERAUD AND V. CHEVALIER

SUMMARY

In 1990-1991 and 2008-2009, two major outbreaks of Rift Valley fever (RVF) occurred in Madagascar. Previous studies suggested that some areas were favourable to enzootic dynamics whereas others were suitable for epizootic dynamics. We aimed to (i) identify environmental factors underlying the enzootic and the epizootic epidemiological patterns, (ii) estimate the annual forces of infection (FOI) from mid-2007 to mid-2013 and describe it's variation over time in these two contrasted situations. We estimated year-specific FOI in both enzootic and epizootic areas using a generalized linear model fitted using data from 2014 sero-survey results obtained from individuals of known age. In enzootic areas, during the 2007 pre-epizootic period the FOI reached 0.11. After the 2008-2009 epizootics, the FOI was low but differed from 0 suggesting an enzootic circulation. By contrast, in epizootic areas, outbreak periods when FOI was high, alternated with periods when FOI was null.

INTRODUCTION

Rift Valley Fever Virus (RVFV) is an arthropod-borne zoonotic virus belonging to the Bunyaviridae family and responsible for outbreaks of Rift Valley Fever (RVF) in Africa, on the Arabian Peninsula and on some islands in the South Western Indian Ocean (SWIO). The virus affects mainly ruminants and humans. Infection causes abortion in pregnant ruminants and acute deaths in newborns. In humans, in the majority of cases, infection is asymptomatic or mild causing fever, headaches and muscle pains. However, in epidemic periods, severe cases occur, characterized by retinitis, encephalitis or haemorrhagic forms that may lead to death. Ruminants are infected through vector bites and probably also by direct contact with infected tissues, such as blood or abortion products (Nicolas et al., 2014, Pepin et al., 2010). Humans are mainly infected through direct transmission with cattle infected tissues (Pepin et al., 2010) although vectorial transmission in humans has been suspected (Pourrut et al., 2010, Nakounné et al., 2000). RVFV circulation has been reported in several eco-climatic areas from the arid region of Western Africa and the Arabian Peninsula to the humid highlands of Madagascar (Chevalier, 2013). Because RVF epidemiology is highly dependent on environmental and climatic factors as well as local practices, the dynamics of RVF transmission seem to be regionally specific. The exploration of the spatio-temporal pattern of RVF outbreaks related to environmental factors, such as sea surface temperature anomalies, coupled with satellite

---

[*] Marie-Marie Olive, CIRAD, AGIRs Unit, Montpellier, France & Institut Pasteur de Madagascar, Virology Unit, Antananarivo, Madagascar. Email: marie-marie.olive@cirad.fr

normalized difference vegetation index data, has allowed the production of predictive models for RVF in Eastern Africa (Linthicum et al., 1999). However, the performances of this predictive model were poor in some countries, such as Madagascar (Anyamba et al., 2010).

Madagascar is a large island in the SWIO with a great diversity of eco-climatic patterns (Cornet, 1974). In 1990-1991 and 2008-2009, two major outbreaks of RVF occurred on the island (Andriamandimby et al., 2010, Morvan et al., 1992, Morvan et al., 1991). Most of the RVF outbreaks were in the Malagasy highlands whereas a recent study showed that the western, north-western and eastern parts of the island are favourable to enzootic transmission (Olive, unpublished data). However, due to the isolation of several Malagasy regions and the limited resources available to implant veterinary surveillance, under-reporting of RVF cases in ruminants was suspected. Gaining a better knowledge of variation in the transmission intensity of RVF should improve surveillance and control planning for this disease in Madagascar. In the absence of reliable case report data, seroprevalence data can be used to estimate the force of infection (FOI) of RVF, a good indicator of its infection dynamics.

Thus, our objectives were to (i) identify environmental factors underlying epizootic RVF epidemiological patterns, (ii) estimate annual FOIs from mid-2007 to mid-2013 and describe variation in FOI over time in both the enzootic and epizootic contrasted situations.

MATERIALS AND METHODS

First, Malagasy environments were characterized using integrative covariates derived from Multiple Factor Analysis (MFA) performed with climatic and landscape factors. Environmental factors associated with enzootic circulation of RVF were previously identified (Olive, unpublished data). To identify environmental factors associated with RVF epizootics, occurrences of RVF cases were analysed using a Generalized Linear Model (GLM) including MFA factors and the isolation of the communes as explanatory variables. Principal Component Analysis (PCA) combined with Hierarchical Clustering (HC) was performed to group Malagasy communes according the environmental factors involved in the epizootic and enzootic epidemiological patterns. Finally, we estimated year-specific FOI in both areas where enzootic and epizootic transmission patterns prevailed using a GLM fitted on data from a serological survey, undertaken in 2014, on cattle of known age.

Multiple Factor Analysis

Environmental variables were selected according to their putative influence on mosquito density and population dynamics. These environmental variables were extracted from several sources presented in Table 1.

Synthetic variables characterizing the environment of communes were computed using a MFA combining the climatic and landscape variables (Escofier & Pagès, 1994). By performing a factor analysis inside each variable category and then between categories, MFA produces a quantitative summary of the initial set of variables taking the form of a set of linear combination of variables, referred to as factors (Escofier & Pagès, 1994). The climatic category included the annual mean of day and night Land Surface Temperature (LST), and the annual mean and seasonality of precipitation (Table 1). The landscape category included the percentage of landscape class (water bodies and vegetation) describing the commune, and the annual mean and seasonality of the Normalized Difference Vegetation Index (NDVI; Table 1). The value of each factor was computed for each of the 1578 Malagasy communes.

Table 1. Environmental variables and their sources

Covariate	MFA category	Source
Mean LST-day	Climatic	Moderate Resolution Imaging Spectroradiometer (MODIS; http://iridl.ldeo.columbia.edu/)
Mean LST-night	Climatic	MODIS(http://iridl.ldeo.columbia.edu/)
Mean precipitation	Climatic	MODIS(http://iridl.ldeo.columbia.edu/)
Seasonality of precipitation	Climatic	MODIS(http://iridl.ldeo.columbia.edu/)
Mean NDVI	Landscape	MODIS(http://iridl.ldeo.columbia.edu/)
NDVI seasonality	Landscape	MODIS(http://iridl.ldeo.columbia.edu/)
Percentage of surface covered by Herbaceous	Landscape	Globcover project (Bontemps et al., 2011)
Percentage of surface covered by Shrub	Landscape	Globcover project (Bontemps et al., 2011)
Percentage of surface covered by Woody/ Trees	Landscape	Globcover project (Bontemps et al., 2011)
Percentage of surface covered by Artificial Surfaces (urban area)	Landscape	Globcover project (Bontemps et al., 2011)
Percentage of surface covered by Crop	Landscape	Globcover project (Bontemps et al., 2011)
Percentage of surface covered by Irrigated area	Landscape	Global Map of Irrigation Areas (GMIA) from AQUASTAT-FAO (Siebert & Frenken, 2014)
Percentage of surface covered by Wetland	Landscape	International Panel on Climate Change (IPCC; (Batjes, 2010)
Percentage of surface covered by Water bodies	Landscape	DIVA-GIS (http://www.diva-gis.org/)
Percentage of surface covered by Marshland	Landscape	Geographical Information Systems at the Royal Botanic Gardens, Kew (Moat & Du Puy, 1997)

Location of RVF outbreaks

Ruminant case data describing the 1990-1991 and 2008-2009 epizootics were collected at the commune administrative level using a bibliography and database maintained by the Institut Pasteur de Madagascar. We considered a commune as affected by RVF when an outbreak occurred in ruminants (abortion and/or high mortality) and was confirmed by laboratory analysis (detection of Immunoglobulin M directed against RVFV by ELISA, detection of RVFV by RT-PCR and/or by virus isolation). Human cases were not considered.

Identification of enzootic and epizootic areas

Firstly, communes were classified according their isolation from the capital city, Antananarivo, where the head of the veterinary services is based. For each commune, we first assessed the distance between the centre of the commune and its capital settlement as well as the accessibility between them (main road, secondary road or no road). We then computed the distance between the commune capital and Antananarivo as well as the accessibility between them. The road data were available from DIVA-GIS (http://www.diva-gis.org/). To classify the communes according to their isolation status, HC combined with PCA was performed using these variables. To identify factors (environmental and isolation) associated with RVF epizootics, occurrence of RVF cases was then integrated as a binomial response in a GLM with MFA factors and isolation status categories as explanatory variables.

Environmental factors associated with enzootic circulation of RVF were previously identified (Olive, unpublished data).

Once the environmental factors associated with epizootic dynamics were identified, HC combined with PCA was performed to group Malagasy communes according to the environmental factors involved in the enzootic and epizootic epidemiological cycles.

Estimation of the year-specific force of infection

A serological serosurvey of cattle of known age was used to estimate year-specific FOI. This survey was performed from March to May 2014 in 15 communes of Madagascar. Cattle sera were analyzed using a commercial ELISA kit (ID-Vet, ID Screen®) to detect antibody directed against RVFV (Kortekaas et al. 2013).

Year–specific FOIs were estimated in the enzootic and epizootic regions. The underlying model was the catalytic model. In this model, it is considered that individuals of age a at the time of sampling in 2014 have been exposed to the following infection forces sequence: $\lambda_{2014}, \lambda_{2014-1}, ..., \lambda_{2014-i}$. According to this model and under the hypothesis that infection does not result in additional mortality, the number of seronegative individuals among sampled individuals of age a follows a binomial distribution B(pa,Na) where Na is the number of individuals of age a sampled and pa is a function of year-specific infection forces as described by Eq. (1).

$$p_a = \prod_{i=1}^{i=a}(1 - \lambda_{2014-i}) \quad (1)$$

The model can be expressed using a generalized linear model with the log of the number of seronegative individuals as the response (poisson family) and one binary explanatory variable for each year prior to 2014 indicating whether the individuals having the focal age were already born in that year and thus exposed to the year-specific FOI. The log of the number of sampled individuals of the focal age was integrated as an offset in the statistical models.

Spatial analyses were computed with the Quantum GIS software and data analyses were performed using R software (QGIS Development Team, 2009; R Development Core Team, 2011).

RESULTS

Multiple Factor Analysis

From the MFA, four factors contributing to 60% of the total variance were selected. Table 2 shows the correlation between each quantitative covariate included in the MFA and each of these four factors:

- factor1 opposed ecosystems with low annual NDVI but high seasonality dominated by herbaceous vegetation and with low surfaces of crops under dry and hot climatic conditions (large positive values) to ecosystems with high annual NDVI but low seasonality including crops under wet and less hot climatic conditions (large negative values). The communes with the largest positive values for factor1 are located in the southern and south-western part of Madagascar while the communes with the largest negative values for factor1 are located in the north-eastern part;

- factor2 opposed ecosystems with low annual NDVI but high seasonality including ligneous vegetation and irrigated areas (rice fields) under climatic conditions characterized by low night temperatures (large positive values) to ecosystems with high annual NDVI but low seasonality including crops under climatic conditions characterized by warmer night temperatures (large negative values). The communes with the largest positive values of factor2 are located in the central highlands. The communes with the largest negative values are mostly located in the eastern part of the island;

- factor3 was a rainfall seasonality index. The highest values of factors3 (highly seasonal rainfall) are observed in the northern, north-western and western part of the island;

- factor4 represented a humid area index (marshlands, wetland and irrigated areas). The highest values are mostly located on the eastern-coast, the north-western and western part of the island.

Location of RVF outbreaks in ruminants

We identified 43 communes affected by RVF in ruminants during the 1990-1991 and 2008-2009 outbreaks, representing 2.7% of the 1578 Malagasy communes (Fig. 1). More than 90% of these communes were located in the Malagasy highlands; the remaining 10% were located in the north and east of Madagascar. The majority of the outbreaks recorded affected cattle (93% cattle vs. 7% small ruminant). Twenty-one communes recorded outbreaks in 1990-1991 and 24 communes recorded outbreaks in 2008-2009. Two communes were affected by RVF during both episodes. Outbreaks in 1990 were reported in March on the eastern-coast, at the end of the rainy season, and RVF was then confirmed in May and June during the dry season. Second episodes occurred during the rainy season in 1991. The outbreaks in 2008 and 2009 occurred during two successive rainy seasons.

Table 2. Correlation between quantitative covariates included in the MFA and factors (factor1, factor2, factor3 and factor4)

	factor1	factor2	factor3	factor4
Mean LST-day	0.92	-0.19	0.,11	/
Mean LST-night	0.50	-0.66	0.14	0.26
Mean precipitation	-0.70	/	0.32	0.31
Seasonality of precipitation	0.17	-0.15	0.82	0.09
Mean NDVI	-0.83	-0.34	/	/
NDVI seasonality	0.63	0.45	0.08	0.08
Herbaceous	0.84	-0.12	-0.24	0.11
Shrubs	0.11	0.40	0.30	-0.17
Wood-trees	-0.33	0.56	0.37	-0.19
Urbanization	/	0.14	-0.30	0.27
Crops	-0.62	-0.61	-0.24	0.10
Irrigated area	/	0.66	-0.08	0.37
Wetlands	/	0.24	-0.39	0.46
Water bodies	/	/	0.07	0.22
Marshlands	/	0.07	0.18	0.71

Identification of enzootic and epizootic areas

Three clusters were used to classify Malagasy communes according to their isolation status: (1) little isolated (n=534); (2) moderately isolated (n=517) and (3) highly isolated (n=527). Thirty-one little isolated communes notified RVF cases whereas 11 cases were recorded in the moderately isolated communes and 1 case in the highly isolated communes.

Occurrence of RVF cases was positively associated with high values of factor2 ($p<0.001$) and high cattle density ($p<0.005$) and was negatively linked to high values of factor4 ($p<0.01$). In addition, communes little isolated declared more cases than others (OR=14, $p<0.05$).

Finally, to classify the location of communes in enzootic or epizootic areas, we performed HC combined with PCA integrating the values of factor2 and factor4. Two clusters were provided by the HC, one with very low values of factor2 which we considered as favourable to RVF enzootic circulation in the southern, north-western and eastern part of the island and one with high values of factor2 which we considered as favourable to RVF epizootics and located in the highlands (Fig. 1). Of the 1578 Malagasy communes, 950 were classified as located in enzootic areas and 628 in epizootic areas.

One commune affected by RVF in 1990 was situated in the enzootic area. Located on the eastern-coast, this commune was the first which recorded RVF in 1990. Two communes that reported cases in 2008-2009 were also situated in the enzootic area. These cases were reported in small ruminants in the north of the island in April 2008. The other communes affected by RVF were situated in the epizootic area.

Estimation of the year-specific force of infection

To avoid over-estimation of seroprevalence due to cattle still having maternal antibodies, we selected animals above 2 years old. Thus, 930 cattle from 2 to 8 years of age were sampled in the 15 Malagasy communes (Table 3). The overall seropositivity rate was 8.7% (CI 95% [7.0-10.7]). Seroprevalence was positively associated with age (χ^2, $p<0.01$). The seroprevalence of cattle born during or before 2008-2009 epizootics (5 years old or more than 5 years old) was 16.3% whereas the seroprevalence of cattle born after 2009 outbreaks (4 years old or less than 4 years old) was 1.5%. These seroprevalences were significantly different (χ^2, $p<0.001$). Six hundred and twenty nine cattle were sampled in enzootic areas and 301 cattle in epizootic areas (Table 3). Overall seroprevalence was not significantly different between enzootic and epizootic areas, however, no cattle below 5 years old were seropositive in epizootic areas, whereas 7 cattle were seropositive in enzootic areas.

Finally, the analysis of serological data showed that the FOI in enzootic areas was not null after the 2008-2009 epizootics with a FOI estimated at 0.005 and 0.025 in 2010 and 2011 respectively (Fig. 2). The highest FOI estimation in enzootic areas (*i.e.* 0.11) was reached during the pre-epizootic period, in 2007 (Fig. 2). By contrast, in epizootic areas, FOI was more variable, being high during the epizootic period (0.082 and 0.12 in 2008 and 2009, respectively) but null during the pre- and post-epizootic periods in 2007 and from 2010 to 2012 (Fig. 2).

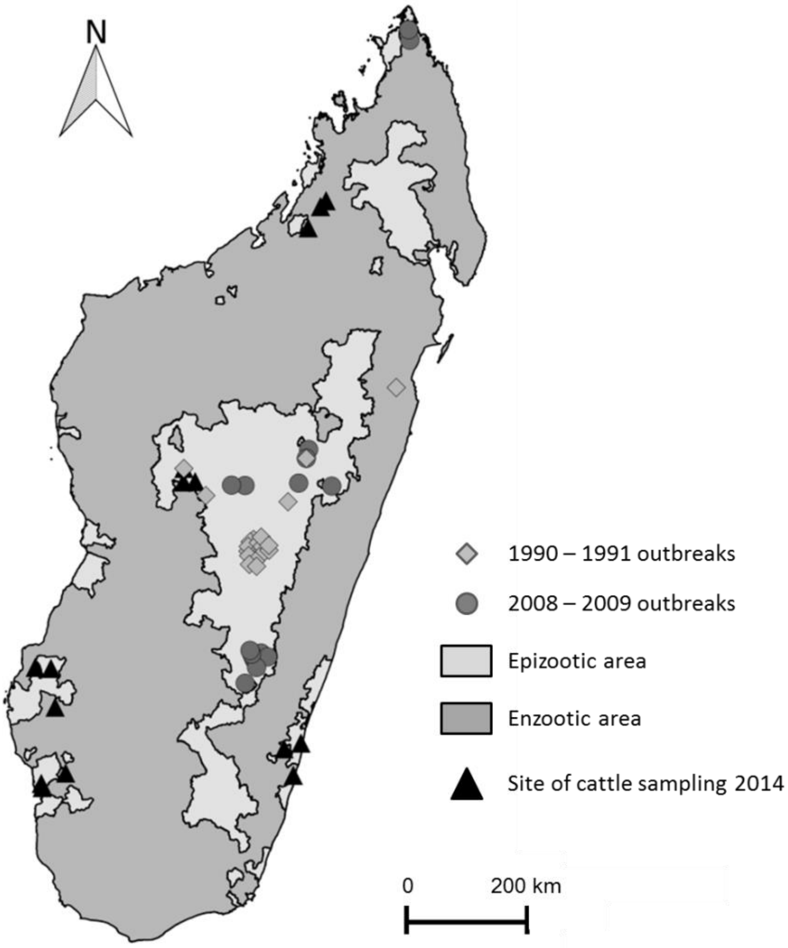


Fig. 1 Localization of communes affected by RVF outbreaks in ruminants, including enzootic and epizootic areas and site of cattle sampling in 2014

Table 3. Seroprevalence (SP) according to age and location (enzootic, epizootic areas and on the overall island)

Age	Year of birth	Enzootic area			Epizootic area			Overall island		
		Positive	Total	SP (%)	Positive	Total	SP(%)	Positive	Total	SP (%)
2	2012	0	110	0.0	0	45	0.0	0	155	0.0
3	2011	3	98	3.1	0	61	0.0	3	159	1.9
4	2010	4	111	3.6	0	50	0.0	4	161	2.5
5	2009	11	116	9.5	6	50	12.0	17	166	10.2
6	2008	13	86	15.1	8	40	20.0	21	126	16.7
7	2007	17	70	24.3	4	27	14.8	21	97	21.6
8	2006	9	38	23.7	6	28	21.4	15	66	22.7
Total		57	629	9.1	24	301	7.0	81	930	8.7

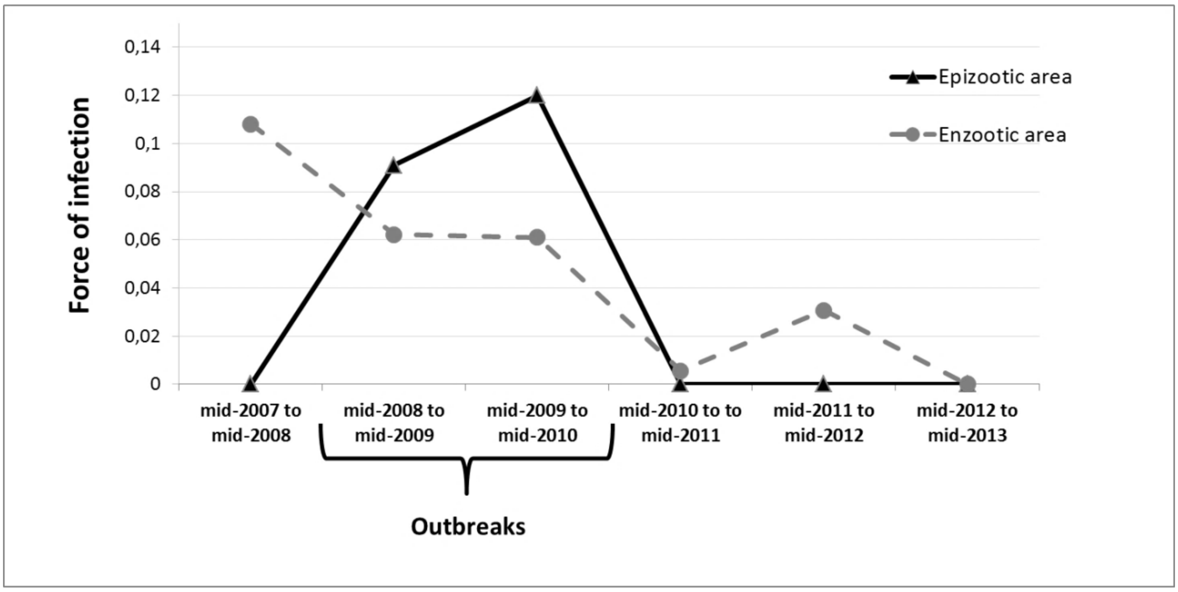


Fig. 2 Estimated force of infection (FOI) from mid-2007 to mid-2013 in enzootic and epizootic areas of Madagascar

DISCUSSION

Using the estimation of the year-specific force of infection from seroprevalence data we were able to assess the dynamics of transmission of RVFV in Madagascar from mid-2007 to mid-2013. This methodology allows us to circumvent biases in case reporting (Imai et al., 2015).

Our results support the hypothesis suggesting two different epidemiological patterns for RVF in Madagascar. The humid environment seems favourable to an RVF enzootic cycle. In these areas, RVFV was probably transmitted at low level during inter-epizootic periods allowing the maintenance of RVF on Madagascar. In the highlands, the seasonal and cold environment seems unsuitable for RVF inter-epizootic maintenance but favourable to outbreaks during the rainy season (Tantely et al., 2013, Andriamandimby et al., 2010). Main cattle markets are located in the Malagasy highlands. Thus, cattle movements from enzootic regions could introduce virus into the highlands, causing outbreaks under specific environmental conditions.

Our sampling allowed the assessment of the dynamics of infection from mid-2007 to mid-2013. However, the analysis of samples collected at previous dates could be useful to assess the dynamics of transmission before the pre-epizootic period in 2007. Our “enzootic vs. epizootic” classification was based on case notification, which we know to be under-estimated. In addition, we chose to classify our communes according to environmental factors associated with the reported cases, which were probably biased. Thus, within future work we will consider others scenarios and estimate the FOI under other hypotheses, such as an existing link between the FOI and different eco-climatic regions.

The seropositive status of animals born after 2008-2009 supports the hypothesis that RVF is still circulating locally after the last epizootic episode, as suggested previously (Gray et al., 2015, Nicolas et al., 2014, Jeanmaire et al., 2011, Chevalier et al., 2011, Zeller et al., 1998). In the enzootic area, the FOI was high the year before the 2008-2009 epidemic. Thus, the identification of an increased intensity of RVF circulation through active surveillance in enzootic areas could be an indicator of RVF pre-epizootic risk. However, further analyses and data are required to explain factors and mechanisms driving RVF epizootics and to confirm this result. Using satellite measurements (sea surface temperatures, rainfall and NDVI) from September 2007 to May 2008, Anyamba et al. (2010) identified the eastern-coast and some areas of the northern and north-western island as at-risk for the 2008-2009 RVF outbreaks. Some of the areas identified by this last study are situated in the enzootic areas of our study. The divergence between the results of the Anyamba et al. (2010) study and our study may be due methodological differences. Indeed, we used landscape data and climatic data from 2001 to 2010 whereas Anyamba et al. (2010) used climatic data prior to the outbreaks.

To our knowledge, this is the first study estimating year-specific FOI for RVF from seroprevalence data in Madagascar. Generally, published models estimating RVF transmission use RVF notification data for which the reliability is heavily dependent upon the quality of the surveillance system (Imai et al., 2015). Using seroprevalence data to estimate the dynamics of transmission therefore seems to be a good alternative when case notifications are skewed. Gaining a better global knowledge of the variation in transmission intensity of RVF will improve surveillance and control planning of RVF in Madagascar.

ACKNOWLEDGMENTS

This study was supported by grants from (i) CIRAD, (ii) the Centre National d'Etudes Spatiales (CNES; grant # 131163/00), and (iii) the Institut Pasteur de Madagascar. Cattle field work in 2014 was funded by the Institut Pasteur of Madagascar (Internal Project). We especially thank the herders who participated in the studies. We also thank those who facilitated in the survey, for example head and regional veterinary services, local veterinarians, heads of villages, local administration authorities and health authorities.

REFERENCES

Andriamandimby, S.F., Randrianarivo-Solofoniaina, A.E., Jeanmaire, E.M., Ravololomanana, L., Razafimanantsoa, L.T., Rakotojoelinandrasana, T., Razainirina, J., Hoffmann, J., Ravalohery, J.-P., Rafisandratantsoa, J.-T., Rollin, P.E. and Reynes, J.-M. (2010). Rift Valley fever during rainy seasons, Madagascar, 2008 and 2009. Emerg. Infect. Dis. 16, 963–970

Anyamba, A., Linthicum, K.J., Small, J., Britch, S.C., Pak, E., de La Rocque, S., Formenty, P., Hightower, A.W., Breiman, R.F., Chretien, J.-P., Tucker, C.J., Schnabel, D., Sang, R., Haagsma, K., Latham, M., Lewandowski, H.B., Magdi, S.O., Mohamed, M.A., Nguku, P.M., Reynes, J.-M. and Swanepoel, R. (2010). Prediction, assessment of the Rift Valley fever activity in East and Southern Africa 2006-2008 and possible vector control strategies. Am. J. Trop. Med. Hyg. 83, 43–51

Batjes, N.H. (2010). IPCC default soil classes derived from the Harmonized World Soil Data Base (Ver. 1.1). Carbon Benefits Project (CBP) and ISRIC – World Soil Information. [cited 2014 Nov 12]. http://www.isric.org/ webdocs/docs/ISRIC_Report_2009_02.pdf

Bontemps, S., Defourny, P., Van Bogaert, E., Arino, O., Kalogirou, V. and Perez, J.R. (2011). GLOBCOVER 2009: Products Description and Validation Report. [cited 2015 June 10]. http://epic.awi.de/31014/16/GLOBCOVER2009_Validation_Report_2-2.pdf

Chevalier, V. (2013). Relevance of Rift Valley fever to public health in the European Union. Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 19, 705–708

Chevalier, V., Rakotondrafara, T., Jourdan, M., Heraud, J.M., Andriamanivo, H.R., Durand, B., Ravaomanana, J., Rollin, P.E. and Rakotondravao, R. (2011). An unexpected recurrent transmission of Rift Valley fever virus in cattle in a temperate and mountainous area of Madagascar. PLoS Negl. Trop. Dis. 5, e1423

Cornet, A. (1974). Essai de cartographie bioclimatique à Madagascar. Not. Explic. 55

Escofier, B. and Pagès, J. (1994). Multiple factor analysis (AFMULT package). Comput. Stat. Data Anal. 18, 121–140

Gray, G., Anderson, B., LaBeaud, D., Heraud, J., Fevre, E., Andriamandimby, S., Cook, E., Dahir, S., deGlanville, W., Heil, G., Khan, S., Muiruri, S., Olive, M., Thomas, L., Merrill, H., Merrill, M. and Richt, J. (In press). Seroepidemiological study of Interepidemic Rift Valley fever virus infection among persons with intense ruminant exposure, Madagascar and Kenya. Am. J. Trop. Med. Hyg.

Imai, N., Dorigatti, I., Cauchemez, S. and Ferguson, N.M. (2015). Estimating Dengue Transmission Intensity from Sero-Prevalence Surveys in Multiple Countries. PLoS Negl. Trop. Dis. 9, e0003719

Jeanmaire, E.M., Rabenarivahiny, R., Biarmann, M., Rabibisoa, L., Ravaomanana, F., Randriamparany, T., Andriamandimby, S.F., Diaw, C.S., Fenozara, P., de La Rocque, S. and Reynes, J.-M. (2011). Prevalence of Rift Valley fever infection in ruminants in Madagascar after the 2008 outbreak. Vector Borne Zoonotic Dis. 395–402

Kortekaas, J., Kant, J., Vloet, R., Cêtre-Sossah, C., Marianneau, P., Lacote, S., Banyard, A.C., Jeffries, C., Eiden, M., Groschup, M., Jäckel, S., Hevia, E. and Brun, A. (2013). European ring trial to evaluate ELISAs for the diagnosis of infection with Rift Valley fever virus. J. Virol. Methods 187, 177–181

Linthicum, K.J., Anyamba, A., Tucker, C.J., Kelley, P.W., Myers, M.F. and Peters, C.J. (1999). Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. Science 285, 397–400

Moat, J. and Du Puy, D. (1997). Madagascar, Remaining Primary Vegetation. Royal Botanic Gardens, Kew (Eds.), London. [cited 2014 Nov 15]. www.kew.org/gis/projects/madagascar/veg_mapping.html

Morvan, J., Rollin, P.E., Laventure, S., Rakotoarivony, I. and Roux, J. (1992). Rift Valley fever epizootic in the central highlands of Madagascar. Res. Virol. 143, 407–415

Morvan, J., Saluzzo, J.F., Fontenille, D., Rollin, P.E. and Coulanges, P. (1991). Rift Valley fever on the east coast of Madagascar. Res. Virol. 142, 475–482

Nakounné, E., Selekon, B. and Morvan, J. (2000). [Microbiological surveillance: viral hemorrhagic fever in Central African Republic: current serological data in man]. Bull. Société Pathol. Exot. 93, 340–347

Nicolas, G., Durand, B., Rakotoarimanana, T.T., Lacote, S., Chevalier, V. and Marianneau, P. (2014). A 3-year serological and virological cattle follow-up in Madagascar highlands suggests a non-classical transmission route of Rift Valley fever virus. Am. J. Trop. Med. Hyg. 90, 265–266

Pepin, M., Bouloy, M., Bird, B.H., Kemp, A. and Paweska, J. (2010). Rift Valley fever virus(Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet. Res. 41, 61

Pourrut, X., Nkoghé, D., Souris, M., Paupy, C., Paweska, J., Padilla, C., Moussavou, G. and Leroy, E.M. (2010). Rift Valley fever virus seroprevalence in human rural populations of Gabon. PLoS Negl. Trop. Dis. 4, e763

QGIS Development Team. (2009). QGIS Geographic Information System

R Development Core Team. (2011). R: A Language and Environment for Statistical Computing. Vienna Austria R Found. Stat. Comput.

Siebert, S. and Frenken, K. (2014). Irrigated areas. In: Atlas of African agriculture research & development. [cited 2014 Nov 12] http://www.fao.org/nr/water/aquastat/irrigationmap/index.stm

Tantely, M.L., Rakotoniaina, J.-C., Tata, E., Andrianaivolambo, L., Razafindrasata, F., Fontenille, D. and Elissa, N. (2013). Biology of mosquitoes that are potential vectors of Rift Valley Fever virus in different biotopes of the central highlands of Madagascar. J. Med. Entomol. 50, 603–610

Zeller, H., Rakotoharinadrasana, H.T. and Rakoto-Andrianarivelo, M. (1998). [Rift Valley fever in Madagascar: infection risks for the abattoir staff in Antananarivo]. Rev. Délevage Médecine Vét. Pays Trop. 51, 17–20

MASS VACCINATION, IMMUNITY AND COVERAGE: MODELLING POPULATION PROTECTION AGAINST FOOT-AND-MOUTH DISEASE IN TURKISH CATTLE

T.J.D. KNIGHT-JONES[*], S. GUBBINS, A.N. BULUT, K.D.C. STÄRK,
D.U. PFEIFFER, K.J. SUMPTION AND D.J. PATON

SUMMARY

Foot-and-mouth disease (FMD) in Turkey is controlled using six-monthly mass vaccination of cattle. However, vaccine protection is undermined by population turnover and declining immunity. We assessed population immunity using a dynamic model of the cattle population in Anatolian Turkey in 2012/13, assuming biannual mass vaccination with a single-dose primary course. Six months after the last round of vaccination almost half the cattle aged <24 months remain unvaccinated. Five months after the last round of vaccination two-thirds of cattle would have low antibody titres (<70% protection threshold). Giving a two-dose primary vaccination course reduces the proportion of 6-12 month old cattle with low titres by 20-30%. Biannual mass vaccination of cattle leaves significant immunity gaps. Improved vaccines are now used. However, without effective movement restrictions and biosecurity, the extent to which FMD can be controlled by vaccination alone remains uncertain.

INTRODUCTION

Vaccines play a crucial role in the control of foot-and-mouth disease (FMD) (Knight-Jones and Rushton, 2013). Whilst FMD has been eradicated in Turkish Thrace, biannual mass vaccination of cattle has failed to control the disease in the rest of the country.

Within Turkey, there is great variation in climate and terrain. Different livestock production systems are found in different settings, and these different production systems have different animal population age structures. This in turn affects the vaccination history, and thus protection, of these populations. Furthermore, this changes with time as animals are born, age and die, and the seasonality of this turnover will vary depending on the setting and the production system. However, population structure is rarely considered in detail during livestock vaccination programmes (Knight-Jones et al., 2014b).

The trivalent vaccine in Turkey used at the time of this study covered serotypes O, A and Asia-1 with a potency of ≥3PD50. The 50% protective dose (PD50) is a measure of vaccine potency assessed in a challenge study. Quality FMD vaccines can induce immunity lasting for ≥6 months after a single dose. Immunity is broader and longer-lasting with a faster onset if vaccine potency is high (Terpstra et al., 1990; Doel, 2003; Brehm et al., 2008; Cox et al., 2010).

---

[*] Theodore J. D. Knight-Jones, formerly Pirbright Institute. Present address: International Livestock Research Institute (ILRI), Lusaka, Zambia. Email: t.knight-jones@cgiar.org

Many ongoing FMD vaccination programmes use vaccines with a shorter duration of immunity and several doses of vaccine are required for sustained protection (Doel, 2005; Knight-Jones et al., 2015; Pay, 1984). When first vaccinated, cattle should receive two doses of vaccine approximately one month apart (a two-dose primary course) (Späth et al., 1995; Doel, 2003; Pay, 1984). However, to save resources, a single-dose primary course is used in many countries, including Turkey, at the time of this study.

Despite ongoing mass vaccination, FMD is highly endemic in Anatolia (approximately 1000 outbreaks were reported in 2013), with multiple serotypes and under-reporting. In Anatolia most farms are smallholdings. Dependency on communal grazing makes it difficult to enforce movement restrictions during FMD outbreaks.

The objective of this study was to quantify population immunity resulting from mass vaccination of cattle in Turkish Anatolia, using data from 2012/13. As we wished to evaluate protection provided by the vaccination programme, immunity from natural infection was not considered. Vaccine immunity and percentage vaccinated were modelled over the annual production cycle. The effects of a single versus a two-dose primary course were estimated.

MATERIALS AND METHODS

We developed a simulation model of FMD population immunity in Turkish cattle. This was undertaken by combining a dynamic model of population structure and vaccine coverage with estimates of post-vaccination immune response (Knight-Jones et al., 2015). Two main sources of data were used to inform the model: (a) demographic data from nationwide cattle surveys, and (b) an extensive field study of post-vaccination serology (Knight-Jones et al., 2015).

As the objective was to assess immunity arising directly from vaccination, maternally derived immunity and immunity from infection were not considered. The study assessed Anatolia and not FMD-free Turkish Thrace, where different vaccines are used.

Cattle population model

The population model was designed to represent cattle age structure in each province. The number of cattle <12 months, 12 to <24 months and ≥24 months on 31st December 2012 was obtained in each province from government census records (Turkish Statistical Institute, 2013). Demographic data taken from separate randomised cross-sectional surveys of cattle, conducted in 2009, 2010 and 2012, covering all provinces, were used to estimate month of birth within age categories. The FMD sero-surveys sampled 96 249 cattle aged 6-24 months (Askaroglu, 2009). For each of the 78 provinces in Anatolia, survey data from cattle 6-17 months old were used to create a distribution of month of birth. Each distribution was then sampled n times, where n was 1% of the number of cattle within each age category for each province. This gave a simulated population of 135 453 cattle representing a random selection from across Anatolian Turkey.

Having sampled the month of birth for the simulated population, the actual day of birth was selected at random from a uniform distribution (e.g. from 1-31 for January). As the age distribution of cattle ≥24 months old was not available, those cattle ≥24 months old were assumed to be equally distributed between the ages of two and five years old.

The simulated population reflected the age-sex structure for each province on 31st December 2012. As the census data used were reported in December, evaluations were performed around this period (Sept to March).

Vaccine coverage

Once the cattle population was simulated, the vaccination history of each animal was derived assuming nationwide biannual mass vaccination since 2007 on the 25th March and 25th September each year (the 2012 average date of autumn vaccination). If a simulated animal was ≥2 months old on a vaccination date it was eligible for vaccination.

To incorporate realistic levels of vaccine coverage (the proportion of eligible cattle vaccinated), district coverage was assumed to vary (Anatolia, Turkey has 904 districts in 78 provinces). For each district, the proportion of eligible cattle typically vaccinated at mass vaccination (vc) was sampled from a BetaPERT distribution (minimum=40%, maximum=100%, most likely=80%), based on Turkish field studies (Knight-Jones et al., 2014a). Whether or not an eligible animal was actually vaccinated, during a particular round of vaccination, was then determined by a Bernoulli distribution, with probability of vaccination vc.

Population immunity

SP prediction models: Antibody levels against FMD virus structural proteins (SP) are a strong correlate of protection and can be measured to assess FMD immunity (Van Bekkum et al., 1969; Pay and Hingley, 1986; Van Maanen and Terpstra, 1989; Barnett et al., 2003; Reeve et al., 2011). Log10 SP antibody titres for serotypes A, O and Asia-1 were predicted for each animal in the simulated population. SP titre measured by liquid phase blocking ELISA (LPBE) was predicted in two steps using regression models fitted to data from an extensive post-vaccination field study performed in Turkey (Knight-Jones et al., 2015). Predictor variables were 1) number of vaccine doses received in a lifetime (which was correlated with age), 2) time since last vaccination and 3) single or two-dose primary course given (Knight-Jones et al., 2015).

Firstly, a GEE (generalised estimator equation) logistic regression model predicted the probability of an animal having a titre equal to or higher than 1:32, which was the lowest dilution tested. This probability was then used as the parameter in a Bernoulli distribution.

In the second step, for each animal predicted to have a titre above the detection threshold (≥1:32), an interval regression model was used to determine expected Log10 (SP titre). For these cattle, final predicted SP titre was determined by drawing a sample from a normal distribution centred on this expected titre, with standard deviation equal to the model residual standard deviation (Gelman and Hill, 2007).

The immunological effect of receiving two doses one month apart at primary vaccination (booster effect) was included for the period after cattle were first vaccinated. After further rounds of vaccination the booster effect was modelled as the effect of having received an extra dose of vaccine in an animal’s lifetime. This was done as data describing the effect of a two-dose primary vaccination on immunity after multiple rounds of subsequent vaccination were not available.

Almost all adult cattle >24 months would have been vaccinated ≥3 times. As cattle vaccinated ≥3 times are usually able to sustain antibody titres throughout the duration of the six month inter-vaccination interval they were combined in one group (Knight-Jones et al., 2015). Unvaccinated cattle were assigned a titre of zero.

Modelling uncertainty: To account for variability and uncertainty, Monte Carlo simulation was used. The population model was simulated 1000 times. For each iteration, the regression parameters used for prediction were sampled from normal distributions with mean equal to the regression coefficient and standard deviation equal to the robust standard error. SP titres were then predicted for each iteration and summary statistics were produced, including the proportion of the simulated population with a titre <1:102. In challenge studies animals with a titre ≥1:102 are likely to be protected against homologous challenge (personal communication A.N. Bulut), with approximately 70% protected (Smitsaart et al., 1998; Robiolo et al., 2006; Robiolo et al., 2010).

To assess the accuracy of SP predictions, titres were predicted for the cattle in the prospective field study used to fit the regression models (Knight-Jones et al., 2015). These predicted titres were then compared with actual titres for the same animals. Analysis was performed using R (R Core Team, 2014). In the results, median predicted values are shown with 95% prediction intervals (PI), i.e. 2.5th to 97.5th percentiles.

Since these studies were completed, the ≥3PD50 vaccine used for mass vaccination has been replaced by a ≥6PD50 vaccine. Immune response data were not available to assess the Turkish ≥6PD50 vaccine, nor were alternative schedules available beyond the use of a booster.

Coverage and immunity over time

The population model was used to assess vaccination and immune status on 25th October 2012 (one month post-vaccination, when antibodies peak) and 14th February 2013 (the maximum time after vaccination that antibody titre was assessed in the post-vaccination field study used to fit the prediction models (Knight-Jones et al., 2015)). Vaccination and immune status were also assessed on the date of autumn and spring vaccination, (25th March 2012 and 25th September 2012). However, as the time since last vaccination on these dates is greater than in the data used to fit the regression models, caution is required when assessing antibody levels on these dates.

The relationship between percentage vaccinated and population immunity was explored graphically and with simple linear regression. For simplicity, this was only done using serotype O without the two-dose primary course, assessed in mid-February.

Vaccine homologous antibody

Post-vaccination sera bind vaccine homologous virus better than they bind other strains of FMD virus. The vaccine in the field study used to fit prediction models was the trivalent FMD vaccine produced by the Sap Institute in Ankara, Turkey, containing FMD strains O Panasia II (O Tur 07), A Iran 05 (A TUR 06) and Asia-1 Sindh-08 (Asia-1 TUR 11). The antigens used in LPBE (O Manisa, A22 IRQ 24/64 and Asia-1 Shamir) are all different to the antigens used in the vaccine (Knight-Jones et al., 2015). However, virus neutralisation (VN) tests were used to estimate the effect of this antigen mismatch on predicted protection as described by Knight-Jones et al. (2015).

The proportion protected was then adjusted according to the difference in the proportion above the 70% protection threshold according to VN and LPBE tests. Adjustments were made using BetaPERT distributions based on the median, 2.5th and 97.5th percentiles shown in Table 2 of Knight-Jones et al. (2015), which represent the proportion above the homologous VN 70% protection threshold according to LPBE titre.

RESULTS

Population structure and vaccine coverage

Age distribution details are included in Table 1. If all of the 94.7% of cattle that are ≥2 months old were vaccinated in spring 2012, then six months later, new births would have increased the percentage of unvaccinated cattle to 18.5% (Fig. 1).

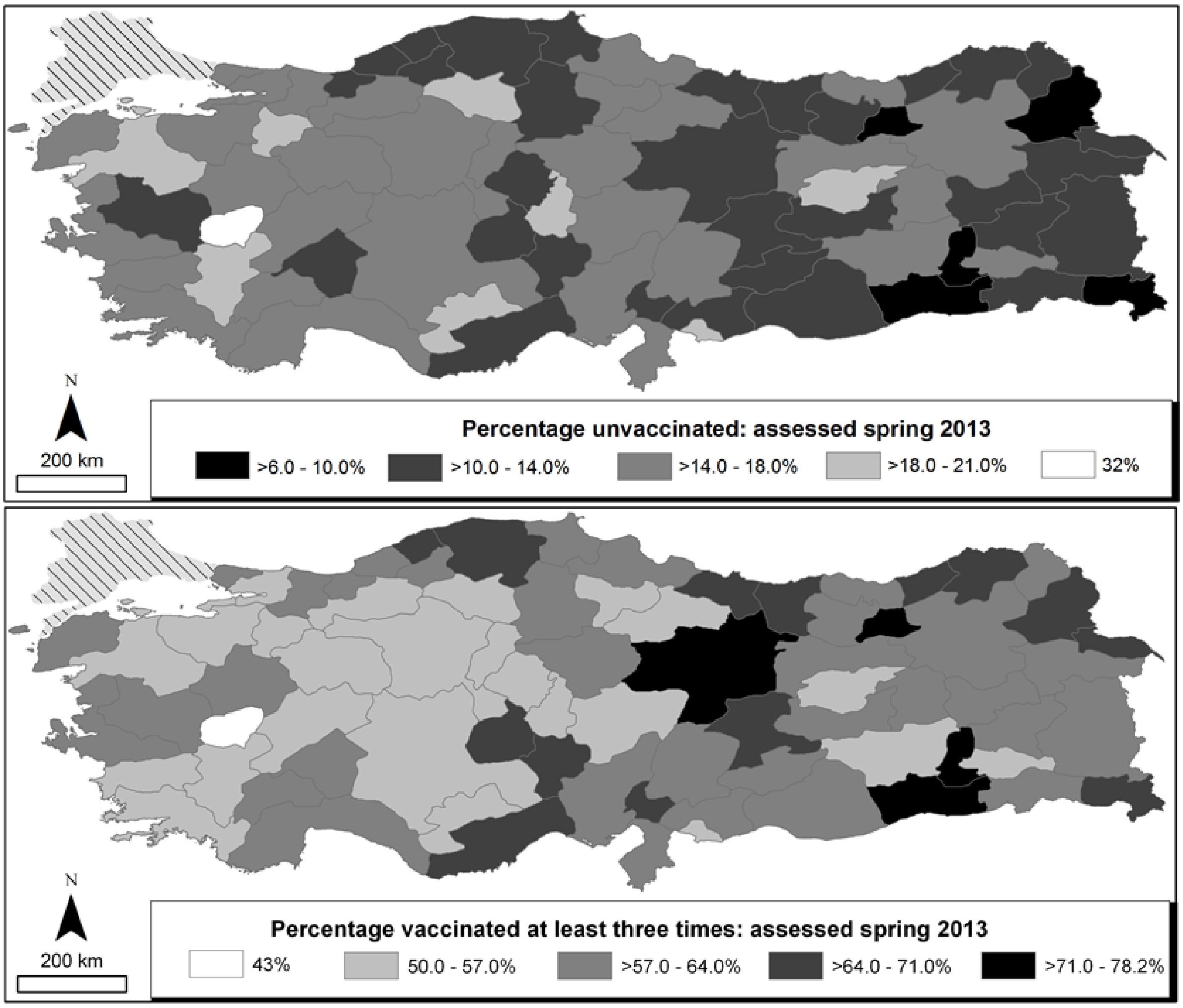


Fig. 1 Maps showing modelled percentage of Turkish cattle previously vaccinated at time of spring 2013 vaccination assuming all eligible cattle were vaccinated each spring and autumn. Maps depict the median percentage (above) unvaccinated and (below) vaccinated at least three times (the number of doses needed for a sustained antibody titre). Thrace not included

Table 1. Median predicted proportion of cattle vaccinated during mass FMD vaccination in Turkey (with 95% PI in brackets), allowing for incomplete coverage of eligible cattle assessed six months after the last round of vaccination [b]. Autumn revaccination (25th September 2012) would vaccinate 74.5% (95% PI: 74.3%-74.8% of eligible cattle, spring revaccination (25th March 2013) would vaccinate 73.2% (95% PI: 73%-73.4%) of eligible cattle. The median total does not necessarily equal the total of the subcategory medians. This, along with rounding, resulted in minor discrepancies with margin totals

		Number of doses	0	1	2	≥3	Total
Evaluated 25th Sep 2012 [c]	Months since last vaccination	Unvaccinated	22% (21.9-22.2)	0%	0%	0%	22% (21.9%-22.2%)
		6	0%	10.4% (10.4-10.5)	13.1% (13.1-13.15)	39.4% (39.2-39.6)	62.9% (62.7-63.1)
		12	0%	3.19% (3.18-3.2)	1.87% (1.8-1.9)	6.7% (6.6-6.8)	11.8% (11.6-11.9)
		≥18	0%	0.77% (0.75-0.79)	0.7% (0.7-0.7)	1.85% (1.7-1.9)	3.3% (3.1-3.4)
	Age (Months)	<6	13.1% (12.9-13.2)	0%	0%	0%	13.1% (12.9-13.2)
		6 - <12	6.8% (6.7-6.9)	4.4% (4.3-4.5)	0%	0%	11.2% (11.1-11.4)
		12 - <18	1.5% (1.4-1.6)	6.1% (6-6.3)	6.1% (6-6.2)	0%	13.7% (13.6-13.9)
		18 - <24	0.5% (0.5-0.6)	2.8% (2.7-2.9)	6% (5.9-6.1)	3% (2.9-3.1)	12.3% (12.2-12.5)
		≥24	0.1% (0.1-0.2)	1% (0.9-1.1)	3.5% (3.4-3.6)	44.9% (44.8-45)	49.6%[a]
		Total	22% (21.9-22.2)	14.5% (14.4-14.6)	15.6% (15.5-15.7)	47.9% (47.8-48.1)	100%
Evaluated 25th Mar 2013 [d]	Months since last vaccination	Unvaccinated	20% (19.8-20.2)	0%	0%	0%	20% (19.8-20.2)
		6	0%	14.4% (14.2-14.6)	10.3% (10.2-10.5)	41.3% (41.1-41.5)	66% (65.8-66.3)
		12	0%	2.4% (2.3-2.4)	2.4% (2.3-2.4)	6.7% (6.5-6.7)	11.4% (11.2-11.6)
		≥18	0%	0.7% (0.7-0.8)	0.4% (0.4-0.45)	1.4% (1.4-1.5)	2.6% (2.5-2.7)
	Age (Months)	<6	11.2% (11-11.3)	0%	0%	0%	11.2% (11-11.3)
		6 - <12	5.6% (5.5-5.7)	7.5% (7.4-7.7	0%	0%	13.2% (13-13.3)
		12 - <18	1.8% (1.7-1.8)	6.6% (6.5-6.7)	3.8% (3.7-3.9)	0%	12.1% (12-12.3)
		18 - <24	0.4% (0.4-0.5)	2.5% (2.5-2.6)	5.9% (5.8-6)	5% (4.9-5.1)	13.9% (13.8-14)
		≥24	0.1% (0.1-0.2)	1.1% (1-1.1)	3.6% (3.5-3.7)	44.8% (44.7-44.9)	49.6[a]
		Total	20% (19.8-20.2)	17.7% (17.5-17.9)	13.3% (13.2-13.5)	49.8% (49.7-50)	100%

[a] Fixed sampling proportion used from census data.

[b] District level coverage was described by a BetaPERT distribution (minimum=40%, maximum=100%, most likely=80%).

[c] i.e. Assessed on day of autumn 2012 vaccination BEFORE vaccination performed.

[d] i.e. Assessed on day of spring 2013 vaccination BEFORE vaccination performed.

When incomplete coverage of eligible cattle is incorporated, about 20% of cattle are unvaccinated six months after the last round of vaccination. An additional 15% of cattle, although vaccinated, had not been vaccinated for ≥12 months. Only about half of all cattle would have been vaccinated ≥3 times since birth (Table 1). Looking at cattle <12 months old, about 82% had not previously been vaccinated at the time of autumn vaccination. For all cattle aged <24 months, 44% would be unvaccinated at the time of autumn vaccination.

Post-vaccination SP titre

The coefficients for the regression models used to predict SP titre reflect the fact that titre increases with prior vaccination and a two-dose primary course, declining with time since vaccination. Predicted titres were very similar to actual titres in validation (see Thesis Table 5-1 and Fig. 5-5 respectively www.researchgate.net/profile/Theodore_Knight-Jones/publications). Many cattle had predicted titres below the antibody detection threshold of 1:32; the rest had SP titres that varied around an average of roughly 1:102 (Fig. 2).

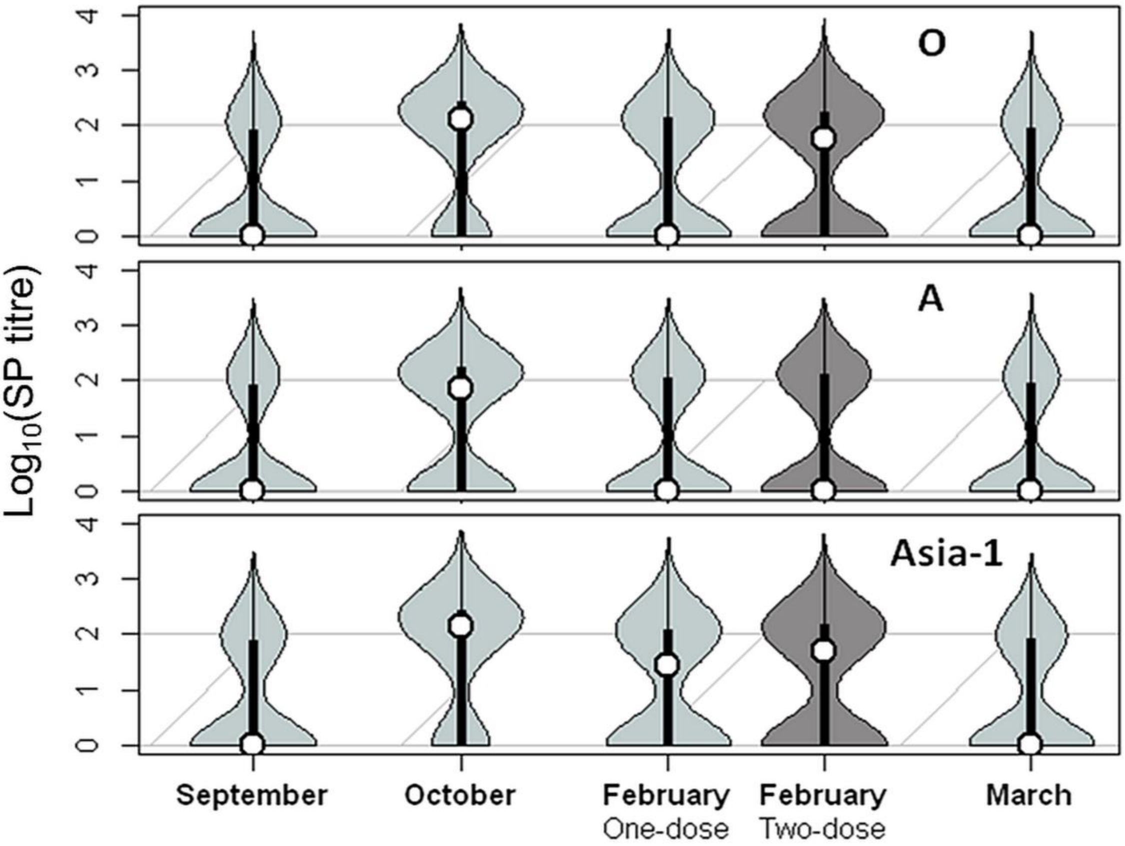
Only at peak antibody response, one month after the last round of vaccination, did more than half the simulated cattle population have a titre ≥1:102 (Fig. 2). A quarter had no detectable antibodies at this time of peak response. Antibody levels were lower for serotype A and largely similar for O and Asia-1. Low serotype A titres were partly a result of differences in vaccine and LPBE antigen (see VN adjustments later).

The beneficial effect of a two-dose primary course occurred mainly in young animals. By mid-February, with a single-dose primary course, roughly 80% of 6 - <12 month old cattle had a titre <1:102 compared to 50-60% with a two-dose primary course (all serotypes). An additional 20-30% were also lifted above the detection threshold (1:32).

Vaccine homologous antibody

Compared to LPBE estimates, protection against vaccine homologous virus, predicted by VN, was higher except for Asia-1. After adjusting LPBE protection estimates to reflect protection against vaccine homologous virus predicted by VN, assessed on 25th October 2012 (one month post-vaccination), 26% (95% PI: 19-34%), 37% (95% PI: 28-48%) and 59% (95% PI: 46-72%) of cattle were expected to be below the 70% protection threshold for serotypes O, A and Asia-1 respectively. The same statistic assessed in mid-February predicted 44% (95% PI: 33-56%), 48% (95% PI: 38-60%) and 74% (95% PI: 60-83%) below the threshold with a single-dose primary course and 39% (95% PI: 29-51%), 44% (95% PI: 34-56%) and 71% (95% PI: 58-81%) if a two-dose primary course were used, again for serotypes O, A and Asia-1, respectively.

Fig. 2 Predicted Log_{10} (LPBE SP titre) for the Turkish cattle population after September 2012 FMD vaccination, for serotypes O (top), A (middle) and Asia-1 (bottom). February 2013 titres (142 days post September vaccination) are shown with and without the routine use of a two-dose primary vaccination course (labelled "Two-dose", and "One-dose" respectively). The proportion of cattle with a particular titre is proportional to the width of the violin-plot. Within the violin-plot is a box-plot showing the median titre (white circle), inter-quartile range and minimum and maximum values. The "protection threshold" titre is indicated ($SP=1:10^2$). Although plots are shown for September and March, they are beyond the limits of the inter-vaccination interval in the data used to fit the prediction models (Knight-Jones et al., 2015) and are thus, speculative



DISCUSSION

The model predicted that the Turkish biannual mass vaccination programme would provide limited protection against FMD. Five months after the last round of vaccination, half to two-thirds of cattle would have low antibody titres. Considering all cattle <12 months old, about 80% would have low titres.

Time since last vaccination and the number of animals that had received three or more vaccinations in their life had a large effect on predicted immunity. With six-monthly vaccination with a single-dose primary course, sustained protection could not be achieved at a young age due to rapidly declining antibodies and the need for multiple doses. Vaccines that induce sustained immunity after one or two doses are required. Although few data are available, following vaccination with ≥6PD50 vaccines, antibody levels have been observed to remain high after a single dose for six months or more (Selman et al., 2006; Pay, 1984). Furthermore, higher potency vaccines are more likely to protect in the event of poor vaccine match (Brehm et al., 2008).

Even if all eligible cattle (≥2 months old) were routinely vaccinated, six months after the last round of vaccination, one-in-five cattle would never have been vaccinated. This is due to the presence of cattle that were too young to be vaccinated at the previous round of vaccination, and from new births. After accounting for incomplete coverage of those eligible for vaccination, only about 40% of cattle would have received >2 doses of vaccine in their life, with the last dose received ≤6 months ago. Two-thirds of cattle <12 months old remain unvaccinated by spring. Spatial clusters of extreme high susceptibility would occur, varying over the annual production cycle. If levels of immunity are low at turn-out for spring grazing, significant transmission would be likely, due to the high levels of contact that occur between cattle from different holdings whilst using communal grazing.

Field studies of the Asia-1 vaccine found reasonable effectiveness during outbreaks. However, incidence in vaccinated cattle was still high (35%) (Knight-Jones et al., 2014a), particularly for young cattle. Using a two-dose primary vaccination course improved immunity in young animals to levels similar to adults. Although the two-dose primary course requires significant additional resources (about 20% more doses), reducing susceptibility of young-stock as soon as possible is vital.

Although the exact interpretation of a predicted protection threshold is uncertain, it provides a useful benchmark measure of immunity. We assessed serological thresholds at which 70% of cattle are protected against generalised FMD lesions after homologous challenge. Protection would be reduced with imperfect vaccine match (Arrowsmith, 1975; Knowles et al., 2009). However, these thresholds are derived after virus is injected into the tongue and protection against field challenge is likely to be greater (Van Bekkum et al., 1969).

The low VN titres seen for Asia-1 vaccine homologous virus were surprising and may reflect within serotype strain differences in the relationship between serology and protection reported by some (Pay and Hingley, 1986; Van Maanen and Terpstra, 1989; Maradei et al., 2008), but not all studies (Barnett et al., 2003). Ultimately, predictions will be influenced by the antigenic match between the vaccine, the test and the challenge virus.

In this study, we extended the serological approach to FMD post-vaccine monitoring, by incorporating estimates of immune response into a dynamic demographic model of vaccine coverage. The model simplification, whereby adult cattle were evenly distributed between ages 2-5 years old, would have minimal impact on estimates of the proportion vaccinated or protected, as adult cattle with multiple vaccinations (≥3 doses) were treated as one group and few cattle are >5 years old (Knight-Jones et al., 2014a). As seen in other studies (Terpstra et al., 1990), increased immunity from additional vaccination beyond three doses was not evident in the field data (Knight-Jones et al., 2015), as long as cattle were recently vaccinated. However, data on the immunity of old cattle (vaccinated many times) were limited (Knight-Jones et al., 2015) and serological predictions for cattle <24 months of age were more robust.

The importance of using vaccines with independent quality assurance and proven potency cannot be emphasised enough. If vaccines are ineffective, veterinary services and farmers bear the burden of mass vaccination without reducing the burden of disease. Loss of confidence in vaccination and reduced participation in future control programmes can have lasting repercussions.

In a process that took many decades, FMD has been controlled successfully using vaccination in South America (Naranjo and Cosivi, 2013) and Europe (Leforban and Gerbier, 2002). There are many factors besides differences in vaccine protection that lie behind this contrast with FMD control in Anatolia. These include high levels of livestock mixing and movements in Anatolia, extensive use of common grazing, high livestock density within villages and limited application of biosecurity measures during outbreaks. Furthermore, livestock carrying new FMD viruses can easily enter Turkey from endemic countries on its Eastern border.

CONCLUSION

Low population immunity after FMD mass vaccination results from a) rapid antibody decay in vaccinated animals and b) a high proportion of animals that have not received a sufficient number of vaccine doses. Using higher potency vaccines and a two-dose primary course would result in longer lasting antibody titres being obtained at a younger age. This is now done routinely across Turkey. This strategy has been accompanied by a dramatic reduction in reported outbreaks (from approx. 1000 in 2013, to 253 in 2014, and 263 in 2015) (Bulut, 2013, 2014, 2015; EC-ADNS, 2015). To what extent this is due to vaccination or post-epidemic natural immunity is not known.

As most cattle have only been vaccinated ≤2 times, a more potent vaccine able to deliver greater immunity after only one or two doses would greatly increase population immunity. However, immunity gaps will still exist as each round of mass vaccination is likely to exclude a quarter of all cattle. Prioritising repeated vaccination of young cattle, with high coverage would help.

Ongoing mass vaccination in Anatolia left large gaps in population immunity and was unable to control FMD in this highly endemic region. Over-reliance on vaccine protection with limited isolation of infected animals is not recommended (Woolhouse et al., 1996; Knight-Jones et al., 2014a; Pay, 1984). The use of this approach with high potency, quality vaccines and a two-dose primary course will increase population immunity and reduce disease burden. However, the extent to which FMD can be controlled by vaccination alone, without effective biosecurity and movement restrictions, is an unanswered question of global significance.

ACKNOWLEDGEMENTS

The authors are grateful to various members of the Turkish state veterinary services for their assistance during the execution of field studies. Particular thanks go to Musa Alkan and Oktay Tezal of the Sap institute. The authors are indebted to Prof Paul Fine for his help initiating this project. This study was funded by BBSRC and EuFMD, and led by The Pirbright Institute.

REFERENCES

Arrowsmith, A.E. (1975). Variation among strains of type A foot-and-mouth disease virus in the Eastern Mediterranean region 1964-1972. J. Hyg. (Lond) 75, 387-397

Askaroglu, H. (2009). EU project for the control of FMD in Turkey, West Eurasia Roadmap FMD Control 2010-2020, 7-9 October, 2009 – Istanbul, Turkey

Barnett, P.V., Statham, R.J., Vosloo, W. and Haydon, D.T. (2003). Foot-and-mouth disease vaccine potency testing: determination and statistical validation of a model using a serological approach. Vaccine 21, 3240-3248

Brehm, K.E., Kumar, N., Thulke, H.H. and Haas, B. (2008). High potency vaccines induce protection against heterologous challenge with foot-and-mouth disease virus. Vaccine 26, 1681-1687

Bulut, A.N. (2013). FMD situation in Turkey. West EurAsia FMD Control-Roadmap 2025, 4th Annual Regional Progress Review Meeting, 2-4th April 2013, Baku, Azerbaijan

Bulut, A.N. (2014). FMD situation in Turkey - 5th West Eurasia roadmap meeting. Astana, Kazakhstan 23-24 April 2014

Bulut, A.N. (2015). FMD situation in Turkey presented at 6th West Eurasia Roadmap Meeting, Almaty, Kazakhstan, 28-30 April 2015 http://www.fao.org/fileadmin/user_upload/eufmd/Roadmap_2015/TurkeyCountryReport.pdf (Date of access: 25/11/2015)

Cox, S.J., Carr, B.V., Parida, S., Hamblin, P.A., Prentice, H., Charleston, B., Paton, D.J. and Barnett, P.V. (2010). Longevity of protection in cattle following immunisation with emergency FMD A22 serotype vaccine from the UK strategic reserve. Vaccine 28, 2318-2322

Doel, T.R. (2003). FMD vaccines. Virus Res. 91, 81-99

Doel, T.R. (2005). Natural and vaccine induced immunity to FMD. Curr. Top. Microbiol. 288, 103-131

EC-ADNS (2015). Animal disease notification system: outbreaks per disease ADNS disease overview report 1st Jan-31st Dec 2015. http://ec.europa.eu/food/animal/diseases/adns/table_11_2015.pdf (Date of access: 07/01/2016)

Gelman, A. and Hill, J. (2007). Data analysis using regression and multilevel/hierarchical models. Pp 150-1. Cambridge University Press, Cambridge

Knight-Jones, T.J.D., Bulut, A.N., Gubbins, S., Stark, K.D., Pfeiffer, D.U., Sumption, K.J. and Paton, D.J. (2014a). Retrospective evaluation of foot-and-mouth disease vaccine effectiveness in Turkey. Vaccine 32, 1848-1855

Knight-Jones, T.J.D., Bulut, A.N., Gubbins, S., Stark, K.D., Pfeiffer, D.U., Sumption, K.J. and Paton, D.J. (2015). Randomised field trial to evaluate serological response after foot-and-mouth disease vaccination in Turkey. Vaccine 33, 805-811

Knight-Jones, T.J.D., Edmond, K., Gubbins, S. and Paton, D.J. (2014b). Veterinary and human vaccine evaluation methods. Proc. Biol. Sci. 281, 20132839

Knight-Jones, T.J.D. and Rushton, J. (2013). The economic impacts of foot and mouth disease - what are they, how big are they and where do they occur? Prev. Vet. Med. 112, 161-173

Knowles, N.J., Nazem Shirazi, M.H., Wadsworth, J., Swabey, K.G., Stirling, J.M., Statham, R.J., Li, Y., Hutchings, G.H., Ferris, N.P., Parlak, U., Ozyoruk, F., Sumption, K.J., King, D.P. and Paton, D.J. (2009). Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. Transbound. Emerg. Dis. 56, 157-169

Leforban, Y. and Gerbier, G. (2002). Review of the status of foot and mouth disease and approach to control/eradication in Europe and Central Asia. Rev. Sci. Tech. 21, 477-492

Maradei, E., La Torre, J., Robiolo, B., Esteves, J., Seki, C., Pedemonte, A., Iglesias, M., D'Aloia, R. and Mattion, N. (2008). Updating of the correlation between lpELISA titers and protection from virus challenge for the assessment of the potency of polyvalent aphtovirus vaccines in Argentina. Vaccine 26, 6577-6586

Naranjo, J. and Cosivi, O. (2013). Elimination of foot-and-mouth disease in South America: lessons and challenges. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 368, 20120381

Pay, T.W.F. (1984). Factors influencing the performance of foot-and-mouth disease vaccines under field conditions in. Applied virology, Academic Press Inc. Orlando, USA & London, (ed Kurstak, E.) 73-86

Pay, T.W.F. and Hingley, P.J. (1986). The use of serum neutralizing antibody assay for the determination of the potency of foot and mouth disease (FMD) vaccines in cattle. Dev. Biol. Stand. 64, 153-161

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/ (Date of access: 25/11/2015)

Reeve, R., Cox, S., Smitsaart, E., Beascoechea, C.P., Haas, B., Maradei, E., Haydon, D.T. and Barnett, P. (2011). Reducing animal experimentation in foot-and-mouth disease vaccine potency tests. Vaccine 29, 5467-5473

Robiolo, B., La Torre, J., Maradei, E., Beascoechea, C.P., Perez, A., Seki, C., Smitsaart, E., Fondevila, N., Palma, E., Goris, N., De Clercq, K. and Mattion, N. (2010). Confidence in indirect assessment of foot-and-mouth disease vaccine potency and vaccine matching carried out by liquid phase ELISA and virus neutralization tests. Vaccine 28, 6235-6241

Robiolo, B., Seki, C., Fondevilla, N., Grigera, P., Scodeller, E., Periolo, O., La Torre, J. and Mattion, N. (2006). Analysis of the immune response to FMDV structural and non-structural proteins in cattle in Argentina by the combined use of liquid phase and 3ABC-ELISA tests. Vaccine 24, 997-1008

Selman, P., Chénard, G. and Dekker, A. (2006). Cedivac-FMD; Duration of Immunity in cattle, sheep and pigs. Open session of the EuFMD, Paphos, Cyprus, 17-19 October 2006 http://www.fao.org/ag/againfo/commissions/docs/research_group/paphos/App31.pdf (Date of access: 21/02/2014)

Smitsaart, E.N., Zanelli, M., Rivera, I., Fondevila, N., Compaired, D., Maradei, E., Bianchi, T., O'Donnell, V. and Schudel, A.A., (1998). Assessment using ELISA of the herd immunity levels induced in cattle by foot-and-mouth disease oil vaccines. Prev. Vet. Med. 33, 283-296

Späth, E.J., Smitsaart, E., Casaro, A.P., Fondevila, N., Fernandez, F., Leunda, M.R., Compaired, D., Buffarini, M. and Pessi, H. (1995). Immune response of calves to foot-and-mouth disease virus vaccine emulsified with oil adjuvant. Strategies of vaccination. Vaccine 13, 909-914

Terpstra, C., Van Maanen, C. and Van Bekkum, J.G. (1990). Endurance of immunity against foot-and-mouth disease in cattle after three consecutive annual vaccinations. Res. Vet. Sci. 49, 236-242

Turkish Statistical Institute (2013). Animal Production Statistics, 2012 - http://www.turkstat.gov.tr/PreHaberBultenleri.do?id=13512 (Date of access: 01/11/2013)

Van Bekkum, J.G., Fish, R.C. and Nathans, I. (1969). Immunologic responses in dutch cattle vaccinated with foot-and-mouth disease vaccines under field conditions: Neutrailizing antibody responses and immunity to O, A and C types. Am. J. Vet. Res. 30, 2125-2129

Van Maanen, C. and Terpstra, C. (1989). Comparison of a liquid-phase blocking sandwich ELISA and a serum neutralization test to evaluate immunity in potency tests of foot-and-mouth disease vaccines. J. Virol. Methods 124, 111-119

Woolhouse, M.E.J., Haydon, D.T., Pearson, A. and Kitching, R.P.P. (1996). Failure of vaccination to prevent outbreaks of foot and mouth disease. Epidemiol. Infect. 116, 363-371

PREDICTIVE MODELLING

DYNAMIC GENERALIZED LINEAR MODELS FOR MONITORING ENDEMIC DISEASES: MOVING BEYOND UNIVARIATE PROCESS MONITORING CONTROL ALGORITHMS

A.C. LOPES ANTUNES*, D. JENSEN, T. HALASA, N. TOFT

SUMMARY

The objective was to use a Dynamic Generalized Linear Model (DGLM) based on a binomial distribution with a linear trend, for monitoring the PRRS (Porcine Reproductive and Respiratory Syndrome sero-prevalence in Danish swine herds. The DGLM was described and its performance for monitoring control and eradication programmes based on changes in PRRS sero-prevalence was explored. Results showed a declining trend in PRRS sero-prevalence between 2007 and 2014 suggesting that Danish herds are slowly eradicating PRRS. The simulation study demonstrated the flexibility of DGLMs in adapting to changes in trends in sero-prevalence. Based on this, it was possible to detect variations in the growth model component. This study is a proof-of-concept, demonstrating the use of DGLMs for monitoring endemic diseases. In addition, the principles stated might be useful in general research on monitoring and surveillance of endemic and (re-)emerging diseases.

INTRODUCTION

New methods for monitoring animal diseases continue to be an active area of research. In the past decade, several studies applied statistical quality control methods for syndromic surveillance in human and veterinary medicine (Buckeridge et al., 2005; Jackson et al., 2007; Dórea et al., 2013). Many of these studies applied univariate process monitoring control algorithms to detect outbreaks of re-emerging diseases. In these cases, control and/or eradication measures are implemented whenever certain threshold levels related to the infection or disease status have been exceeded. However, the term “monitoring” can also be used to describe actions, where a continuous process of collecting data on animal diseases is ongoing, but without any instant control activities (Salman, 2003).

For endemic diseases, it is common to implement control and eradication programmes at herd and regional levels to reduce the economic impact of diseases. Often, these programmes are based on laboratory diagnostics. One example is the Danish monitoring programme for Porcine Reproductive and Respiratory Syndrome (PRRS).

---

*Ana Carolina Lopes Antunes, National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Denmark. Email: aclan@vet.dtu.dk

Despite disease control efforts in Denmark, PRRS continues to contribute towards the economic losses of the industry since its first diagnosis in 1992. PRRS monitoring is primarily based on serological testing performed on regular basis from herds that have the Specific Pathogen Free System (SPF) certificate (Specific Pathogen Free System (SPF-SuS), 2015). The frequency of testing depends on the SPF herd type, being performed once a month for breeding herds and once a year for finisher herds. The SPF herds represent about 40% of all Danish swine (SPF-SuS, 2015). For non-SPF herds, PRRS diagnostic test are not mandatory and different reasons might explain the variation in frequency of laboratory testing. Thus, diagnostic laboratory submissions of PRRS are collected based on different purposes and frequencies in Denmark.

For disease monitoring, the resulting time series are characterized by observational noise as a result of the variation in the disease prevalence and of the number of samples and herds tested over time. Furthermore, its randomness and non-stationary nature are difficult to model. In these cases, it is necessary to use models with a more dynamic structure, where it is possible to add trends, cyclic patterns and also allow the parameters to change over time. State space models are one possible approach in which relevant prior knowledge and current information are combined. While state space models have been adopted in herd management (Jensen et al., 2015; Madsen & Kristensen, 2005; Ostersen et al., 2010), their use has been underutilized in veterinary sciences for diseases surveillance purposes. In the literature, there are few studies using these type of models for disease monitoring and surveillance in humans (Cao et al., 2014; Cowling et al., 2006).

The objective was to use a state space model for monitoring the PRRS sero-prevalence in Danish swine herds. The binomial DGLM with a linear growth was described and its performance for monitoring control and eradication programmes based on changes in PRRS sero-prevalence was explored. This study is a proof of concept, demonstrating the use of DGLMs for monitoring endemic disease, but the principles stated might also be useful in general research on monitoring and surveillance of endemic and (re-)emerging diseases.

MATERIALS AND METHODS

Data source

Laboratory submission data stored in the National Veterinary Institute – Technical University of Denmark (DTU Vet) information management system and in the Laboratory for Swine Diseases-SEGES Pig Research Centre (VSP-SEGES) were used to determine the weekly PRRS sero-prevalence in Danish swine herds from January 2007 to December 2014.

Each laboratory submission consisted of individual blood samples collected from the same herd on the same day from different animals. Only submissions where at least 2 individual blood samples were tested by serological tests including Blocking Enzyme-Linked Immunosorbent Assay (ELISA) and/or Immunoperoxidase monolayer assay (IPMA) for one or both PRRSV (Porcine Reproductive and Respiratory Syndrome Virus)strains were included in the analysis. These serological tests used were a DTU Vet “in-house” ELISA (Sørensen et. al, 1997) and IPMA (Bøtner et al., 1994). Furthermore, diagnostic test results performed at VSP-SEGES were based on IDEXX PRRS X3 Ab ELISA test (IDEXX, Ludwigsburg, Germany). Results from experimental studies were excluded from the analysis.

Herds were classified as PRRS sero-positive when at least 2 individual blood samples in each submission tested PRRS positive, independently of the PRRS strain. The between-herd PRRS sero-prevalence was calculated weekly as the proportion of PRRS positive herds from the total number of herds tested for PRRS.

Modelling

A binomial DGLM with a linear growth as described by West and Harrison (1997) was used to model the data. The general purpose of the DGLM is to estimate the underlying parameter vector from the observed data (θ) combined with any prior information available at time 0 (D_0) before any observation is made. This can be achieved sequentially where the estimated value is updated each time a new value (PRRS sero-prevalence) is obtained. In this case, the conditional distribution of θ_t given by D_t ($\theta_t|\mathrm{D}_t$) was estimated. These models can be used to estimate a one-step forecast of the mean, allowing for a comparison with the actual observed PRRS sero-prevalence. Moreover, the linear growth component includes a time-varying slope (or local linear trend), allowing the system to adapt to a possible positive or negative growth for each t.

In general, the DGLM consists of an observation equation (Eq. 1) and a system equation (Eq. 2):

$$\mathrm{g}(p_t) = F_t'\theta_t \tag{1}$$

$$\theta_t = G_t\theta_{t-1} + W_t \tag{2}$$

Equation 1 describes how the values of an observation (PRRS sero-prevalence) derive from $\mathrm{g}(p_t)$, depends on an unobservable parameter vector (θ) for time t based on a linear function. For the model specification, $\mathrm{g}()$ is the identity function. Equation 2 describes the dynamic properties of the unobservable parameter vector θ. In this study, the transposed design matrix (F_t') has the structure presented in Table 1, in order to estimate underlying values of PRRSV sero-prevalence according to Eq 1. The system matrix (G) used to update the mean of the PRRSV sero-prevalence for each time step taking into account the trend. Both matrix structures were constant for each t (week). The variance-covariance matrix (W_t) describes the evolution of variance and covariance of each parameter for each time step. Rather than estimating (W_t), the system variance was modelled using a discount factor (see Eq. 4).

Table 1. Matrices structure used in Eq. 1 and 2

F_t'	G_t
$\begin{bmatrix}1 & 0\end{bmatrix}$	$\begin{bmatrix}1 & 1\\0 & 1\end{bmatrix}$

The DGLM update for each time step t was performed as follows:

a) the posterior distribution for θ_{t-1} was expressed by a prior mean (m_{t-1}) and a variance (C_{t-1}), ($\theta_{t-1}\,|D_{t-1}$)~$[m_{t-1}, C_{t-1}]$;

b) the prior distribution for Θ_t $(\Theta_t \mid D_{t-1}) \sim [a_t, R_t]$ was made based on the prior mean (a_t) and prior variance (R_t) which were calculated as described in Eq. 3 and 4. The specification of the variance components was specified using a discount factor (δ);

$$a_t = G_t m_{t-1} \tag{3}$$

$$R_t = \frac{1}{\delta} G_t C_{t-1} G_t' \tag{4}$$

c) the prior distribution for Y_t $(Y_t \mid D_{t-1}) \sim [f_t, q_t]$ was calculated based on the forecast mean (f_t) and forecast variance (q_t) (Eq. 5 and 6);

$$f_t = F_t' a_t \tag{5}$$

$$q_t = F_t' R_t F_t \tag{6}$$

d) the posterior mean (f_t^*) and variance (q_t^*) were calculated as described in Eq. 7 and 8. In this case, it was assumed that the prior probability p (PRRS sero-prevalence) of a binomial distribution was Beta(α, β). If κ successes (PRRS positive herds) out of n trials (number of herds tested for PRRS) were observed, the posterior p, given the new observation was Beta$(\alpha_t + \kappa_t, \beta_t + n_t - \kappa_t)$. The parameters α_t and β_t were calculated according to Eq. 9 and 10.

$$f_t^* = \frac{\alpha_t + \kappa_t}{\alpha_t + \beta_t + n_t} \tag{7}$$

$$q_t^* = \frac{f_t^*(1 - f_t^*)}{\alpha_t + \beta_t + n_t + 1} \tag{8}$$

$$\alpha_t = f_t \left(\frac{f_t(1 - f_t)}{q_t} - 1 \right) \tag{9}$$

$$\beta_t = (1 - f_t) \left(\frac{f_t(1 - f_t)}{q_t} - 1 \right) \tag{10}$$

e) the posterior distribution for Θ_{t-1} in a) was calculated based on its mean matrix m_t and its variance-covariance matrix C_t as demonstrated in Eq. 11 and 12.

$$m_t = a_t + R_t F_t (f_t^* - f_t)/q_t \tag{11}$$

$$C_t = R_t - R_t F_t F_t' R_t (1 - q_t^*/q_t)/q_t \tag{12}$$

Model initialization: Reference analysis was used to estimate the initial parameters $D_0 \sim [m_0, C_0]$ as described by West and Harrison (1997). We defined the matrices K_t and H_t and the vectors k_t and h_t for the first two observations $p_{1:2}$.

For $t = 1$, the initial parameters were defined as H_1=0, h_1=0, $K_1 = H_1 + F_1 F_1'$ and $k_1 = h_1 + F_1 p_1$. For $t = 2$, the vectors and matrices were updated as described in Eq. 13 to Eq. 16.

$$H_2 = G_2^{-1\prime} K_1 G_2^{-1} \tag{13}$$

$$h_2 = G_2^{-1\prime} k_1 \tag{14}$$

$$K_2 = H_2 + F_2 F_2' \tag{15}$$

$$k_2 = h_2 + F_2 p_2 \tag{16}$$

Then, the prior distribution for $t = 3$ was calculated according to Eq. 17 and 18.

$$m_2 = K_2^{-1} k_2 \tag{17}$$

$$C_2 = K_2^{-1} \tag{18}$$

System variance: The DGLM model was run based on different discount factors (δ) ranging from 0.1 up to 1 by increments of 0.01. The discount factor which minimized the sum of the squared forecast errors based on the first two years of the data was chosen for the analysis.

Monitoring model components: The values obtained from the m vector for each time step t were used to decompose the time series and obtain the model growth (PRRS sero-prevalence trend). The variance on the growth parameter was calculated from the C matrix and used to calculate 95% confidence intervals (CI).

Simulated scenarios: PRRS sero-prevalence baseline was simulated for 8 years, in which the number of positive herds (X) per week was drawn from a binomial distribution ($X \sim bin(\mathrm{n}, \mathrm{p})$) with a probability (p) (PRRS sero-prevalence) and a sample size (n) equal to the number of Danish herds tested for PRRS per week between 2007 and 2014. The weekly sero-prevalence was calculated as the simulated number of sero-positive herds divided by the total weekly number of herds tested. The first 104 weeks were simulated with a constant initial prevalence of 0.24, corresponding to the average PRRS sero-prevalence in Danish herds observed based on the laboratory diagnostic data from 2007 to 2014. In the first scenario (Scenario A), a constant decrease from p=0.24 to p=0.10 during 4 years followed by constant sero-prevalence was simulated. The second scenario (Scenario B) represented a decrease in the sero-prevalence from p=0.24 to p=0.10 during 2 years, followed by an increase to p=0.18 during the subsequent 2 years.

The sensitivity (Se) and timeliness were used to evaluate the performance of the DGLM to detect significant changes in the simulated scenarios. The Se was defined as the proportion of simulations in which significant changes in the model growth component from zero were found. Timeliness was defined as the number of weeks between a change in the PRRS sero-prevalence (decrease, increase, constant) was simulated and detected.

Convergence rate: A total of 20,000 simulations of weekly PRRS sero-prevalence with a constant decrease from 0.24 to 0.05 over 5 years were carried out. The number of iterations needed to reach a stable variance in the average time to detect significant changes (convergence) was determined visually by plotting the variance of the average timeliness with a stepwise increase of 100 iterations up to 20,000 iterations against the number of iterations. Stable results were observed when using only 10,000 iterations and hence all further simulations were run with 10,000 iterations.

All analyses were performed using R (version 3.1.1) (R Core Team, 2014).

RESULTS

Data description

A total of 56,341 laboratory submissions from 5,390 Danish swine were included in the analysis. The average weekly number of herds tested for PRRSV was 130 (min=9, max=206); the mean weekly number of PRRS positive herds was 31 herds (min=0, max= 60). The weekly average PRRS sero-prevalence was 0.24 (min=0, max=0.38). The yearly average of PRRS sero-prevalence declined from 0.28 in 2007 to 0.20 in 2014, with an average decrease of 0.01 per year.

Model initialization and discount factor

Table 2 shows the posterior C_2 and m_2 matrices obtained from the reference analysis and used as priors for the DGLM model for $t = 3$. The discount factor which minimized the sum of forecast errors for the data was δ=0.98.

Table 2. Priors for t = 3 obtained from the reference analysis

m_2	C_2
$\begin{bmatrix} 0.30 \\ 0.07 \end{bmatrix}$	$\begin{bmatrix} 1 & 1 \\ 1 & 2 \end{bmatrix}$

Modelling and decomposing DGLM

Results show a declining trend of PRRS sero-prevalence between 2007 and 2014. Significant decreases (95% CI excluding zero) were detected mainly in the last 6 months of 2007; end of 2008 to the first semester of 2010 and from the last quarter of 2010 until the beginning of 2013 (Fig. 1). No significant increases in PRRS sero-prevalence were observed and all values for the growth component were below 0.

Simulated scenarios

The simulated scenarios are represented in Fig. 2 and 3. The results for the simulation study are presented in Table 3. Significant changes in the model growth component from zero were found in both scenarios. However, the DGLM detected changes in the growth with a higher sensitivity for decreasing changes when compared to constant growth in the time series. The lowest sensitivity was found for Scenario A when the PRRS sero-prevalence became constant after the decrease, with the DGLM growth component being non-significantly different from zero in 39.02% of the simulations.

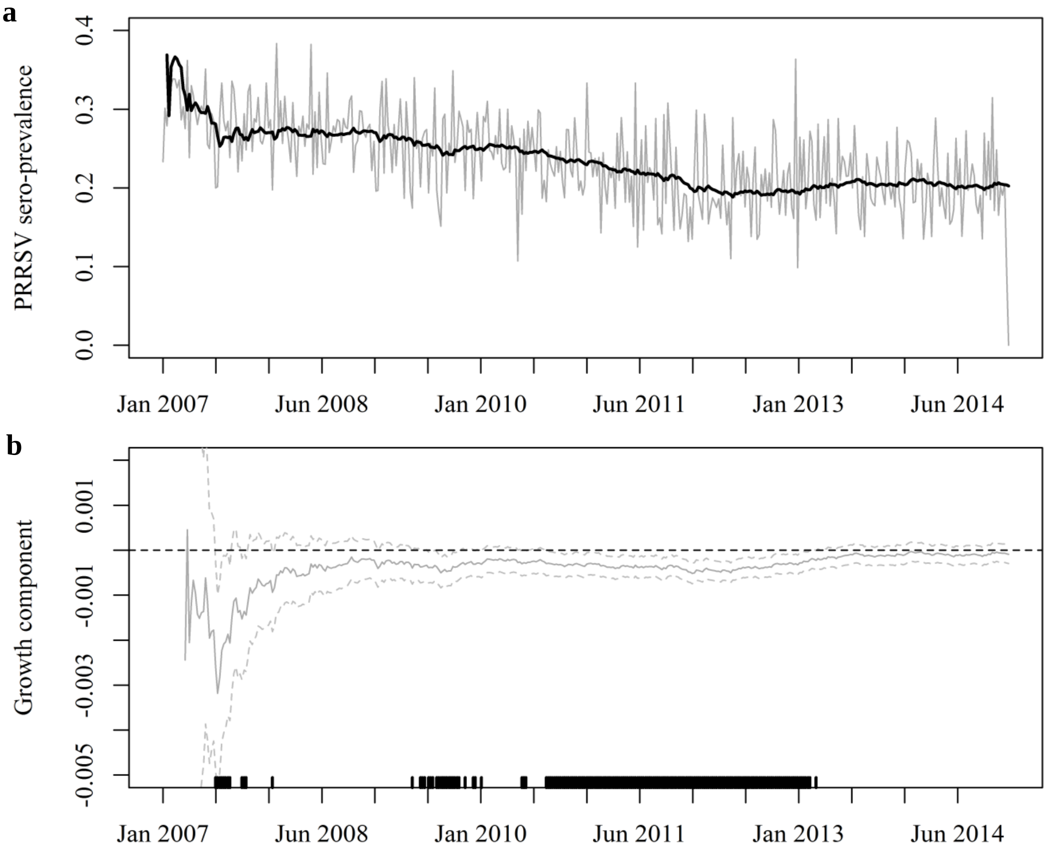


Fig. 1 Using a DGLM to monitor PRRS sero-prevalence in Danish swine herds from 2007 to 2014. Results show the weekly PRRS sero-prevalence and the filtered mean (black) (a) and the corresponding DGLM growth component (b). The black rugs indicate were the growth component is significantly different from zero

Table 3. Timeliness (weeks) and Se for the simulated scenarios

	Scenario A		Scenario B	
Intervention	Decrease	Constant	Decrease	Increase
Timeliness (median)	47	96	27	146
(min-max)	(0-89)	(57-106)	(0-56)	(110-257)
Se (%)	100	39.02	100	99.64

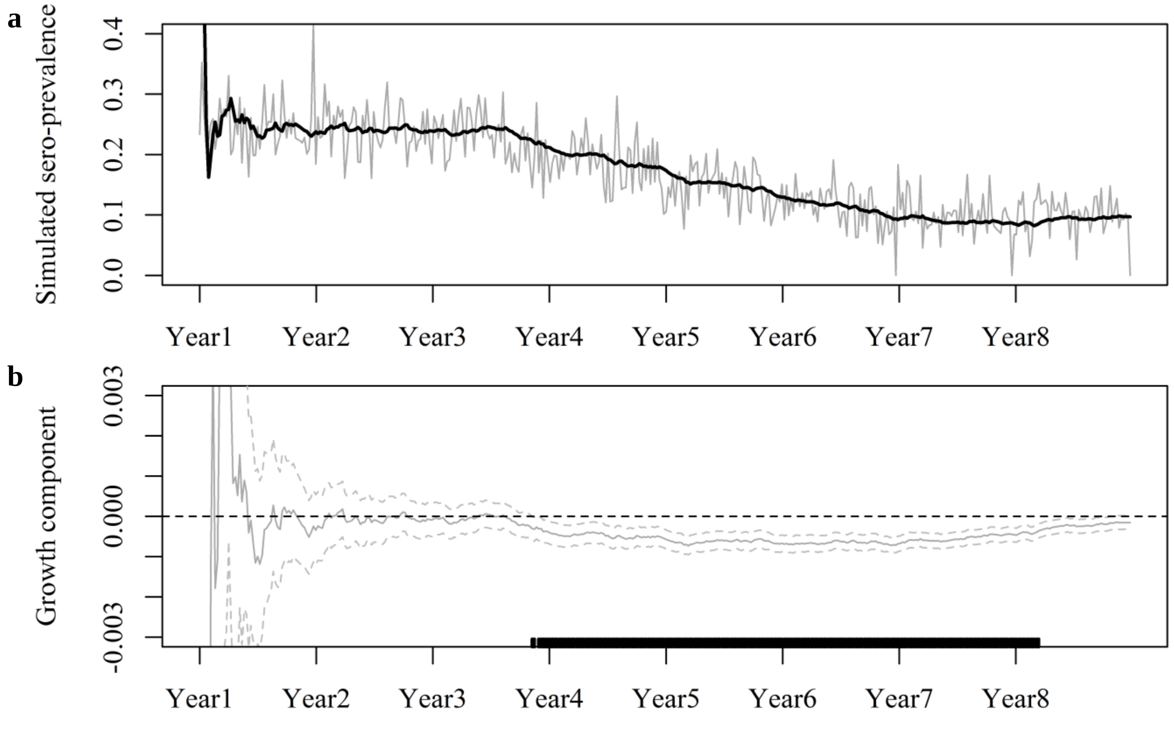


Fig. 2 Simulated control program Scenario A. PRRS sero-prevalence was constant during 104 weeks, followed by a decrease to 0.10 during 208 weeks and then a constant prevalence. The DGLM filtered mean (black line) (a) and the corresponding DGLM growth component (b) (grey lines) are presented. The black rugs indicate a significant negative the growth component based on 95% CI

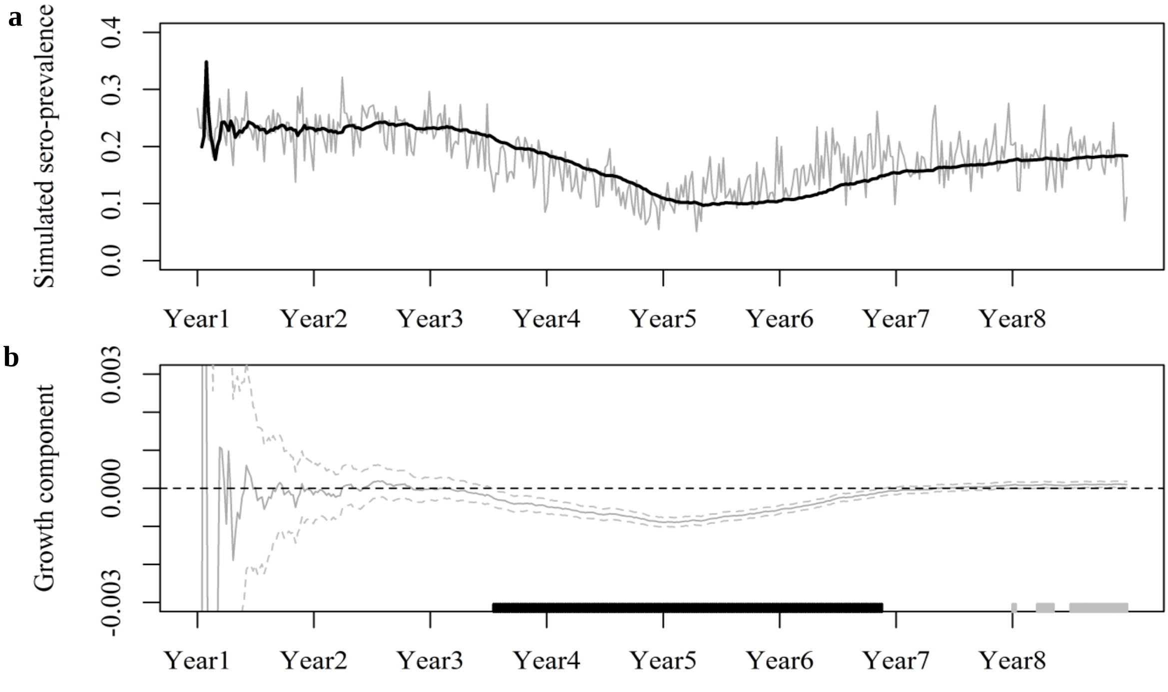


Fig. 3 Simulated control program Scenario B. PRRS sero-prevalence was constant during 104 weeks, followed by a decrease to 0.10 during 104 weeks and an increase up to 0.18 during 104 weeks. The DGLM filtered mean (black line) (a) and the corresponding DGLM growth component (b) (grey lines) are presented. The black and grey rugs indicate significant declines and increase in the growth component based on 95% CI, respectively

DISCUSSION

The objective of this study was to use a binomial DGLM with a linear growth component for monitoring PRRS sero-prevalence in similar contexts to the Danish Pig Industry. The same model can be used for monitoring other prevalence data. These types of models can also be derived for Poisson distribution for monitoring count data, such as the number of samples submitted for analysis etc. Moreover, an ordinary Dynamic Linear Model (DLM) can be used if the data are normally distributed. They also allow for modelling interventions as well as changes in level shift through multi-process models (Thysen, 1993). The DGLM provide a flexible framework in which it is possible to include different data sources in a multivariate process as shown by (Jensen et al., 2015). Moreover, the use of this method allows monitoring of trends and also other components of time series such as seasonal, regression and autoregressive effects components which have a wide interest in biomedical time series applications (West & Harrison, 1997).

As no information on PRRS outbreaks and eradication programmes is available for Danish swine herds, a simulation study was conducted. One limitation of this study is related to the simulation approach used; the simulated sero-prevalence was based on a binomial distribution. The variation in the number of herds tested had an impact on the simulated prevalence contributing to the variation (noise in the baseline). As a consequence, the timeliness to detect interventions showed a wide range of values and the sensitivity was not similar for all interventions. One approach to overcome this issue could be to aggregate the data on a monthly basis, thus reducing the noise in the baseline and possibly improve the performance of the model to adapt to changes in the trend.

The DGLM model was able to detect changes in both scenarios. However, it is important to notice that decreases were larger compared to the increases, corresponding to an absolute decay in sero-prevalence of 0.145 and absolute increase of 0.08. For scenario B, significant positive changes in the model growth component were found after a period in which non-significant changes were found. These justify the longer time needed to detect increases. The variation in the growth parameter was monitored based on 95% CI's. Different approaches could be, *e.g.* Shewart control charts, cumulative sensitivities, V-mask (Montgomery, 2013) or target values, which might yield improved the performances.

In a Bayesian framework the choice of priors is critical for making inference. Reference analysis was used to initiate the DGLM model. From a practical point of view, when a system is set up, the number of observations is low to make the influence of the priors significant. In this case, the use of "non-informative" priors can be used. This method offers an easily applied default analysis (West & Harrison, 1997) when running a DGLM. However, it can be seen from the simulated scenarios that the DGLM takes 3 months to adapt to the data. For this reason, it is important to have historical data (retrospective analysis) to train the model when setting up a monitoring system.

The systems variance was defined based on a discount factor, expressing the decay of information in the system. Defining δ=0.98 implies a small systems variance with a very slow adaptation to new observations. This value was defined using the same method described in Kristensen et al. (2010), where δ should optimized for the performance of the model in making forecasts, *i.e.*, minimizing the forecast errors for the first two years of data (retrospective analysis). In recent literature (Bono et al., 2012; Jensen et al., 2015), the Expectation-Maximization algorithm (Dempster et al., 1977) was used to define the W variance-covariance matrix. This approach offers a general approach to iterative computation of maximum-likelihood estimates when the observations can be viewed as incomplete data. The use of a discount factor provides a parsimonious approach when compared to the full estimation of W.

In summary, results show a declining trend on PRRS sero-prevalence between 2007 and 2014 suggesting more Danish herds are eradicating PRRS. The simulation study highlighted that DGLM are flexible models able to adapt to changes in the time series. It was possible to detect variations in the growth component of simulated scenarios. This study is a proof of concept, demonstrating the use of DGLMs for monitoring endemic disease, but the principles stated might also be useful in general modelling, monitoring and surveillance of (re)emerging diseases. Further analysis to compare the performance of the DGLM, including different components, to other models will be investigated in future studies.

ACKNOWLEDGEMENTS

The authors would like to thank to VSP-SEGES for providing the serological test results.

REFERENCES

Bono, C., Cornou, C. and Kristensen, A.R. (2012). Dynamic production monitoring in pig herds I: Modeling and monitoring litter size at herd and sow level. Livestock Science 149, 289–300. doi:10.1016/j.livsci.2012.07.023

Buckeridge, D. L., Switzer, P., Owens, D., Siegrist, D., Pavlin, J. and Musen, M. (2005). An evaluation model for syndromic surveillance: assessing the performance of a temporal algorithm. MMWR. Morbidity and Mortality Weekly Report 54 Suppl, 109–115. doi:su5401a18

Bøtner, A., Nielsen, J. and Bille-Hansen, V. (1994). Isolation of porcine reproductive and respiratory syndrome (PRRS) virus in a Danish swine herd and experimental infection of pregnant gilts with the virus. Veterinary Microbiology 40(3-4), 351–360. doi:10.1016/0378-1135(94)90122-8

Cao, P.-H., Wang, X., Fang, S.-S., Cheng, X.-W., Chan, K.-P., Wang, X.-L., Lu, X.,Wu, C.-L.,Tang, X.-J., Zhang, R.-L., Ma, H.-W., Cheng, J.-Q., Wong, C.-M. and Yang, L. (2014). Forecasting influenza epidemics from multi-stream surveillance data in a subtropical city of China. PloS One 9(3), e92945. doi:10.1371/journal.pone.0092945

Cowling, B.J., Wong, I. O.L., Ho, L.-M., Riley, S. and Leung, G.M. (2006). Methods for monitoring influenza surveillance data. International Journal of Epidemiology 35(5), 1314–1321. doi:10.1093/ije/dyl162

Dempster, A.P.A., Laird, N.M.N. and Rubin, D.D.B. (1977). Maximum likelihood from incomplete data via the EM algorithm. Journal of the Royal Statistical Society Series B Methodological, 39(1), 1–38. doi:http://dx.doi.org/10.2307/2984875

Dórea, F.C., McEwen, B.J., Mcnab, W.B., Revie, C.W. and Sanchez, J. (2013). Syndromic surveillance using veterinary laboratory data: data pre-processing and algorithm performance evaluation. Journal of the Royal Society, Interface / the Royal Society 10(83), 20130114. doi:10.1098/rsif.2013.0114

Dupuy, C., Morignat, E., Dorea, F., Ducrot, C., Calavas, D. and Gay, E. (2015). Pilot simulation study using meat inspection data for syndromic surveillance: use of whole carcass condemnation of adult cattle to assess the performance of several algorithms for outbreak detection. Epidemiology and Infection 143(12), 2559–2569. doi:10.1017/S0950268814003495

Jackson, M.L., Baer, A., Painter, I. and Duchin, J. (2007). A simulation study comparing aberration detection algorithms for syndromic surveillance. BMC Medical Informatics and Decision Making 7(1), 6. doi:10.1186/1472-6947-7-6

Jensen, D.B., Cornou, C., Toft, N. and Kristensen, A.R. (2015). A multi-dimensional dynamic linear model for monitoring slaughter pig production. In 7th European Conference on Precision Livestock Farming (pp. 503–512). Milan. doi:10.1017/CBO9781107415324.004

Kristensen, A.R., Jørgensen, E. and Toft, N. (2010). Herd Management Science II. Advanced topics. p92

Madsen, T.N. and Kristensen, A.R. (2005). A model for monitoring the condition of young pigs by their drinking behaviour. Computers and Electronics in Agriculture 48(2), 138–154. doi:10.1016/j.compag.2005.02.014

Montgomery, D. (2013). Introduction to statistical quality control. (7th Ed.). Arizona State University: John Wiley and Sons, Inc. 234-276,416-447p

Ostersen, T., Cornou, C. and Kristensen, R. (2010). Detecting oestrus by monitoring sows' visits to a boar. Computers and Electronics in Agriculture 74(1), 51–58. doi:10.1016/j.compag.2010.06.003

R Core Team. (2014). R. A Language and Environment for Statistical Computing. Vienna, Austria. Retrieved from https://www.r-project.org

Specific Pathogen Free System (SPF-SuS). (2015). Specific Pathogen Free System. Retrieved May 12, 2015, from https://www.spf.dk/

Sørensen, K. J., Bøtner, A., Smedegaard Madsen, E., Strandbygaard, B. and Nielsen, J. (1997). Evaluation of a blocking Elisa for screening of antibodies against porcine reproductive and respiratory syndrome (PRRS) virus. Veterinary Microbiology 56(1-2), 1–8. doi:10.1016/S0378-1135(96)01345-4

Thysen, I. (1993). Monitoring Bulk Tank Somatic Cell Counts by a Multi-Process Kalman Filter. Acta Agriculture Scandinavia (43), 58 – 64

West, M. and Harrison, J. (1997). Bayesian Forecasting and Dynamic Models (2nd Ed.). New York, USA: Springer. 136-137,p516-560

BAYESIAN ESTIMATION OF WEATHER-DRIVEN PROCESSES TO MODEL IXODES *RICINUS* POPULATION DYNAMICS

J. CAT[*], T. HOCH, K. CHALVET-MONFRAY

SUMMARY

The vector tick *I. ricinus* population dynamic is driven by weather conditions, mainly temperature and relative humidity. Both variables are of major concern in the context of climate warming. Providing relevant predictions for present and future tick activity time patterns requires integrating the joint effect of temperature and relative humidity on the life cycle biological processes in population dynamic models (PDM). A PDM of *I. ricinus* was established in which the biological processes varied with temperature and relative humidity time series. The PDM focused on host-questing and survival. Both were a priori modelled from expert knowledge and literature data respectively. The parameters associated with each process were then posteriori estimated based on observed field and laboratory data, using a Bayesian inference method. The posteriori parameters can be integrated in a PDM for simulations so as to assess their ability to fit to observed field time series.

INTRODUCTION

The tick *Ixodes ricinus* is a major vector of diseases in pets, cattle and humans across Europe. In particular, *I. ricinus* can transmit babesiosis, louping ill virus and anaplasmosis in cattle and dogs, as well as Lyme borreliosis and tick-borne encephalitis in human (Estrada-Peña et al., 2010). It is the most common tick in Europe and like other arthropods is sensitive to the weather conditions in its habitat. Its life cycle is driven by weather conditions, mainly temperature and humidity. Consequently, over their area of distribution, *I. ricinus* populations display seasonal activity patterns that are characteristic of each climatic area (Korenberg, 2000). The ongoing climate warming in Europe will probably modify these activity patterns. In this context, tick-borne disease present and future risk assessment requires weather-based modeling of the activity time patterns based on observed field data. In this context, both present and future tick-borne disease risk assessments require firstly weather-based models that are competent to reproduce the presently activity time patterns observed in the field.

The *I. ricinus* life cycle is composed of three stages: larvae, nymph and adult. Three phases can be distinguished per stage: the moulting phase, when the tick develops to the next stage, the active phase, when the emerged tick quests for a host at the top of the soil vegetation, and the feeding phase. Over the active phase, ticks quest for a host to feed on if the weather conditions are appropriate. This questing behavior can be reversed: if the weather conditions are too dry or hot, ticks enter the soil to protect themselves from desiccation. Weather can act

---

[*] Julie Cat, UR0346 Animal Epidemiology Unit, INRA - VetAgro Sup Campus vétérinaire de Lyon - 1 avenue Bourgelat – 69280 Marcy-l’Etoile, France. Email: julie.cat@vetagro-sup.fr

on the life cycle at different scales: while development and survival are considered to vary between days, host-questing may vary at the intra-day scale. The population vector activity at a given moment can be assessed by catching the questing active ticks in the field, particularly by the cloth-dragging method. Such field data are the first step to assess the changes in the intensity of exposure to tick bite over time.

Furthermore, population dynamic models aim at producing time patterns for the active population size, while they account for each step of the life cycle. The simulated time patterns of the active population size is thereafter usually compared to the tick count time patterns observed in the field (Gardiner & Gray, 1986, Mount et al., 1997, Ogden et al., 2006, Hoch et al., 2010, Dobson et al., 2011). The biological processes of the *I. ricinus* life cycle such as development, survival and questing ability depend on several weather variables. The effects of these weather variables on some of the biological processes were reported from laboratory experiments. Some of the existing tick population dynamic models integrated weather variables as inputs on the parameters associated with the biological processes. For instance, the development rate defined by Campbell (1948) as a quadratic function of temperature was used in several models (Randolph et al., 2002, Hoch et al., 2010, Hancock et al., 2011). Survival also varied according to weather covariates in the model developed by Gardiner and Gettinby (1983). Host-seeking was already modelled as a function of temperature (Mount et al., 1997, Hoch et al., 2010).

Though the existing population dynamic models that have been developed for the genus Ixodes largely included temperature as a weather input variable, relative humidity has not been included in most of the models. However, relative humidity may represent a major determinant of the seasonal trends in the genus *Ixodes* tick populations. Indeed, relative humidity has a great impact on survival among genus *Ixodes* as already evidenced by Milne (1950) and Stafford (1994). Its influence on the questing ability was also evidenced by Perret and others (2003). More generally, the weather conditions experienced by ticks in their natural habitats are defined by the joint effect of temperature and humidity. Moreover, most of the population dynamic models were fitted on observed tick counts obtained in Atlantic areas (Gardiner & Gray, 1986, Randolph et al., 2002, Gray et al., 2009, Dobson et al., 2011, Hancock et al., 2011), characterized by a mild climate without extreme summer or winter weather conditions. For instance, no population dynamic model was fitted in the southern margin of the *I. ricinus* distribution area in Europe. Indeed in the present Continental and semi-Continental climate areas characterised by hot and dry summers, models have to be particularly sensitive to the more extreme seasons, particularly summers, in order to provide realistic pictures of the population dynamics.. In order to provide scenarios under conditions of climate warming, models must be relevant to address the greater dryness and more frequent heatwave events that may be projected over Europe in the future (Barros et al., 2014). Finally, in the existing tick population dynamic models the observed time series have only been used to validate the models by comparing the observed and simulated population dynamics. In tick population dynamic modeling the observed field time series have not been used to develop the models, i.e. to determine the model parameters.

Bayesian estimation can enable integration of both i) the joint contribution of expert knowledge on the effect of weather on a given biological process, and ii) observed field data in parameter estimation. Bayesian estimation was already used for answering ecological questions from observed field data, when most of the population dynamic was hidden but known in theory, such as estimating fish (Rivot et al., 2004) or tick (Bord et al., 2014) population sizes.

The objective was to set up a population dynamic model of *I. ricinus* in which the biological processes that drive the population dynamics varied with weather time series, including both temperature and humidity. Survival and questing were identified as the biological processes most sensitive to relative humidity.. This study presents a method for modeling both the survival and host-questing processes including previous knowledge and observed data.

MATERIALS AND METHODS

The Bayesian estimation approach required to identify and distinguish i) the prior knowledge of each process, from ii) the observed dataset to be used for posterior parameter estimation. Survival and host-questing both vary in a complex way in relation to the joint effects of temperature (T) and relative humidity (RH). Consequently, a first major step was to model their response to each variable. This represented the prior setting step, which consisted of translating the biology-based shape observed graphically into a mathematical expression, as a function of T and RH. This resulted in a prior model per process, in which a prior distribution was attributed to each model parameter (slope, asymptote, intercept, etc.). Prior distributions were chosen so as to catch the variability in the data at each T-RH condition. Finally, the posterior distributions for each parameter were determined using Bayesian inference. For this step, a second set of observed data was used. Both mortality rate and host-questing probability relationships with T and RH displayed a ceiling and a floor at their upper and lower margins. This is why logistic, log-logistic and Weibull models were investigated for prior modeling as they ensure asymptotical behaviours at the upper and lower limits of the ranges of values.

Host-questing

Bayesian inference model structure: Host-questing (HQ) behaviour is supposed to vary at the intra-day scale, while the active population size varies at the intra-season scale. This was already highlighted for other vectors such as the mosquito (Chalvet-Monfray et al., 2007). Consequently, for the Bayesian inference on host-questing activity the day and hour levels have to be distinguished. Among a given population of ticks in their active phase on day $N_{A,i}$, at the hourly weather condition n a number of ticks $N_{Q,i,n}$ is questing, according to a HQ probability p_n. The weather condition n is defined by the two parameters $(T_{i,h},\ RH_{i,h})$, i.e. the hourly temperature and relative humidity at the questing time h on day i. $N_{A,i}$ and p_n allow defining the binomial distribution $N_{Q,i,n} \sim \mathcal{B}(N_{A,i}, p_n)$. The active population size can be considered constant over the entire day, because intra-day mortality can be neglected (reference for this?). A normal distribution was attributed to $logit(p)$ so that $logit(p_n) \sim \mathcal{N}(logit(\hat{p}_n), \sigma^2)$ where $\hat{p}_n$ the expected value was obtained by prior modeling. However, the questing population $N_{Q,i,n}$ can only be partially assessed by field sampling. As previously defined by Bord et al. (2014) the cloth-dragging sampling method allows catching a proportion of the questing ticks $N_{C,i,n}$, which is the population caught on the cloth under the weather condition occurring at the collection time. $N_{C,i,n}$ has a binomial distribution such that $N_{C,i,n} \sim \mathcal{B}(N_{Q,i,n}, \tau)$ where τ is the sampling rate associated to the cloth-dragging method. The observed dataset was composed of the caught population $N_{C,i,n}$, and the couple $(T_{i,h}\ ,\ RH_{i,h})$ defining the weather condition n.

Defining prior model shape from expert knowledge data: Beugnet and others (2009) defined *I. ricinus* aggressiveness as the response to T and RH in an activity matrix. For each class of joint T and RH an activity index ranging from 0 to 100 was attributed based on expert judgement. This base was considered to set up prior modeling of the weather-induced changes in host-questing probability at the intra-day level. Three-dimensional representation of this activity matrix is displayed in Fig. 1.

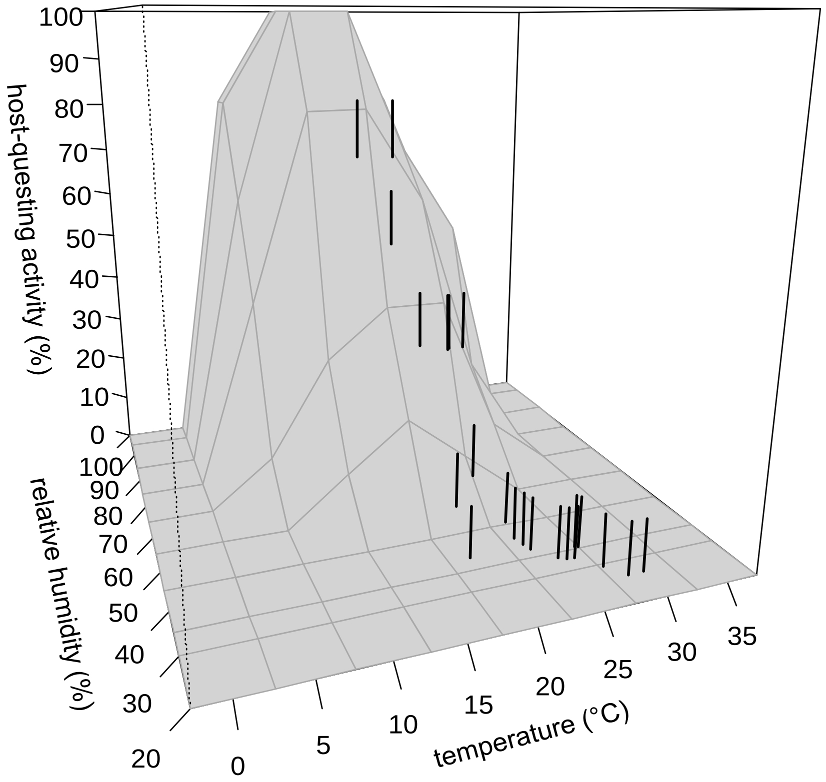


Fig. 1 Prior knowledge of the joint effect of temperature (T) and relative humidity (RH) on the proportion of host-questing *I. ricinus* ticks. Solid surface: activity index adapted from the expert-based matrix elaborated by Beugnet et al. (2009). Solid black lines: T-RH conditions at which field observed data on host-questing activity were available for Bayesian estimation

Three-dimensional graphical analysis of the behaviour of this activity index was performed. Figure 1 showed that host-questing decreased from the ceiling host-questing activity $K_m = 100$ at an optimal temperature to the floor 0 value both at the upper and lower limits of the temperature range. The shapes on each side of the optimal T were modelled as logistic functions. The contribution of T was modelled with two logistic functions. A logistic model was considered to reproduce the sharp decrease in host-questing probability with decreasing RH as seen in Fig. 1. The host-questing activity value was finally written as

$$HQ = K_m\left(1 - \frac{1}{1+e^{\beta(T-\theta_1)}}\right)\left(1 - \frac{1}{1+e^{b(T-\theta)}}\right)\frac{1}{1+ae^{-\alpha RH}} \tag{1}$$

where θ and θ_1 the T50 values, β and b the associated slopes. θ and $\theta_1$were estimated graphically at 27 °C and 3°C. α was the slope of the logistic curve that defined the relationship with RH, and a was a parameter that delayed the raise in activity at the floor activity value. It was determined graphically that the slope α varied with temperature.

Thus, the temperature-varying slope α was written as

$$\alpha = \alpha_m \left(1.5 - \frac{1}{1+e^{g(T-\theta_2)}}\right) \qquad (2)$$

so that α was equal to the maximal slope value α_m below the temperature value θ_2, with g the associated slope. For temperature values above θ_2, the slope α decreased with temperature. To obtain the expected host-questing probability value $\hat{p}_n$, HQ was divided by 100. The prior estimates attributed to each parameter due to graphical analysis are summarized in Table 1. The prior model shape obtained with these values is displayed in Fig. 2.

Table 1. Prior distributions and values for the parameters in the host-questing model

Parameter	Prior estimate	Prior distribution
K_m	100	-
β	0.65	$\mathcal{U}(0.5,0.8)$
b	0.35	$\mathcal{U}(0.2,0.4)$
θ	27	-
θ_1	3	-
a	100	-
α_m	0.1	$\mathcal{U}(0.05,0.2)$
g	0.1	-
θ_2	15	-
$N_{A,1}$, $N_{A,2}$, $N_{A,3}$, $N_{A,4}$, $N_{A,5}$	200	$\mathcal{U}(100,500)$
$N_{A,6}$, $N_{A,7}$	100	$\mathcal{U}(80,200)$
τ	0.5	$\mathcal{U}(0.1,0.6)$
σ	0.1	-

Observed field data for posterior parameter estimation: Several tick collections were carried out at different times during the same day over summer 2015, on a site of collection in Central-Eastern France (Cat et al., unpublished results). Genus *Ixodes* ticks were caught and counted on ten fix strips every two hours, each strip being a one-by-ten meter surface. . Up to four collections were performed per day between 7 a. m. and 2 p.m.. Simultaneously, hourly weather data were recorded by a local weather station located on the collection site. Seven days were investigated from June 4th to Aug 5th 2015, up to four collections per day were done. A total of 22 tick count values were obtained. The weather conditions corresponding to each tick count are displayed on Fig. 1.

Defining prior distributions: Prior distributions were attributed for some parameters. Uninformative uniform distributions were defined for β, b and α_m (Table 1). A fixed value was attributed to a and g to allow convergence. To account for the dependency between each T50 value θ, θ_1 and θ_2 and its associated slope in the logistic expression, T50 values were fixed at 27°C, 3°C and 15°C respectively. A uniform prior distribution was attributed to the sampling rate τ for which the upper and lower boundaries were adapted from Bord (2014). Due to the population dynamic, the active population size is supposed to decrease from the beginning summer in June to August. To simulate the changes over the observation period two different prior distributions were set for $N_{A,i}$ depending on the date of the collection day i. The final host-questing model structure is displayed in Fig. 3. Posterior distributions were determined using OpenBUGS software (Lunn et al., 2000). Three Monte-Carlo Markov Chains were run (MCMC), 30,000 samplings were performed per chain.

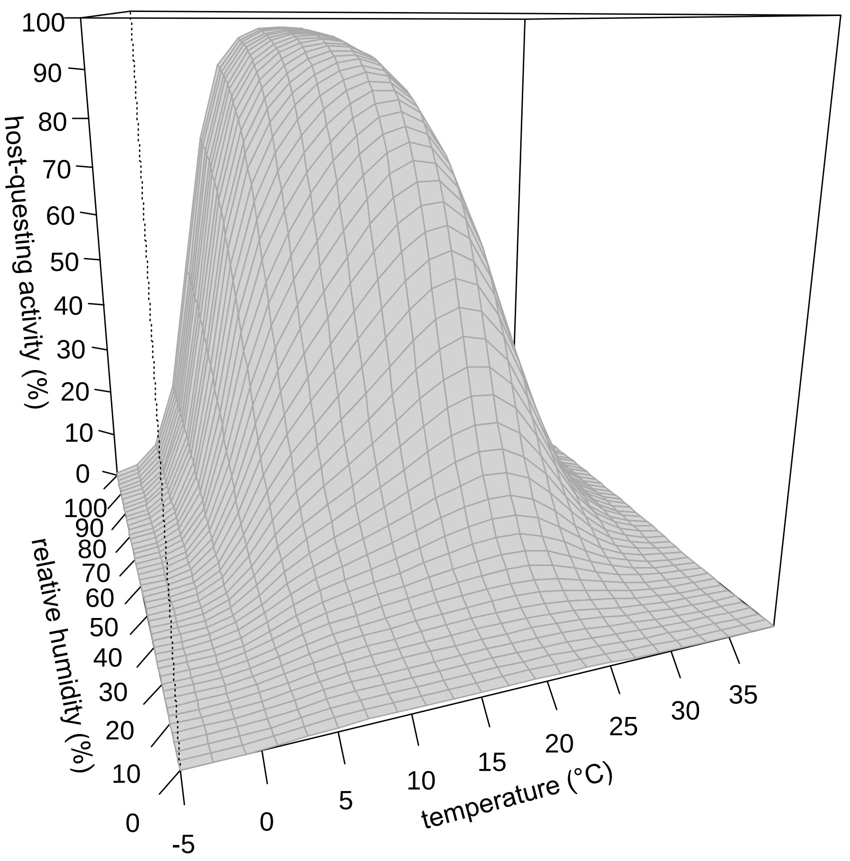


Fig. 2 Prior modeling of the effect of temperature and relative humidity on the proportion of host-questing I. ricinus ticks. Solid surface: host-questing proportion determined by Eq. (1). The parameter values used for calculation are the estimate values reported in Table 1

Survival

Literature data for prior and posterior parameter estimation: Milne (1950) reported survival durations of female I. ricinus ticks under several controlled conditions in laboratory of T (5, 11, 19 and 25°C) and RH (0, 50, 70, 85 and 95%). Five replicates were performed for each T-RH condition. These data were adapted into mortality rates, i.e. inverse survival durations, as displayed in Fig. 4. The mean mortality rates obtained per each T-RH joint conditions were used for prior modeling.

Defining prior model shape: Figure 4 showed that mortality rate μ decreased sharply with increasing RH, and displayed a floor at the highest RH values that seemed similar for all the T conditions. The assumption was made that the effect of RH was the same for 100 % as for 95 %. A ceiling value μ_m could be highlighted, which varied according to the T conditions. In order to model the influence of RH several candidate models were reviewed among logistic, Weibull 1 and Weibull 2 models. These models are usually applied for dose-response modeling in ecotoxicology (Ritz, 2010). All are made of four parameters: a floor value d, a ceiling value μ_m, a slope λ and the parameter r which corresponds to 50 % of the maximal response. Fitting using non-linear regression modeling was performed for each candidate model in R software version 2.15.0 (2015). The models were compared with the residual sum-of-squares (RSS). Previously, the nlstools package (Baty et al., 2014) was used to visualize the initial values to set. For this need, $\log_{10}(\mu)$ was used rather than μ to allow a normal error distribution in the non-linear regression. Accounting for the high variability in the mortality rates, the mean floor value $\log_{10}(d)$ was assumed equal to be -2.5, corresponding to $\log_{10}\left(\frac{1}{300}\right)$. This corresponded to a mean survival duration of 300 days at the highest RH value of 100 %. The ceiling value $\log_{10}(\mu_m)$ was estimated graphically at -0.8, corresponding to $\log_{10}\left(\frac{1}{6.3}\right)$. This corresponded to a mean survival duration of 6.3 days at the lowest RH conditions between 50 and 0 %. The initial values used for non-linear regression were λ = -0.1, $\log_{10}(\mu_m)$ = -0.8 and r = 80 for logistic and Weibull 1 models. Values remained the same for Weibull 2 models except for λ = 0.1 (positive value for λ).

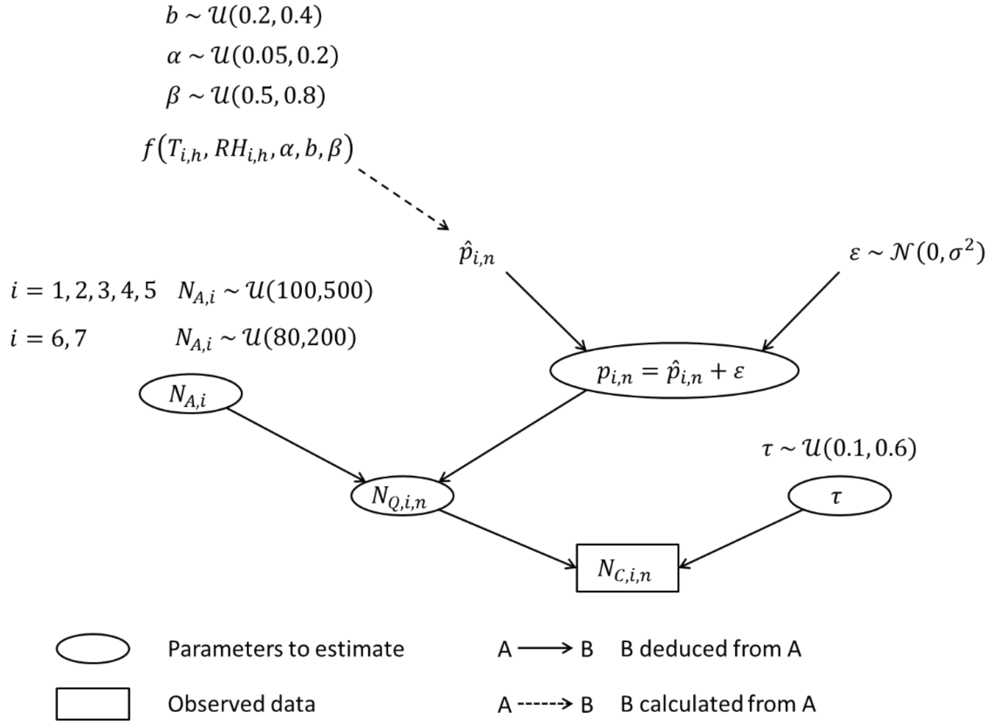


Fig. 3 DAG (Directed Acyclic Graph) for the host-questing model

As displayed in Fig. 4 $\log_{10}(\mu_m)$ increased with T. It was assumed that $\log_{10}(\mu_m)$ reached floor and ceiling values at the lower and upper T limits.

RESULTS

Host-questing

Inference on host-questing probabilities: The highest probability was found on the first collection on day 1 (Fig. 5 median = 0.571, 2.5th percentile (pc) = 0.364, 97.5th pc = 0.810). The changes in tick counts over the day resulted in decreasing host-questing (HQ) probabilities over time during the collection day. Wide posterior distributions were found for some

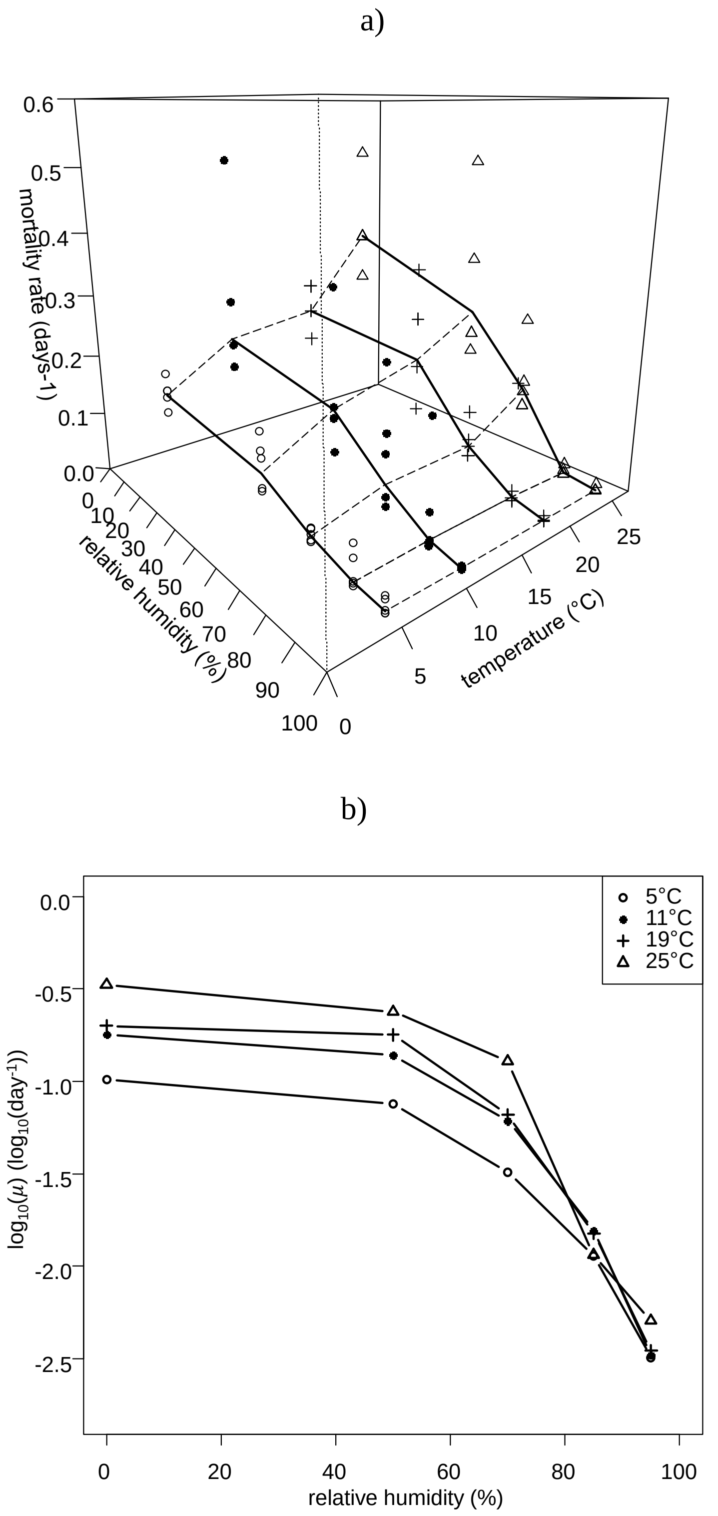


Fig. 4 Mortality rates of *I. ricinus* female ticks adapted from Milne (1950). a) Joint effect of temperature (T) and relative humidity (RH). b) Decimal logarithm of the mean mortality rates. Solid black lines: variation in mean mortality rate with RH. Dashed black lines: variation in mean mortality rate with T. Dots: observed mortality rates per joint T-RH condition for T = 5 °C (filled circles), 11°C (solid circles), 19°C (crosses) and 25°C (filled triangles)

probabilities. The widest distribution was found on the first collection on day 3 (2.5th pc = 0.30, 97.5th pc = 0.81). The wide ranges found on some probabilities indicated that the MCMC converged only roughly to a modal value. Over day 5 ticks underwent very hot and dry conditions (Cat et al., unpublished results). Whereas the HQ activity was scored zero in the activity matrix displayed in Fig. 1 for the first collection of day 5, the probability ranged from 0.138 (2.5th pc) up to 0.404 (97.5th pc) (median value 0.241). Days 6 and 7 were respectively in the end of July and beginning of August. Whereas this period is classically considered to havelow to absent questing activity, a high probability was estimated on the 1st collection of each day (median day 6 = 0.375, day 7 = 0.379). These posterior values were very close to the score obtained with the activity matrix that was equal to 0.4 for both. Probabilities remained high later in the morning (second and third collections), whereas the expert-based activity was scored zero for the corresponding T-RH conditions.

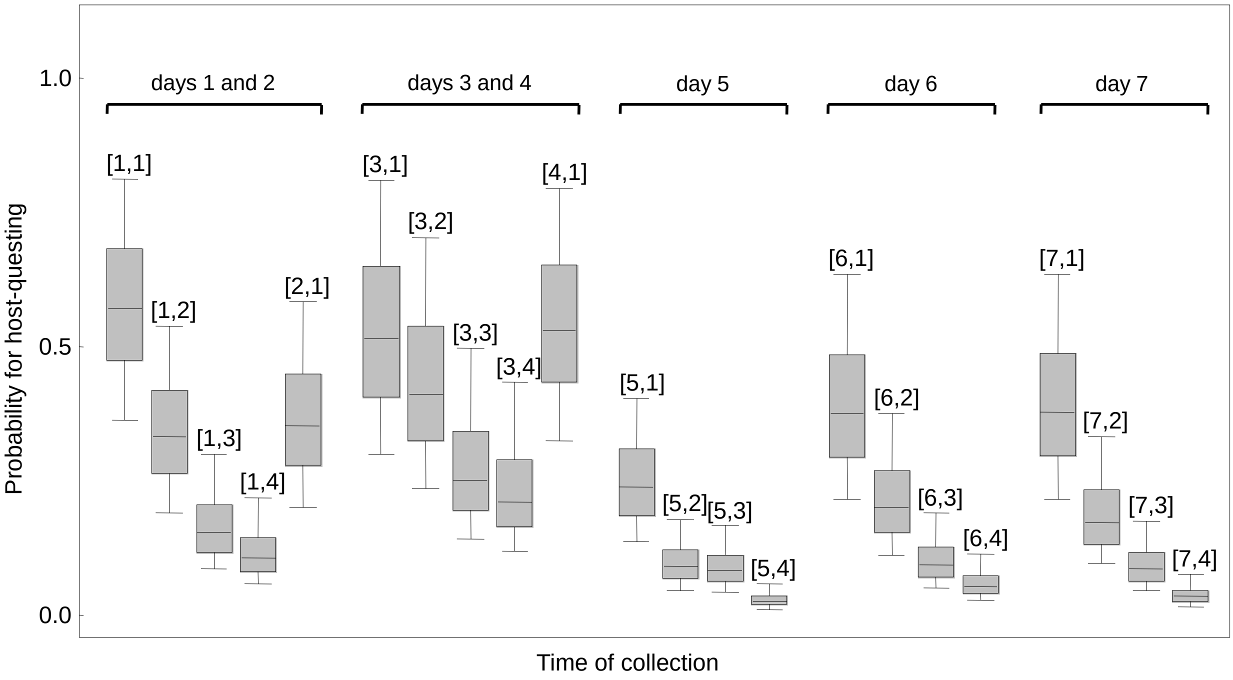


Fig. 5 Posterior distributions of the weather-varying host-questing probability per sampling period during summer 2015. Brackets: chronological order of the collections on each day, e.g. [1, 2] means second collection on day 1

Inference on model parameters: While α_m prior distribution was set at 0.1, the posterior distribution dropped to the lower limit of the prior distribution (Table 2, median = 0.062, 2.5th pc = 0.05, 97.5th pc = 0.082), resulting in an accurate estimate. b values converged from the starting value set at 0.35 (median value = 0.244, 2.5th pc = 0.202, 97.5th pc = 0.341). None convergence of the MCMC was obtained for parameter β, thus the posterior distribution was very similar to the prior uniform distribution $\mathcal{U}(0.5, 0.8)$ (posterior distribution 2.5th pc = 0.509, 97.5th pc = 0.791). τ posterior estimation dropped to the lower limit of the prior uniform distribution assigned (median = 0.142, 2.5th pc = 0.101, 97.5th pc = 0.275).

Table 2. Posterior estimates for the host-questing model parameters

Parameter	Mean	Median	2.5th percentile	97.5th percentile
β	0.651	0.652	0.509	0.791
b	0.252	0.244	0.202	0.3414
α_m	0.0632	0.062	0.0504	0.0818
τ	0.156	0.142	0.101	0.275

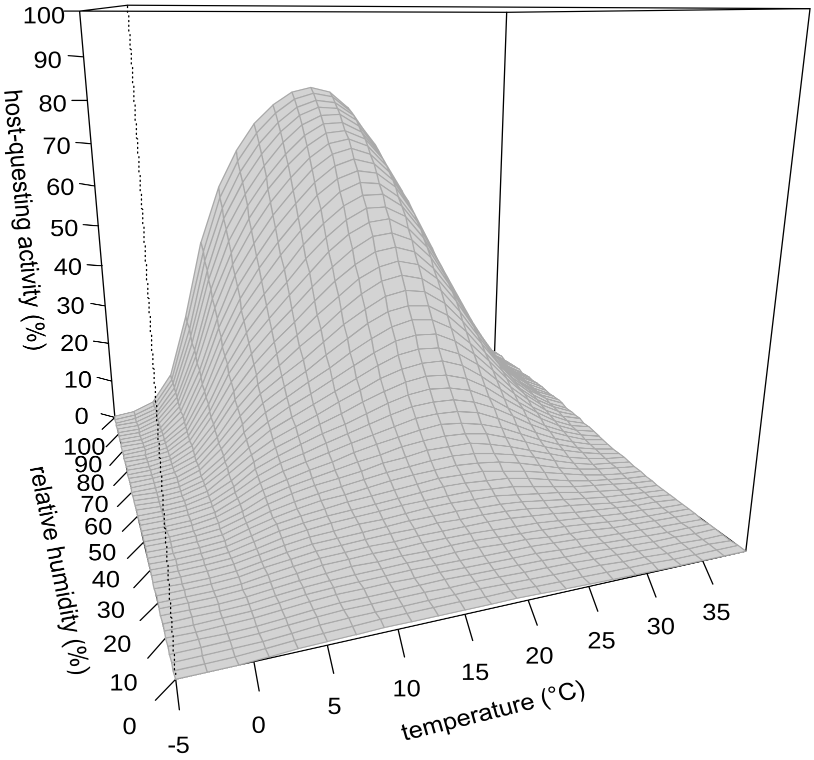


Fig. 6 Host-questing activity model accounting for the posterior parameters obtained. Parameters α_m and b were replaced in Eq. (1) by their respective posterior mean estimates obtained by Bayesian estimation from field data. The other parameters remained as reported in the prior host-questing model, values indicated in Table 1

Figure 6 displayed the effect of the posterior mean estimates of α_m and b respectively on the HQ activity (Table 2). When compared to the prior HQ model in Fig. 2, the increase in activity with increasing RH was less sharp than in the prior model. While the prior model overestimated activity for low RH in the expert model (Fig. 1), the posterior model reproduced the very low activity for low RH that was stated by the expert model. The posterior model displayed non-zero mild activity for temperatures around 30°C and RH values between 60 and 100 %. The maximum activity K_m = 100 was not reached with the posterior model but was approximately 80 %.

Survival

The parameter estimates were significant for the three candidate models. The Weibull-2 model displayed the minimal residual sum-of-squares (RSS) value (Logistic RSS = 0.587, Weibull 1 RSS = 0.656, Weibull 2 RSS = 0.50). Consequently the contribution of RH was modelled as

$$\log_{10}(\mu_m) + \left(\log_{10}(d) - \log_{10}(\mu_m)\right)\left[1 - e^{-e^{\lambda(RH-r)}}\right] \quad (3)$$

The associated parameter estimates are summarized in Table 3. The estimates were very close to the starting values (λ = 0.1, r = 80 %, $\log_{10}(\mu_m)$ = -0.8.

Table 3. Non-linear regression model of the mortality rates defined by Milne (1950)

Parameter	Estimate	Standard error	p-value	
λ	0.0913	0.0156	1.95e-05	***
r	83.97	1.34	< 2e-16	***
$\log_{10}(\mu_m)$	-0.747	0.0727	1.04e-08	***

DISCUSSION

In this work the effect of the temperature (T) and relative humidity (RH) weather variables on two biological processes of the I. ricinus tick life cycle was modelled from literature and observed data. For the host-questing behaviour, expert-based literature data on I. ricinus aggressiveness were used to propose a prior model that accounted for the joint effect of T and RH on the host-questing probability. Prior values of the model parameters were estimated graphically, and then Bayesian inference was performed using observed field data on hourly changes in I. ricinus host questing activity. This allowed provision of posterior estimates on both i) the host-questing probabilities at the observed weather conditions, and ii) the model parameters. The observed data on tick questing were obtained in extreme hot and dry heatwave-like conditions for which questing was stated to be absent by the expert knowledge. Though, significant HQ probabilities were estimated for these conditions. For survival, literature data on survival durations tested in T-RH controlled conditions were used to propose a prior model for the joint effect of T and RH on the associated mortality rates. Non-linear regression was performed to first define the relationship between RH and the mean mortality rate at each T-RH condition tested. A Weibull 2 model was finally determined.

Methodological contribution

The work presented here provided a significant methodological insight for tick population dynamic modelling. First, the model scheme presented represents a major tool to exploit the distinct sources of knowledge on tick biology for modelling the population dynamic, whether coming from expert, laboratory controlled trials or field observations. Each source of knowledge is a major element to develop a model. Indeed, field data usually provide information on the seasonal vector activity, but not on the hidden biological processes that drive the life cycle, nor on the proper past weather history of each tick. Laboratory experiments can allow studying tick response to stimuli such as temperature in controlled trials. However, they reflect only partially the weather conditions experienced by ticks on field. On the other hand, experiments on tick biology in field controlled conditions are very few in literature.

Moreover they often provide limited insight into tick response to weather, given that tick populations are difficult to control, resulting in poorly reliable data. Thus laboratory data and expert knowledge remain important in the case of ticks, and more generally in the case of vector ecology. Second, the methods of non-linear regression method and Bayesian inference exploited here were rarely used for studying the I. ricinus population dynamics.

Contribution for closeness to tick biology

The work presented here addressed the importance of some processes of the tick biology that are often neglected in population dynamic modelling, whereas they vary with weather and are determinant processes for the population dynamics.. Tick biology depends on weather variables that can contribute to life cycle processes. First, with the method presented the RH can be included as an input variable in the population dynamic model, as its impact on some biological processes is modelled and therefore integrated. Second, data on weather-varying survival were exploited in the current work, whereas previous reports had not included these. Finally, the intra-day scale was considered for modelling host-questing, accounting for its variability over the day, whereas it has previously been considered only at the day scale (Mount et al., 1997, Hoch et al., 2010).

Assessing the influence of host-questing observed data

The α_m parameter was the RH-related maximal slope as in Eq. (2) and was estimated lower than the prior value. This result is consistent with the posterior values estimated for HQ probability at low RH conditions, which were higher than the prior ones and therefore influenced the model shape upwards, resulting in a smaller RH-varying slope. It is worth noting that α_m was very sensitive to inference as it varied a lot from the prior to the posterior estimate, whereas b posterior estimates approximately converged to the starting value and thus were less sensitive to inference. The β slope affected the HQ probability for the range of temperature values below the optimal temperature, i.e. below approximately 15°C. As no observed data were available for this temperature range this makes sense that β prior was not sensitive to inference. This finding highlights that field data on the changes in host-questing should be obtained for colder and wetter conditions such as experienced in winter, so as to perform again β estimation. The posterior model did not display maximal activity value at 100 % but rather at 80 % (Fig. 6). This may be due to the decrease in α_m value from the prior value assigned to the posterior estimate: the slope decreased, thus the ceiling value couldn't be reached as previously. This result may also be an indirect consequence of the uninformative large prior information that was set for Bayesian inference: as it provided inaccurate values on the active population size, HQ probability determined from the tick counts may have been underestimated. This finally had an impact on the posterior parameter estimates. More accurate prior information and more numerous observed data may allow correcting this effect.

Dealing with unknowns on weather effects

We considered together nymphs and adults without distinction, for both host-questing and survival. However, making the distinction would require either data on each stage for I. ricinus, or extrapolation from other species such as I. scapularis. For instance, only data on survival durations in controlled conditions in I. ricinus female were available. It would be tempting to use the Stafford data (Stafford, 1994) on I. scapularis larvae and nymph survival durations for producing separate prior models for larvae and nymph stages in I. ricinus. Indeed, survival data in controlled conditions are very sparse for I. ricinus. Though, this remains challenging as I. ricinus and I. scapularis weather condition preferences may be different and these differences have not been assessed. Regarding host-questing in I. ricinus, expert parasitology knowledge that would distinguish nymph and adult responses to T and RH could be set. In contrast to HQ expert data, for the survival data no information was available in relation to the extreme temperature values. Mortality rate is expected to reach a ceiling value for extreme temperature values. Ogden and others (2004) observed high mortality at extreme temperature values above 30°C and below 0°C. However, one should be careful when stating the effect of extreme values, as these conditions may be very different to the temperature experienced by the ticks due to the protecting effect of soil. This would in particular result in overestimated mortality rates in the winter.

Improving prior and posterior estimation

The active population size prior distributions were uninformative ones with theoretical boundary values. More precise posterior distributions might be obtained by adding more informative prior distributions and data. First, the seasonal changes in the active population size should be simulated with an existing population dynamic model by a discrete distribution such as a binomial distribution function. This would provide more informative prior distributions on the active population size. Secondly, I. ricinus abundance at the collection site the field data came from should be determined, so as to get closer to the true abundance. Indeed, tick abundance can greatly vary between sites, due to host abundance and type of habitat (James et al., 2013). Removal sampling experiments by cloth-dragging have already been performed for estimating tick abundance (Tälleklint-Eisen & Lane, 2000). Finally, more data on the intra-day changes in the number of host-questing ticks should be obtained.

Simulating present and future population dynamics

The modelled biological processes could be integrated into existing population dynamic models. Then both former and new models could be run with weather time series as input, so as to compare how well the respective simulated time series for active population size fitted the observed field tick count time series. This would help to determine how well integrating the biological processes would increase the ability for the population dynamic model to reproduce the observed field time series. Sensitivity analysis of T and RH should be performed for both models. This would allow i) comparison of the behaviour of each model, and ii) assessment of the influence of the weather-driven biological processes on the simulated time series. Regarding the present exposure to tick assessment, the quality assessment of the models developed should be performed for a location with contrasting seasons such as within Continental climate areas, where extreme weather conditions occur either in winter or in summer. Regarding the future exposure to tick assessment, a population dynamic model that includes T and RH on survival and questing will probably be relevant to assess climate warming scenarios. Though, its ability to fit to field time series should be tested in a location where hot, dry summers and mild winters occur, while still within the I. ricinus distribution area. For instance this is the case of South-Western France.

ACKNOWLEDGEMENTS

We thank Marie-Laure Delignette-Muller for advising on the statistical modelling and Bayesian inference. This work was supported by the metaprogramme Adaptation of Agriculture and Forests to Climate Change (ACCAF) of the French National Institute for Agricultural Research (INRA).

REFERENCES

Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J.-P. and Delignette-Muller, M.-L. (2014). A toolbox for nonlinear regression in R: the package nlstools. J. Stat. Softw 66, 1-21

Beugnet, F., Chalvet-Monfray, K. and Loukos, H. (2009). FleaTickRisk: a meteorological model developed to monitor and predict the activity and density of three tick species and the cat flea in Europe. Geospatial Health 4, 97–113

Bord, S., Druilhet, P., Gasqui, P., Abrial, D. and Vourc'h, G. (2014). Bayesian estimation of abundance based on removal sampling under weak assumption of closed population with catchability depending on environmental conditions. Application to tick abundance. Ecol. Model. 274, 72–79

Campbell, J.A. (1948). The life history and development of the sheep tick, *Ixodes ricinus* L., in Scotland under natural and controlled conditions. Edinburgh, UK: University of Edinburgh.

Chalvet-Monfray, K., Sabatier, P. and Bicout, D.J. (2007). Downscaling modeling of the aggressiveness of mosquitoes vectors of diseases. Ecol. Model. 204, 540–546

Dobson, A.D.M., Finnie, T.J.R. and Randolph, S.E. (2011). A modified matrix model to describe the seasonal population ecology of the European tick *Ixodes ricinus*. J. Appl. Ecol. 48, 1017–1028

Estrada-Peña, A., Farkas, R., Jaenson, T.G., Madder, M., Pascucci, I., Salman, M. and Tarrés-Call, J. (2010). Scientific opinion on geographic distribution of tick-borne infections and their vectors in Europe and the other regions of the Mediterranean Basin: EFSA Panel on Animal Health and Welfare (AHAW). EFSA J. pages etc

Gardiner, W.P. and Gettinby, G. (1983). A weather-based prediction model for the life-cycle of the sheep tick, *Ixodes ricinus* L. Vet. Parasitol. 13, 77–84

Gardiner, W.P. and Gray, J.S. (1986). A computer simulation of the effects of specific environmental factors on the development of the sheep tick *Ixodes ricinus* L. Vet. Parasitol. 19, 133–144

Gray, J.S., Dautel, H., Estrada-Pena, A., Kahl, O. and Lindgren, E. (2009). Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. 2009, 593232–Article ID 593232

Hancock, P.A., Brackley, R. and Palmer, S.C.F. (2011). Modelling the effect of temperature variation on the seasonal dynamics of *Ixodes ricinus* tick populations. Int. J. Parasitol. 41, 513–522

Hoch, T., Monnet, Y. and Agoulon, A. (2010). Influence of host migration between woodland and pasture on the population dynamics of the tick *Ixodes ricinus*: A modelling approach. Ecol. Model. 221, 1798–1806

James, M.C., Bowman, A.S., Forbes, K.J., Lewis, F., McLeod, J.E. and Gilbert, L. (2013). Environmental determinants of *Ixodes ricinus* ticks and the incidence of *Borrelia* *burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. Parasitology 140, 237–246

Korenberg, E.I. (2000). Seasonal population dynamics of *Ixodes* ticks and tick-borne encephalitis virus. Exp. Appl. Acarol. 24, 665–681

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

Milne, A. (1950). The ecology of the sheep tick, *Ixodes ricinus* L.; microhabitat economy of the adult tick. Parasitology 40, 14–34

Mount, G.A., Haile, D.G. and Daniels, E. (1997). Simulation of blacklegged tick (Acari : Ixodidae) population dynamics and transmission of *Borrelia burgdorferi*. J Med Entomol 34, 461–484

Ogden, N.H., Lindsay, L.R., Beauchamp, G., Charron, D., Maarouf, A., O'Callaghan, C.J., Waltner-Toews, D. and Barker, I.K. (2004). Investigation of relationships between temperature and developmental rates of tick *Ixodes scapularis* (Acari: Ixodidae) in the Laboratory and Field. J. Med. Entomol. 41, 622–633

Ogden, N.H., Maarouf, A., Barker, I.K., Bigras-Poulin, M., Lindsay, L.R., Morshed, M.G., O'Callaghan, C.J., Ramay, F., Waltner-Toews, D. and Charron, D.F. (2006). Climate change and the potential for range expansion of the Lyme disease vector *Ixodes scapularis* in Canada. Int. J. Parasitol. 36, 63–70

Perret, J.L., Guerin, P.M., Diehl, P.A., Vlimant, M. and Gern, L. (2003). Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*. J. Exp. Biol. 206, 1809–1815

R: A Language and Environment for Statistical Computing. http://www.R-project.org/

Randolph, S.E., Green, R.M., Hoodless, A.N. and Peacey, M.F. (2002). An empirical quantitative framework for the seasonal population dynamics of the tick *Ixodes ricinus*. Int. J. Parasitol. 32, 979–989

Ritz, C. (2010). Toward a unified approach to dose-response modeling in ecotoxicology. Environ. Toxicol. Chem. 29, 220–229

Rivot, E., Prévost, E., Parent, E. and Baglinière, J.L. (2004). A Bayesian state-space modelling framework for fitting a salmon stage-structured population dynamic model to multiple time series of field data. Ecol. Model. 179, 463–485

Stafford, K.C. (1994). Survival of immature *Ixodes scapularis* (Acari: Ixodidae) at different relative humidities. J. Med. Entomol. 31, 310–314

Tälleklint-Eisen, L. and Lane, R.S. (2000). Efficiency of Drag Sampling for Estimating Population Sizes of *Ixodes pacificus* (Acari: Ixodidae) Nymphs in Leaf Litter. J. Med. Entomol. 37, 484–487

Barros V.R., Field C.B., Dokken D.J., Mastrandrea M.D., Mach K.J., Bilir T.E., Chatterjee M., Ebi K.L., Estrada Y.O., Genova R.C., Girma B., Kissel E.S., Levy A.N., MacCracken S., Mastrandrea P.R. and White L.L. (2014). Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. In, Intergovernmental Panel on Climate Change, contribution of Working Group II to the Fifth Assessment Report., Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press, p 688

RISK FACTORS AND PREDICTIVE MODELS FOR EQUINE FRACTURES IN THOROUGHBRED FLAT RACING IN NORTH AMERICA

S.P. GEORGOPOULOS[*], T.D.H. PARKIN

SUMMARY

The aim of this paper is to identify the risk factors associated with fractures as well as to develop and validate the performance of predictive models for fractures in flat horse racing of Thoroughbreds in North America. The analysis is based on 230,034 Thoroughbreds that made 2,201,152 starts (91% of all official racing starts over the time period) in the USA and Canada from 1st January 2009 to 31st December 2014. Multivariable logistic regression was used to identify plausible risk factors. Risk factors were selected through a stepwise process with a forward bidirectional elimination approach using Akaike's Information Criterion (AIC). Logistic regression models were also used as predictive models along with two machine learning models: Improved Balanced Random Forests (IBRF) and Artificial Neural Networks (ANN). The models were trained on races from the period 2009 – 2013 and the races from 2014 were used for performance evaluation.

INTRODUCTION

The aim of this work was to quantify the risk of fatal and non-fatal fractures for individual horses on entering a race in North America so that horses at particular risk can be identified and the risk of injury reduced. Data included for analysis were from the Equine Injury Database (EID) for the years 2009 – 2014. The EID contains information for most races that took place in North America during that period and is a near census collection of the available data.

One of the objectives of the study was to identify, for Thoroughbred flat racing in the USA and Canada, the association between equine fractures and plausible risk factors. Those risk factors were related to the horse; such as gender, age, expected performance using the horse's odds, time spent training with the same trainer, prior racing history, and prior injuries and scratches, as well as, related to the race; such as surface type, country and race distance.

Recent studies investigating equine injuries across different countries and jurisdictions have identified associations between injuries and similar risk factors. Age, gender, race distance, surface type and conditions, race type, field size, season, prior racing history, distance galloped in training, exercise history, prerace inspection by regulatory veterinarians and shoe

---

[*] Stamatis Panagiotis Georgopoulos, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, 464 Bearsden Road, Glasgow G61 1QH, UK. Email: s.georgopoulos.1@research.gla.ac.uk

characteristics have been identified to be associated with equine injuries (Peloso et al., 1994) (Estberg et al., 1995; Estberg et al., 1996a; Estberg et al., 1996b; Kane et al., 1996; Cohen et al., 1997; Estberg et al., 1998; Estberg et al., 1998; Kane et al., 1998; Cohen et al., 2000; Hernandez et al., 2001; Williams et al., 2001; Parkin et al., 2004a; Parkin et al., 2004b; Hernandez et al., 2005; Parkin et al., 2005; Henley et al., 2006; Parkin et al., 2006; Boden et al., 2007; Lyle et al., 2012). Despite the geographical differences and differences in practices across those jurisdictions and in some cases the lack of consistency in the specific outcome chosen to be modelled, these studies provided a starting point from which to expand on the risk factor analysis of equine fractures in North America. Risk factors specific to the North American jurisdiction have also been included such as a horse having previously entered the veterinarian's list, a list used by association and regulatory veterinarians to provide horses with illness, injury or soundness issues a brief respite from racing. Furthermore, since the information in the EID did not include training exercise the number of starts a horse had prior to a race might be a proxy for increased cumulative exercise. Moreover, information on prerace inspection by regulatory veterinarians was not available but the number of times a horse had been previously scratched (taken out of the race before it started) has been included as a risk factor.

Another objective of the study was to make use of logistic regression and machine learning models to quantify the probability of a Thoroughbred sustaining a fracture during flat racing. Since machine learning techniques have been used with success for tasks ranging from classification to robotics and natural language processing (Bengio, 2009), they may also be useful for predicting equine injuries in flat racing. The project's classification problem was a highly abstract one where the road from input to output was not known. Furthermore, the response, an equine fracture, was an extremely rare result, at about 0.2% of the starts, producing an imbalanced, hard to predict, output. However, by making use of the large dataset available in the EID over a 6-year period, it was considered that the machine learning methods could be efficiently used to produce a satisfactory classification result.

The information contained in the EID could be used to identify risk factors, to make predictions on a horse's risk level and ultimately improve racing conditions in North America and notwithstanding differences across countries, be useful for other horse racing jurisdictions as well.

MATERIALS AND METHODS

The analysis reported in this paper is based on data available in the EID for the years 2009 to 2014. The Equine Injury Database contains information on flat racing injuries of Thoroughbreds from racetracks across the United States and Canada and was officially launched by *The Jockey Club* in 2008. Race start data were supplied by *The Jockey Club* and covered all tracks that voluntarily contributed to the EID in each year. The injury reports were recorded into the EID by veterinarians at the participating racetrack. The analysis for those six years contained information on 230,034 Thoroughbreds racing at 89 racecourses. All official starts were used to calculate historical information for each horse but only the 2,201,152 starts from the racecourses reporting injuries to the EID were used in the models. The starts reported to the EID represented 91% of starts from all official racing in the USA and Canada for that period.

Risk factor analysis

The first step of the risk factor analysis was specifying the outcome variable. The outcome used in this analysis was any type of fracture the horse might have sustained, identified from veterinary records within the EID. These included both fatal (i.e. the horse was subject to euthanasia) and non-fatal fractures. The analysis described below was performed with the outcome at the level of the start and all starts made by horses that did not result in fracture were included as controls or unaffected horses.

Then the association between the possible risk factors and the outcome variable was evaluated with a univariable logistic regression model for each risk factor. The risk factors that were found to be significantly associated with a p-value less than 0.20, were chosen to be included in a stepwise selection process. The p-value threshold of 0.20 was chosen to prevent the exclusion of a predictor that only becomes evident when possible confounders have been accounted for (Dohoo et al., 2003). A forward bidirectional elimination approach using Akaike's Information Criterion (AIC) was utilised for the stepwise selection. AIC allows comparisons between the competing models. Maximum likelihood is used to compare between models with different risk factors, with an added penalty for complexity which depends upon the number of parameters used to fit the data. This penalty can be seen as an allowance made for parsimony. The best model, following Occam's razor, is the one offering the highest information gain with least complexity (Bozdogan, 1987). The final multivariable logistic regression model included the risk factors selected through this stepwise selection.

This process was repeated to create another multivariable logistic regression model on a sub-sample of the population consisting only of starts from horses that had been in racing for at least six months. This model was created to assess the risk factors that summarise historical racing information prior to each race (Table 2).

Predictive models

The first predictive model used was a multivariable logistic regression model. The model was trained using the same methodology used in the risk factor analysis. Predictions for horses that had been in racing for at least six months were obtained from a separate model specific to that population using variables that summarised historical racing information prior to each race.

A second predictive model was trained using the Improved Balanced Random Forests (IBRF) algorithm proposed by Xie and others (2009). The IBRF algorithm is based on Random Forests developed by Breiman (2004) and specifically on Balanced Random Forests an approach suggested by Chen and others (2004) to accommodate for imbalanced outcomes. For the model, 1000 conditional inference trees (Strasser & Weber, 1999) were trained on samples, randomly drawn with replacement, numbering A*N positive outcomes and (2-A)*N negative outcomes, where N is the number of all positive outcomes and A is a number randomly and uniformly selected for each sample, between 0.9 and 1.1. Positive and negative cases were then weighted by 1/A and 1/(2-A) respectively. The resulting prediction was the averaged outcome from the ensemble of trees.

A final predictive model, based on an implementation of Artificial Neural Networks (ANN) (Bishop, 1995) was created. Given a big enough dataset, simple network architectures (Ciresan et al., 2010) and architectures without unsupervised pre-training (Ciresan et al., 2012) can achieve good competitive results. The ANN used was feed-forward, trained using the backpropagation algorithm (Rumelhart et al., 1986) and had one fully connected hidden layer with 500 nodes. The activation function used for the nodes of the networks was the sigmoid hyperbolic tangent, that converges faster than the logistic sigmoid (Glorot & Bengio, 2010; LeCun et al., 2012). From the variables available in the EID, those that were found to be associated with equine fractures, signified by a p-value of less than 0.2 on univariate logistic regression models were used in the input layer of the models. The input variables were transformed as suggested by LeCun and others (2012). Categorical variables were given values of either -1 or 1. For those with more than two levels, dummy variables were created. Numerical variables were centred at zero and normalised Training of the ANN was conducted using mini-batches of 10,000 observations each. This stochastic learning method was chosen because it is faster and it usually leads in better solutions as it easier for the model to avoid local minima (LeCun et al., 2012). The model was trained for 1000 epochs.

RESULTS

Risk factor analysis

The results from the final multivariable model for all horses are shown in Table 1. A total of 18 risk factors were included in the model.

Table 1. Final multivariable model for risk factors for fractures – all horses

Risk factor	Odds ratio	95% CI	P-value
(Intercept)	0.002	0.002 - 0.003	< 0.001
Age (years)	1.027	1.001 - 1.053	0.043
Country			
Canada	Ref	Ref	Ref
USA	1.363	1.194 -1.557	< 0.001
Entered the vet list			
No	Ref	Ref	Ref
Yes	1.569	1.430 - 1.722	< 0.001
Entered the vet list (30 days prior race)			
No	Ref	Ref	Ref
Yes	0.843	0.691 - 1.028	0.091
Entered the vet list (180 days prior race)			
No	Ref	Ref	Ref
Yes	1.316	1.170 - 1.481	< 0.001
First Start			
No	Ref	Ref	Ref
Yes	0.735	0.640 - 0.843	< 0.001
Gender			
Mare/Gelding	Ref	Ref	Ref
Stallion	1.476	1.365 - 1.596	< 0.001
Low purse race (<= $7500)			
No	Ref	Ref	Ref
Yes	0.767	0.699 - 0.842	< 0.001

Table 1. Cont’d

Risk factor	Odds ratio	95% CI	P-value
Months in racing	0.987	0.982 - 0.991	< 0.001
No. of previous injuries	1.340	1.170 - 1.535	< 0.001
No. of previous non-vet scratches	1.037	1.018 - 1.056	< 0.001
Odds rank in race	0.942	0.931 - 0.953	< 0.001
Purse change since previous race			
None	Ref	Ref	Ref
Drop	1.073	0.994 - 1.157	0.070
Raise	0.937	0.861 - 1.020	0.132
Sharp Drop	1.090	0.965 - 1.230	0.167
Sharp Raise	1.097	0.957 - 1.258	0.184
Race distance (furlongs)	0.938	0.916 - 0.960	< 0.001
Start with new trainer			
No	Ref	Ref	Ref
Yes	1.101	0.997 - 1.215	0.058
Surface			
Synthetic	Ref	Ref	Ref
Dirt	1.356	1.221 - 1.505	< 0.001
Turf	1.293	1.137 - 1.472	< 0.001
Time with same trainer (months)	0.989	0.984 - 0.994	< 0.001
Training with first trainer			
Yes	Ref	Ref	Ref
No	1.080	0.993 -1.174	0.074

For the population of horses that have raced for at least six months (Table 2), it was possible to include historical information of up to six months prior to a race and it was found that the more starts horses made up to a month prior to the race the less the risk per start of sustaining a fracture (Odds ratio (OR): 0.786, p: < 0.001). On the other hand, horses with more starts between 60 and 90 days prior to a start were at more risk per start (OR: 1.092, p: 0.001). Similarly, more starts between 90 and 180 days prior to a start were associated with an increased odds (OR: 1.068, p: < 0.001). Also, horses were at greater risk the higher their wins-to-starts ratio was in the periods of up to a month prior to the race (OR: 1.233, p: 0.002), 30 to 60 (OR: 1.244, p: 0.001), 60 to 90 (OR: 1.138, p: 0.051) and 90 to 180 (OR: 1.236, p: 0.013) days prior to a race.

Predictive models

The performance of each predictive model was evaluated by calculating the Area Under the Receiver Operating Characteristic Curve(AUC), as suggested by Bradley (1997), who considers the AUC to be one of the best ways to evaluate a classifier’s performance. Also, the evaluation method had to take into account both possible outcomes since the response is extremely imbalanced response and it is trivial for a classifier to achieve an accuracy of over 99.7% by simply predicting a 0% probability of an equine fracture at every start. All models were trained using the data available from the period 2009 - 2013. The data from 2014 were used to evaluate the predictive performance of each model.

Table 2. Final multivariable model for risk factors for fractures – horses over 6 months in racing

Risk factor	Odds ratio	95% CI	P-value
(Intercept)	0.001	0.001 - 0.002	< 0.001
Age (years)	1.044	1.009 - 1.080	0.014
Average speed in previous race (m/s)	1.033	0.999 - 1.067	0.055
Country			
Canada	Ref	Ref	Ref
USA	1.418	1.179 - 1.707	< 0.001
Entered the vet list			
No			
Yes	1.585	1.426 - 1.763	< 0.001
Entered the vet list (30 days prior race)			
No	Ref	Ref	Ref
Yes	0.838	0.656 - 1.070	0.156
Entered the vet list (180 days prior race)			
No	Ref	Ref	Ref
Yes	1.326	1.158 - 1.518	< 0.001
Gender			
Mare/Gelding	Ref	Ref	Ref
Stallion	1.606	1.447 - 1.783	< 0.001
Low purse race (<= $7500)			
No	Ref	Ref	Ref
Yes	0.738	0.658 - 0.827	< 0.001
Months in racing	0.988	0.984 - 0.993	< 0.001
Months since last start	0.964	0.940 - 0.988	0.004
No. of previous injuries	1.325	1.149 - 1.526	< 0.001
No. of previous non-vet scratches	1.026	1.006 - 1.046	0.010
No. of previous vet scratches	0.960	0.915 - 1.008	0.104
No. of starts (Present – 30 days prior race)	0.786	0.735 - 0.840	< 0.001
No. of starts (60 - 90 days prior race)	1.092	1.039 - 1.148	0.001
No. of starts (90 - 180 days prior race)	1.068	1.044 - 1.093	< 0.001
Odds rank in race	0.946	0.931 - 0.961	< 0.001
Purse change since previous race			
None	Ref	Ref	Ref
Drop	1.053	0.959 - 1.156	0.283
Raise	0.865	0.778 - 0.962	0.007
Sharp Drop	1.117	0.959 - 1.300	0.154
Sharp Raise	0.969	0.809 - 1.160	0.732
Race distance (furlongs)	0.918	0.890 - 0.946	< 0.001

Table 2. Cont'd

Risk factor	Odds ratio	95% CI	P-value
Start with new trainer			
No	Ref	Ref	Ref
Yes	1.107	0.983 - 1.248	0.094
Surface			
Synthetic	Ref	Ref	Ref
Dirt	1.457	1.261 - 1.683	< 0.001
Turf	1.290	1.084 - 1.535	0.004
Time with same trainer (months)	0.988	0.983 - 0.993	< 0.001
Wins/starts (Present – 30 days prior race)	1.233	1.078 - 1.411	0.002
Wins/starts (30 – 60 days prior race)`	1.244	1.029 - 1.108	0.001
Wins/starts (60 – 90 days prior race)	1.138	0.999 - 1.297	0.051
Wins/starts (90 – 180 days prior race)	1.236	1.046 - 1.460	0.013

The Neural Network produced marginally better classification results than the other algorithms (62.7%). The second best algorithm was the multivariable logistic regression (62.5%) followed by the Improved Balanced Random Forests (61.9%). To get the 95% confidence interval for the AUC scores a bootstrap with 10,000 iterations was used on the racing starts of 2014. All the models produce a statistically significant prediction compared to a random model (50%). Effectively though, there was no statistically significant difference regarding the performance of the models as the 95% confidence intervals overlap considerably (Table 3).

Table 3. Predictive models evaluation

Model	Area Under Curve	95% CI
IBRF	61.9%	60.1% - 64.4%
Logistic Regression	62.5%	60.5% - 64.7%
Neural Network	62.7%	60.6% - 64.8%

DISCUSSION

Previous studies have shown that the odds of musculoskeletal injury, injury of the suspensory apparatus and of the superficial digital flexor tendon was between five and 14 times greater among horses assessed to be at increased risk by regulatory veterinarians conducting pre-race examinations (Cohen et al., 1997). In this study it was found that the risk of sustaining a fracture was higher if the horse has entered the veterinary list at some point during its career. It was also found that the risk further increases if the horse enters a race within six months following being placed on the veterinary list; if it enters a race within one month of being on the vet list this increase was smaller. Horses that are able to enter a race within one month of entering the veterinary list probably had a less severe condition compared to horses that returned to racing within six months. Also, horses that have previously been scratched, not by the track veterinarian but for other reasons, were at a slightly higher risk for each previous scratch.

Stallions were found to be at a higher risk of sustaining a fracture compared with mares and geldings. A case-control study of deceased Thoroughbred horses in California (Anthenill et al., 2007) showed that stallions were at a higher risk of having a fracture of the forelimb proximal sesamoid bones. Stallions have also been found to be at higher risk of sustaining a catastrophic musculoskeletal injury (Estberg et al., 1998), a non-fatal superficial digital flexor tendon injury (Takahashi et al., 2004), or any form of fatal injury (Estberg et al., 1996). A case-control study of serious musculoskeletal injuries of Thoroughbreds in Australia (Bailey et al., 1997) on the other hand found no significant association between gender and injuries. However, this study compared only males and females and it is unclear if geldings were included in the male category, potentially confounding the result.

There have been a number of studies that found a significant association between the age of the horse and the risk of injury (Estberg et al., 1996; Bailey et al., 1997; Cohen et al., 1997) (Cohen et al., 2000; Parkin et al., 2004; Perkins et al., 2005). In our study a higher risk of injury was also found for older horses.

It was also found that horses that were expected to perform well in a race were at higher risk. A strong indication that competitive performers were at higher risk is suggested by the impact of the odds rank risk factor. Low purse races also seem to be safer. Purse in this context might be a proxy for the quality of the horses, thus lowering the risk, as well as the competitiveness of the race. A similar finding of higher risk for the more competitive stakes races was shown in a case-control study of serious musculoskeletal injuries of Thoroughbreds in Australia (Bailey et al., 1997).

Regarding the racing history of horses, previous studies have shown an almost eightfold increase in the risk of sustaining a superficial digital flexor tendon injury for Thoroughbreds that took a break of more than two months (Cohen et al., 1997). Another study (Perkins et al., 2005) found that horses with no starts were at a higher risk of sustaining a non-fatal injury to the superficial digital flexor tendon and to the suspensory apparatus. The current study also showed that for the month immediately prior to the race, the more starts, the lower the risk for the horse but the association changed for the periods of two months up to six months prior to the race. These findings also suggest that there was an increased risk for horses that took a break of more than on month from racing.

A case-control study of Thoroughbreds racing in California (Estberg et al., 1996) found that an increase in cumulative exercise and race distance over the previous two months was associated with an increased risk of fatal skeletal injury. Another study (Anthenill et al., 2007) found that an increase in the number of workouts increased the risk of proximal sesamoid bone fractures. Our study did not include training exercise but the increase in risk for horses that had more starts in the periods 60 to 90 and 90 to 180 days prior to the race might in this context be a proxy for increased cumulative exercise.

Moreover, it was found that the more time a horse has been with a trainer the lower the risk. It is also suggested that the risk is lower for horses training with their first trainer and that for the start immediately after a change in trainer the risk was higher although these results had a p-value slightly larger than 0.05 and was not statistically significant.

From the risk factors directly related to the type of the race the one with the highest impact was found to be racing surface. Turf surfaces had a higher risk than synthetic and dirt surfaces had an even higher risk. This is in accordance with a previous study of equine fatalities (Arthur, 2010) that reported that dirt had the highest incidence rate followed by turf and synthetic surfaces. The difference was statistically significant between dirt and synthetic but not between turf and dirt or turf and synthetic. It is likely that the reason the current study identified a significant difference is mostly due to the greater statistical power provided by the very large cohort available for analysis. Another study (Mohammed et al., 1991) also found that dirt courses are associated with higher risk compared to turf. Regarding race related factors, it was also found that starts from races in the USA were at higher risk compared to starts from races in Canada.

This is the first study to make extensive use of the data available from 2009 to 2014 in the Equine Injury Database regarding fracture injuries. This risk factor analysis has assessed factors, plausibly linked with fractures, during flat horse racing in the USA and Canada and has identified risk factors with a statistically significant association with fractures. Furthermore, the predictive models were able to use the information available in the data to identify horses at higher risk.

It is considered likely that the results report unbiased estimates for the effect of those risk factors as as the statistical analysis included 91% of starts from all official racing in the USA and Canada for that period. The roughly 9% of starts from tracks not reporting to the EID could be a small source of bias.

It is important to recognise that the outcome chosen to model in this work was fractures and no attempt was made to differentiate between different causes of injury. As risk factors for different fracture types have been shown to differ (Parkin et al., 2004; Anthenill et al., 2007), it is likely that some of these have not been identified in the current analysis. Over recent years, the different types of injury sustained have become more accurately reported to the EID. This will enable future analyses to use more specific outcome variables that will identify risk factors that are unique to a more specific injury type.

Furthermore, it is acknowledged that statistical significance does not necessarily mean clinical significance. Even when the model identified a risk factor that increases the risk significantly or through the use of predictive models a horse with a higher than average risk was identified, the vast majority of starts (99.8%) did not result in a fracture.

In conclusion, the results of this study could help identify horses at high risk on entering a race and inform the design and implementation of preventive measures aimed at minimising the number of Thoroughbreds sustaining fractures during racing in North America. In collaboration with The Jockey Club the investigators are working on identifying the most suitable way to translate these results into advice for stakeholders.

ACKNOWLEDGEMENTS

This work was funded through an Industry Partnership PhD provided by The Jockey Club and the University of Glasgow.

REFERENCES

Anthenill, L.A., Stover, S.M., Gardner, I.A. and Hill, A.E. (2007). Risk factors for proximal sesamoid bone fractures associated with exercise history and horseshoe characteristics in Thoroughbred racehorses. Am J Vet Res, 68, 760-771

Arthur, R.M. (2010). Comparison of Racing Fatality Rates on Dirt, Synthetic, and Turf at Four California Racetracks. Proceedings of the Annual Convention of the AAEP, 405-408

Bailey, C.J., Reid, S.W.J., Hodgson, D.R., Suann, C.J., Rose, R.J. (1997). Risk factors associated with musculoskeletal injuries in Australian Thoroughbred racehorses. Prev Vet Med, Volume 32, 47-55

Bengio, Y. (2009). Learning Deep Architectures for AI (Foundations and Trends in Machine Learning). Now Publishers Inc., 1-127

Bishop, C.M. (1995). Neural networks for pattern recognition. New York: Oxford University Press, 116-160

Boden, L.A., Anderson, G.A., Charles, J.A., Morgan, K.L., Morton, J.M., Parkin, T.D., Clarke, A.F., Slocombe, R.F. (2007). Risk factors for Thoroughbred racehorse fatality in flat starts in Victoria, Australia (1989-2004). Equine Vet. J., Volume 39, 430-437

Bozdogan, H. (1987). Model selection and Akaike's Information Criterion (AIC): the general theory and its analytical expressions. Psychometrica, 52, 345-370

Bradley, A. P. (1997). The Use of the Area Under the ROC Curve in the Evaluation of Machine Learning Algorithms. Pattern Recognition, 30(7), 1145-1159

Breiman, L. (2004). Consistency For a Simple Model of Random Forests, California: University of California at Berkeley, pages

Chen, C., Liaw, A. and Breiman, L. (2004). Using Random Forest to Learn Imbalanced Data, University of California Berkeley, pages?

Ciresan, D.C., Meier, U., Gambardella, L.M. and Schmidhuber, J. (2010). Deep Big Simple Neural Nets Excel on Handwritten Digit Recognition, arXiv, 3207-3220

Ciresan, D., Meier, U. and Schmidhuber, J. (2012). Multi-column Deep Neural Networks for Image Classification., pages?

Cohen, N.D., Berry, S.M., Peloso, J.G., Mundy, G.D., Howard, I.C. (2000). Association of high-speed exercise with racinf injury in Thoroughbreds. Journal of the Am Vet Med J 216, 1273-1278

Cohen, N.D., Peloso, J.G., Mundy, G.D., Fisher, M., Holland, R.E., Little, T.V., Misheff, M.M., Watkins, J.P., Honnas, C.M., Moyer, W. (1997). Racing-related factors and results of prerace physical inspection and their association with musculoskeletal injuries incurred in thoroughbreds during races. J Am Vet Med Assoc, 211, 454-463

Dohoo, I., Martin, W. and Stryhn, H., (2003). Veterinary Epidemiologic Research. Charlottetown: AVC Inc., 321-322

Estberg, L., Gardner, I.A., Stover, S.M., and Johnson, B.J. (1998). A case-crossover study of intensive racing and training schedules and risk of catastrophic musculoskeletal injury and lay-up in California Thoroughbred racehorses. Prev Vet Med, 33, 159-170

Estberg, L., Gardner, I.A., Stover, S.M., Johnson, B., Case, J.T., and Ardans, A. (1995). Cumulative racing-speed exercise distance cluster as a risk factor for fatal musculoskeletal injury in Thoroughbred racehorses in California. Prev Vet Med, 24, 253-263

Estberg, L., Stover, S., Gardner, I., Drake, C., Johnson, B., Ardans, A. (1996a). High-speed exercise history and catastrophic racing fracture in thoroughbreds. J Am Vet Med Assoc, 57, 1549-1555

Estberg, L., Stover, S., Gardner, I., Johnson, B., Case, J., Ardans, A., Woods, L. (1996b). Fatal musculoskeletal injuries incurred during racing and training in thoroughbreds. J Am Vet Med Assoc, 208, 92-96

Estberg, L., Stover, S., Gardner, I., Johnson, B., Jack, R., Case, J., Woods, L. (1998). Relationship between race start characteristics and risk of catastrophic injury in thoroughbreds: 78 cases (1992). J Am Vet Med Assoc, 212, 544-549

Glorot, X. and Bengio, Y. (2010). Understanding the difficulty of training deep feedforward neural networks. Sardinia, 249-256

Henley, W., Rogers, K., Harkins, L. and Wood, J.L. (2006). A comparison of survival models for assessing risk of racehorse fatality. Prev Vet Med, Volume 74, 3-20

Hernandez, J.A., Scollay, M.C., Hawkins, D.L., Corda, J.A., and Krueger, T.M. (2005). Evaluation of horseshoe characteristics and high-speed exercise history as possible risk factors for catastrophic musculoskeletal injury in Thoroughbred racehorses. Am J Vet Res, Volume 66, 1314-1320

Hernandez, J., Hawkins, D.L. and Scollay, M.C. (2001). Race-start characteristics and risk of catastrophic musculoskeletal injury in Thoroughbred racehorses. J Am Vet Med Assoc, Volume 218, 83-86

Kane, A.J., Stover, S.M., Gardner, I.A., Bock, K.B., Case, J.T., Johnson, B. J., Ardans, A.A. (1998). Hoof size, shape, and balance as possible risk factors for catastrophic musculoskeletal injury of Thoroughbred racehorses. Am J Vet Res, Volume 59, 1545-1552

Kane, A.J., Stover, S.M., Gardner, I.A., Case, J.T., Johnson, B.J., Read, D.H., and Ardans, A.A. (1996). Horseshoe characteristics as possible risk factors for fatal musculoskeletal injury of thoroughbred racehorses. Am J Vet Res, 57, 1147-1152

LeCun, Y., Bottou, L., Orr, G.B. and Muller, K.R., (2012). Efficient BackProp. In: Neural networks: Tricks of the trade. Berlin, Heidelberg: Springer, 9-48

Lyle, C.H., Blissitt, K.J., Kennedy, R.N., McGorum, B.C., Newton, J.R., Parkin, T.D., Boden, L.A. (2012). Risk factors for race-associated sudden death in Thoroughbred racehorses in the UK (2000–2007). Equine Vet J, 44, 459-465

Mohammed, H.O., Hill, T. and Lowe, J. (1991). Risk factors associated with injuries in Thoroughbred horses. Equine Vet J, 23, 445-448

Parkin, T.D., Clegg, P.D., French, N.P., Proudman, C.J., Riggs, C.M., Singer, E.R., Morgan, K. (2005). Risk factors for fatal lateral condylarfracture of the third metacarpus/metatarsus in UK racing. Equine Vet J, 37, 192-199

Parkin, T.D., Clegg, P.D., French, N.P., Proudman, C.J., Riggs, C.M., Singer, E.R., Morgan, K. L. (2004). Horse-level risk factors for fatal distal limb fracture in racing Thoroughbreds in the UK. Equine Vet J, 36, 513-519

Parkin, T.D., Clegg, P.D., French, N.P., Proudman, C.J., Riggs, C. M., Singer, E.R., Morgan, K.L. (2004). Race- and course -level risk factors for fatal distal limb fracture in racing Thoroughbreds. Equine Vet J, 36, 521-526

Parkin, T.D., Clegg, P.D., French, N.P., Proudman, C.J., Riggs, C.M., Singer, E.R., Morgan, K. L. (2006). Analysis of horse race videos to identify intra-race risk factors for fatal distal limb fracture. Prev Vet Med, 74, 44-55

Peloso, J.G., Mundy, G.D. and Cohen, N. (1994). Prevalence of, and factors associated with, musculoskeletal racing injuries of thoroughbreds. J Am Vet Med Assoc Volume 204, 620-626

Perkins, N.R., Reid, S.W.J. and Morris, R.S. (2005). Risk factors for injury to the superficial digital flexor tendon ans suspensory apparatus in Thoroughbred racehorses in New Zealand. NZ Vet J, 53, 184-192

Rumelhart, D.E., Hinton, G.E. and Williams, R.J. (1986). Learning internal representations by error propagation. In: Parallel distributed processing: explorations in the microstructure of cognition. Cambridge, MA, USA: MIT Press, 318-362

Strasser, H. and Weber, C. (1999). On the Asymptotic Theory of Permutation Statistics. Mathematical Methods of Statistics, Volume 8, 220–250

Takahashi, T., Kasashima, Y. and Ueno, Y. (2004). Association between race history and risk of superficial digital flexor tendon injury in Thoroughbred racehorses. J Am Vet Med Assoc, 225, 90-93

Williams, R.B., Harkins, L.S., Hammond, C.J. and Wood, J. (2001). Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and National Hunt racing during 1996, 1997 and 1998. Equine Vet J, 33, 478-486

Xie, Y., Li, Ngai, E.W.T. and Ying, W. (2009). Customer churn prediction using improved balanced random forests. Expert Systems with Applications, Volume 36, 5445-5449

SHEEP EPIDEMIOLOGY

A QUANTITATIVE AND QUALITATIVE ANALYSIS OF ATTITUDES TOWARDS SUSTAINABLE PARASITE CONTROL PRACTICES FROM SCOTTISH SHEEP FARMERS

C.M. JACK[*], E. HOTCHKISS, N.D. SARGISON, L. TOMA, C. MILNE AND D.J. BARTLEY

SUMMARY

This study aims to identify and demonstrate the impact of factors influencing the adoption of sustainable parasite control practices by Scottish sheep farmers. The use of both focus group and questionnaire methods were employed to elicit attitudes regarding ‘best practice’ recommendations. Quantitative statistical analysis of questionnaire responses using structural equation modelling identified a number of significant influences on the adoption of sustainable practices such as the confirmation of anthelmintic resistance on a holding, level of farming experience and underpinning roundworm control knowledge. Focus group discussions highlighted the need to simplify recommendations given to farmers as well as addressing the issue of conflicting advice concerning appropriate parasite control strategies. By identifying some of the key influences on farmers’ decision-making on roundworm control, this can allow the tailoring of advice to target issues affecting acceptance by the sheep farming community.

INTRODUCTION

The sustainable control of gastro-intestinal nematode parasites remains one of the main perennial endemic disease pressures that livestock farmers face. Gastro-intestinal nematodes impact on health, welfare and production efficiency of livestock. For over 50 years parasite control strategies have heavily relied on suppressing nematode populations with frequent use of highly efficacious, broad spectrum anthelmintics (Bartley, 2008). The effectiveness of these treatments is threatened by the emergence of nematode populations that are resistant to one or more of the drug classes available (Bartley et al., 2004; Sargison et al 2007). It is therefore increasingly apparent that steps toward maintaining sustainable productivity in the growing face of anthelmintic resistance (AR) is required by farmers.

In response to growing concerns of AR development, the Sustainable Control of Parasites in Sheep (SCOPS) initiative was set up in 2003, in order to promote practical recommendations for producers and advisors regarding ‘best practice’ approaches to parasite control (Abbott et al., 2012). These recommendations are summarised into eight guidelines, which outline measures to prevent the introduction and reduce the development of AR, in order to preserve the effectiveness of current and future anthelmintics. There are numerous channels for the dissemination of recommendations such as through animal health advisors (e.g. veterinarians,

---

[*] Corin Jack, The Moredun Group, Pentlands Science Park, Bush Loan, Penicuik, Midlothian, Scotland, UK. Email: corin.jack@moredun.ac.uk

suitably qualified persons and researchers), online/printed publications as well as promotion at agricultural events.

Various questionnaire surveys have been published on the parasite management practices of sheep farmers from around the world, as well as within the UK (Coles, 1997; Sargison et al., 2002; Bartley, 2008; McMahon et al., 2012; Morgan et al., 2012). Such studies have highlighted the variable adoption of sustainable roundworm control practices, and emphasised the need to improve promotion and perception of these practices if sustainable parasite control is to be generally accepted. In recent years the rapidly growing application of socio-psychological research methods in behavioural science has highlighted their influence on animal health decision making. These studies have investigated behaviours relating to a wide range of disease agents, across most livestock species (Wauters & Rojo-Gimeno, 2014; Vande Velde et al., 2014). However a limited amount of work has investigated how these factors may influence parasite control behaviours.

The challenging nature of sustainable worm control extension favoured a mixed methods approach towards identifying the various factors that may influence stakeholder's engagement and adoption of 'best practice' approaches to parasite management. The factors influencing endorsements of such control schemes are diverse and unique to each individual and enterprise. This study aims to use both qualitative and quantitative approaches to investigate the influence of socio-psychological factors on the overall adoption of SCOPS practices as well as practices that are known risk factors for the development of AR.

MATERIALS AND METHODS

Focus group

Focus groups were arranged to elicit qualitative data on barriers and motivators to implementation of best practice worm control advice, with the aim that this data would inform the design of a quantitative questionnaire. The participants of the focus groups were selected by regional advisors of the Moredun Foundation, which is a charitable organisation with a remit to provide information on issues effecting ruminant health and productivity. The advisors arranged farmer meetings in their respective geographical regions to discuss the topic of roundworm control and uptake of sustainable worm control practices. The general selection criteria for attendees was to include between 10-15 sheep producers, preferably from a range of backgrounds and farming systems, in order to achieve a cohort to represent a cross section of the sheep farming industry. The proposed period for conducting the focus groups was January to February 2014. The timings were chosen due to the 'relative quietness' in the work schedule of most farmers at this time of year in order to try and maximise attendance and engagement.

The format of the focus groups followed a defined structure as outlined in Fig.1. A number of different recording and stimuli materials were used to suit the different components of the talk, primarily with the use of an audience response system (ARS; Turning Point, Turning Point Technologies). ARS is an electronic voting system allowing each participant to give a real-time, anonymous response to a given question. The type of response options varied between single, multiple and ranked responses depending on the purpose of the question. For example if farmers were required to assign their perceived importance to the eight SCOPS guidelines, a ranked question was chosen. After each question the electronic responses were collated and graphically presented in order to allow participants to observe the findings of the group and stimulate discussion. The first half of the meetings (topics 1-3) centred on common anthelmintic treatment scenarios based around the sheep farming calendar. This allowed participants to become familiarised with the ARS, as well as time to recollect personal experiences and listen to opinions of others. The second half of the meetings (topics 4-6) were firstly used to familiarise participants with the SCOPS recommendations, in order to then discuss the perceived motivators and barriers to adoption of these practices, and to evaluate their importance and ease of use by participants. The discussions during the meetings were recorded using a voice recorder and then manually transcribed prior to analysis using the qualitative data analysis software N-Vivo (version 10; QSR International). The ARS responses were also recorded and used to support the qualitative findings. The qualitative data was analysed using a combination of thematic and framework analysis as described by Barbour (2008) to identify common themes, and themes were then compared between focus group meetings.

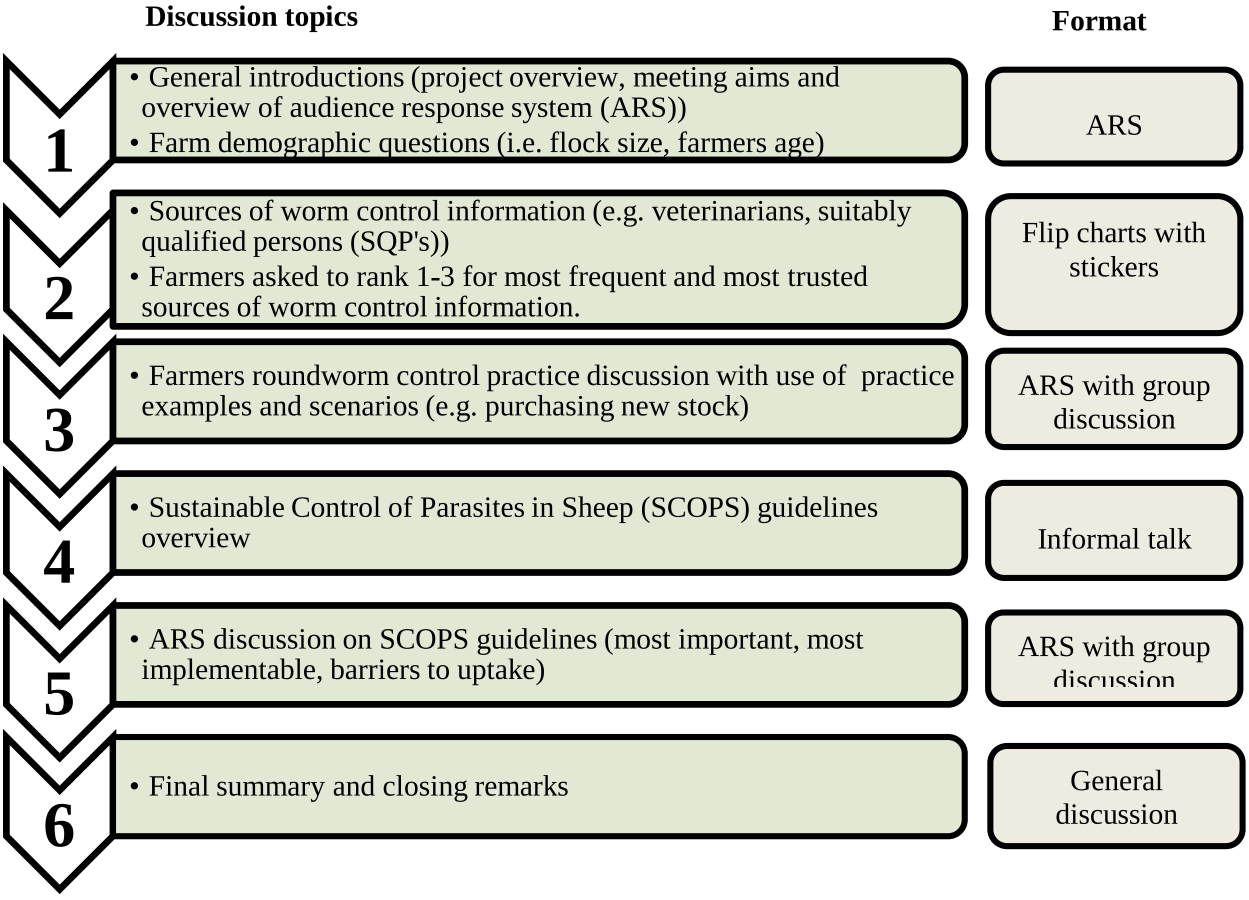


Fig. 1 Flow chart representation of the focus group format

Quantitative attitudinal survey design

A questionnaire was devised based on a range of different source material including a combination of common themes highlighted from the farmer focus group meetings, devisors own parasite management expertise and comparable questionnaire literature related to disease management. For the purpose of this study, the use of components from multiple theoretical frameworks was incorporated into the survey design.

The design was built around five main components which were arranged in the following order and were comprised of the following topics: 1) farmer demographics and enterprise characteristics, 2) general roundworm control/AR attitude statements, 3) open-ended sense questions, 4) SCOPS attitudinal statements, and 5) parasite control behaviours. The first section included closed-ended questions relating to demographical information (age, education and years earning a living as a farmer), as well as details of the farming system (e.g. enterprise type, flock size, land typography, farming priorities). The second section included broader questions relating to attitudes towards general parasite control that weren't specific toward a particular control measure (such as the perceived importance of roundworm, risk perception of AR). The third section included three open-ended questions which were used to gauge the level of the respondent's knowledge and understanding on the topic of roundworm control and AR. The fourth section focussed on attitudinal statements which were framed around the eight SCOPS guidelines (Abbott et al., 2012). This fourth section formed the largest component of the questionnaire, including around 60% of all questionnaire items. The final section included the outcome behavioural control measures of interest, which were linked to the proceeding attitudinal items.

Survey implementation

Farmer contact details were obtained from the Scottish Government (Rural and Environment Science and Analytical Services Division; RESAS) by the use of agricultural census data. This information resource allowed the random selection of farms from around Scotland which could be stratified to suit the purposes of the study. The selection criteria used to target farms of interest included, premises with flocks with more than 50 breeding ewes and other sheep (1 year old and over) for breeding, and at least 25 ewes used for breeding in the previous season. This was to avoid sampling from particular smallholdings where the motives for rearing livestock were not financially driven. Furthermore, based on a quota sampling strategy, a target of 400 completed telephone interviews was established. The sampling frame was further stratified regionally by animal health divisional office (AHDO) in order to ensure a proportional population sample from each region.

Opt-out letters were sent out to farmers two weeks prior to the implementation of the survey. The letters outlined the aim of the study, the estimated interview duration, the voluntary nature of the survey and gave assurance that any publication of results would ensure anonymity. If the recipient did not reply to the opt-out letter within the specified time it was considered that they had implicitly agreed to participate in the telephone interview, allowing contact details to be gathered and for them to be contacted.

A pilot study with six farmers was conducted before undertaking the main survey. This informed the modification of questionnaire items ensuring no ambiguity of questions by respondents and suitability of items for the telephone survey format. Additionally, the lengths of the interviews were monitored to ensure that interview times were not excessive, in order to achieve optimum completion rates.

The interviews were not audio-recorded but responses were documented by the interviewer and compiled in a Microsoft Excel spreadsheet. Farmers were assured that all information provided would remain completely anonymous in any subsequent reports or publications and that they and their enterprises would not be individually identifiable. Any farmers wishing to opt out after the data was collected were able to do so.

Statistical analysis

Factor analysis: Initial exploratory factor analysis was performed on ordinal (Likert scale) items in order to identify and evaluate inter-relationships. Based on their interactions, the total numbers of observed variables were condensed into a smaller set of unobserved (latent) factors. In the development of the proposed models, items within the 'general attitudes' to roundworm control section were used. The procedures for the assessment of factor loadings (correlation coefficients) and reliability analysis (Cronbach alpha) were conducted as described by Hair et al. (2004) Based on the study sample size (400) and in order to achieve statistical significance for each value with a statistical power of 80 per cent, a minimum threshold of ±0.30 factor loading was used. Consequently any values above ±0.30 were identified as significant loadings and therefore required further evaluation. Factor loadings greater than ±0.30, or loadings that demonstrated significant loadings across more than one factor i.e. cross-loading, were not included within the resultant factor, but assessed for inclusion as an individual regression item. Once the significant loadings and non-significant/cross-loadings were identified, the factor model was re-specified and reanalysed, in order to obtain an empirically and conceptually appropriate factor structure.

Endogenous variables were formulated into two ordinal scores by summating the total number of SCOPS recommended practices that respondents reported to adopt within the behaviour section of the questionnaire (maximum possible of 10). In addition a score was derived for the number of AR selective practices stated in the same section, with a maximum possible of 7.

Structural equation modelling (SEM): In order to examine the inter-relationships between the observed and unobserved (latent) variables in the proposed theoretical model, the analysis was performed using the multivariate analysis technique known as structural equation modelling. This technique comprises two parts; the first is the measurement model which represents the relationships between the specified indicators and their latent constructs. The second is the structural model which then examines the causal relationships between the model constructs. The Lavaan package (Rosseel, 2012) within R studio was chosen for the purposes of the SEM analysis. The WLSMV model estimator was used, which uses diagonally weighted least squares (DWLS) to estimate the model parameters. The resulting model output was evaluated for goodness of fit by using the following model fit indices as detailed by Vande Velde et al. (2014): Minimum Function Test Statistic (MFTS) divided by the degrees of freedom (DF) (< 2.0), the Comparative Fit Index (CFI), Tucker-Lewis Index (TLI) (CFI, TLI > 0.90), Root Mean Square Residual (RMSR) (< 0.08), and Standard Root Mean Square Residual (SRMR) (< 0.10).

RESULTS

Focus groups

The focus group meetings were held in the following order with the corresponding number of attendees: Moredun, Midlothian (n=14), Kirriemuir, Angus (n=14), Cupar, Fife (n=19) and Grantown-on-Spey, Morayshire (n=9). The demographic characteristics of participants and farm types varied considerably between meetings. The majority of participants were mixed livestock and arable enterprises (61%). The majority of participants had medium to large sized flocks of between 150-800 breeding ewes (59%). The Moredun meeting had the highest proportion of under 35’s (45%) compared to the other groups. Overall, most attendees ages ranged between 36 and 55 (62%).

From the analysis of both the ARS responses and verbal discussions, a range of issues were raised regarding each of the recommended parasite control guidelines. The most commonly identified barriers to adoption among the eight guidelines included the perceived complexity of a number of practices and conflicting advice regarding best practice recommendations.

The issue of conflicting advice was mentioned in relation to differences between information sources:

> *‘Conflicting advice from you lads will tell us something, you'll read in the press something, and then the vets will tell you something else’ (Kirriemuir)*

> *‘One vet will be different from the other’ (Cupar)*

...Also in regards to changes in parasite control advice over time.

> *‘When I was fresh out of college it was use one wormer at one time and another form the next time and that went on. And you dosed onto clean pasture and now apparently that’s, and I'm not that old...now that’s the completely wrong thing to do....’ (Moredun)*

Another important barrier to the uptake of the SCOPS recommended practices that was highlighted was complexity. One of the problems identified was the applicability of having a universal set of guidelines, considering the wide variety of farming enterprises present:

> *‘The thing is, it’s easy if you see it black or white but everybody's farms are different and it’s not a one model fits all...you have to adapt to everybody's situation’ (Moredun)*

> *‘That's key like, there’s no set guidelines can apply to every farm around, it doesn't work that way’ (Grantown-on-Spey)*

Also the addition of incorporating faecal egg count testing for assessing treatment effectiveness and identifying when to treat animals was also seen as adding an extra dimension of complexity:

> *‘I think you need to be involved in taking samples and testing, which would add to the complexity of making a decision’ (Cupar)*

The awareness of the principle of parasite refugia was low amongst all groups of participants, and therefore was the area that required the most explanation and clarification:

> *‘It’s a really hard thing to get your head around. I wouldn't know enough about it’ (Moredun)*

Although complexity and conflicting advice were among the highest overarching barriers to the adoption of SCOPS practices, each of the guidelines had its unique set of barriers. Other barriers included in the discussion were perceived resource requirements (time, labour, facilities, and expense) as well as attitudes (perceived need and impact on production).

Questionnaire descriptive statistics

The total number of opt-out letters received from the original 1,930 farmers contacted was 427 (22%), leaving 1,503 farmers eligible to be contacted. The survey was conducted between the 5th January 2015 and 18th February 2015 with the target of 400 completed interviews achieved. The following numbers of interviews were conducted from each region of Scotland: 65 in the South East, 76 in the South West 74 in Central region, 92 in the North West, 46 in the North East, and 47 in the Islands. The average duration of interviews was 21 minutes, ranging between 12 to 48 minutes.

The majority of farmers (69%) sampled in the survey were aged 51 years and over, with only 3% of the respondents aged between 18 and 35, which is consistent with the 2015 agricultural census age profiles (National statistics., 2015). The number of years earning a living as a farmer however was more normally distributed with the highest proportion of respondents stating their years earning between 31 and 40 years (28%). The education level of most respondents shows that most had received further education (64%), with approximately 15% studying at an agricultural college.

In regards to the farming enterprises, almost two thirds of respondents' farms were situated on either upland or hill grazing land, with over half of the sample population comprised of mixed livestock farmers, and a quarter of farmers with sheep only. The proportional flock sizes as indicated by numbers of breeding ewes were more orientated towards small to medium sized flocks (i.e. <500 ewes), with a quarter of farms with larger flocks (>500 ewes).

Model validation

Both of the models proposed consisted of two single-indicator latent variables (Roundworm control knowledge & Confirmed presence of AR) and 6 multiple-indicator latent variables (detailed in Table 1). The exploratory factor analysis established acceptable factor loadings i.e. >0.70 for all multiple-indicator latent variables. Additionally, the reliability analysis shown in Table 1 demonstrated suitable measures (α = >0.60) between all sets of indicators with the exception of 'Risk perception'. Both models reflected a goodness of fit with the observed data as indicated by the following model fit indices. The SCOPS model; MFTS/DF= 1.61, CFI (0.94), TLI (0.93), RMSEA (0.04). The AR model; MFTS/DF = 1.67, CFI (0.93), TLI (0.92), RMSEA (0.04).

Table 1. Description of latent constructs with corresponding indicators and Cronbach alpha reliability measures (α). The 5-point Likert scale corresponds to 1 = Strongly disagree; 2 = Disagree; 3 = Unsure; 4 = Agree; 5 = Strongly agree

Latent variable	Indicator and statement	α	Value and labels	Variable type
Experience	What is your age	0.76	1 = 18-35; 2 = 36-50; 3 = 51-65; 4 = over 65	Categorical
	How many years have you been earning a living as a farmer		1 = 10 years or less; 2 = 11-20; 3 = 21-30; 4 = 31- 40; 5 = 41-50; 6 = over 51	Categorical

Table 1. Cont'd

Latent variable	Indicator and statement	α	Value and labels	Variable type
Roundworm control knowledge	Knowledge score	-	0 = none correct; 1 = one correct; 2 = two correct; 3 = three correct	Ordinal
Confirmed presence of AR	Do you have confirmed drug resistance		1= Yes 2= No	Binary
Roundworm control importance	**1. -** Roundworm control is important on my farm	0.88	5-point Likert scale [a]	Ordinal
	2. - My roundworm control strategy improves the productiveness of my animals		5-point Likert scale [a]	Ordinal
	3. - Roundworm control is important for the profitability of my farm		5-point Likert scale [a]	Ordinal
	4. - Roundworm control is important for the health & welfare of my animals		5-point Likert scale [a]	Ordinal
Self-efficacy	**1. -** I am confident in my ability to detect problems associated with roundworms	0.61	5-point Likert scale [a]	Ordinal
	2. - I make time to implement practices that could improve my roundworm management		5-point Likert scale [a]	Ordinal
Risk perception	**Susceptibility** Wormer resistance is a problem in my region	0.59	5-point Likert scale [a]	Ordinal
	Severity Wormer resistance is a threat to my farming business		5-point Likert scale [a]	Ordinal
Vet service (Pros)	**1. -** Working with my vet could improve my roundworm control strategy	0.88	5-point Likert scale [a]	Ordinal
	2. - Working out a roundworm control strategy with my vet is cost effective		5-point Likert scale [a]	Ordinal
	3. - Working out a roundworm control strategy with my vet ensures I get reliable advice		5-point Likert scale [a]	Ordinal
Vet service (Cons)	**1. -** Roundworm control advice provided by vets is too complex	0.81	5-point Likert scale [a]	Ordinal
	2. - Roundworm control advice provided by vets is difficult to implement		5-point Likert scale [a]	Ordinal

The outcome 'number of SCOPS practices adopted' followed a normal distribution, with mean number of reported SCOPS practices equal to 4 (range 0-9). The outcome "AR selective practices" also followed a normal distribution, with a mean number of 3 AR selective practices reported by respondents (range 0-7).

The standardised estimates of the models are shown in Fig. 2 and 3. The SCOPS model (Fig. 2) explained 0.80 of the variance in the uptake of SCOPS behaviours. The factors 'Roundworm control knowledge', 'Experience' and 'Confirmation of AR' were shown to have significant influence on the uptake of SCOPS practices. These factors share a similar degree of influence on the uptake of SCOPS practices, with 'Experience' having a negative influence on the uptake of SCOPS. The AR model (Fig.3) explained 0.85 of the variance in the number of AR selective behaviours. In contrast to the SCOPS model, the AR model shows a greater range of factor effects on the outcome behaviours, with the factor 'Roundworm control knowledge' having the least significant influence on adoption of AR selective practices and 'Confirmation of AR' having the greatest negative impact. Negative attitudes to veterinary roundworm services also demonstrated a significant positive influence on the adoption of AR selective practices.

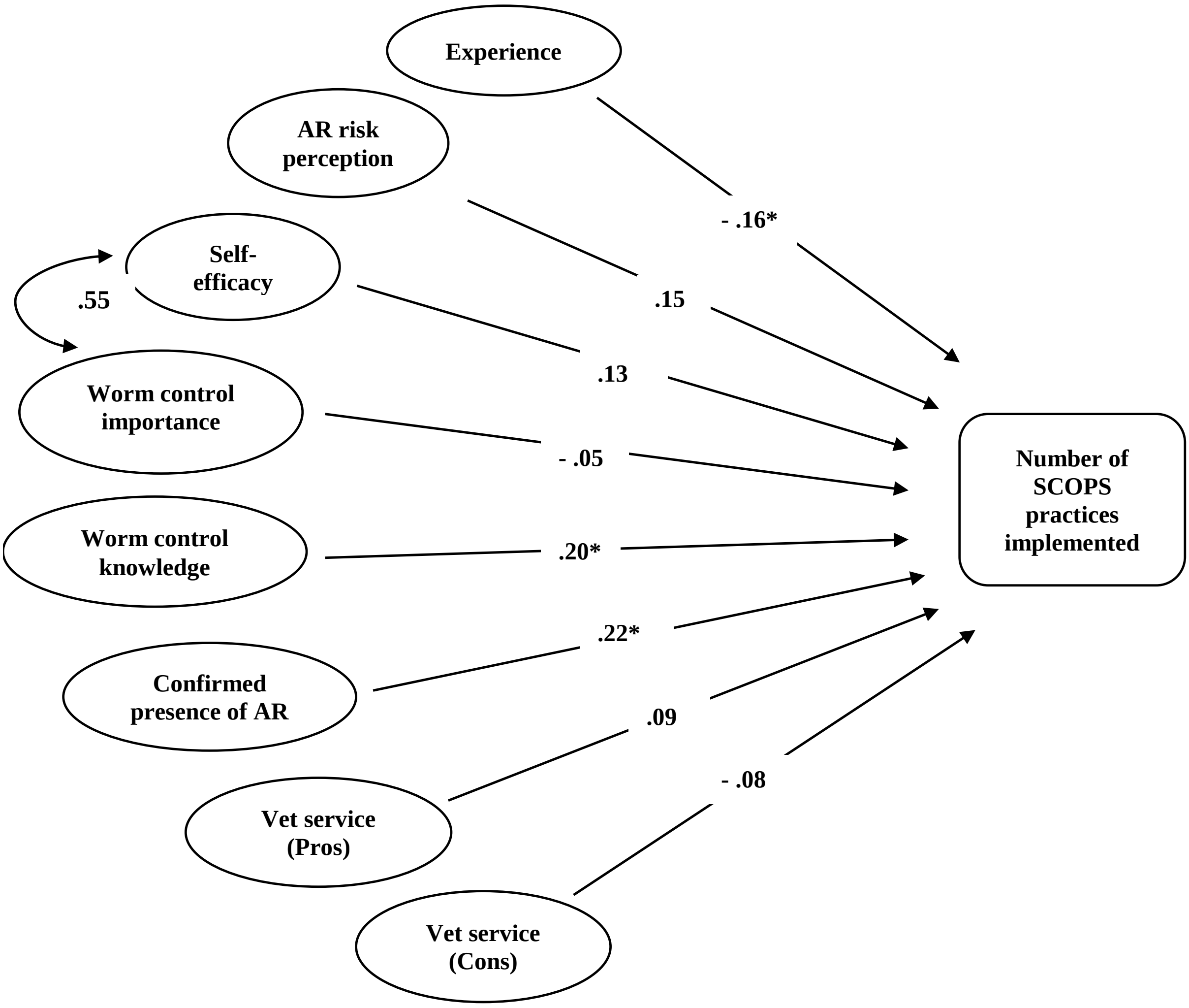


Fig. 2 Structural model representing latent influences on uptake of SCOPS practices with numbers corresponding to standardised coefficient estimates between latent variables and the observed outcome variable (* P= <0.05)

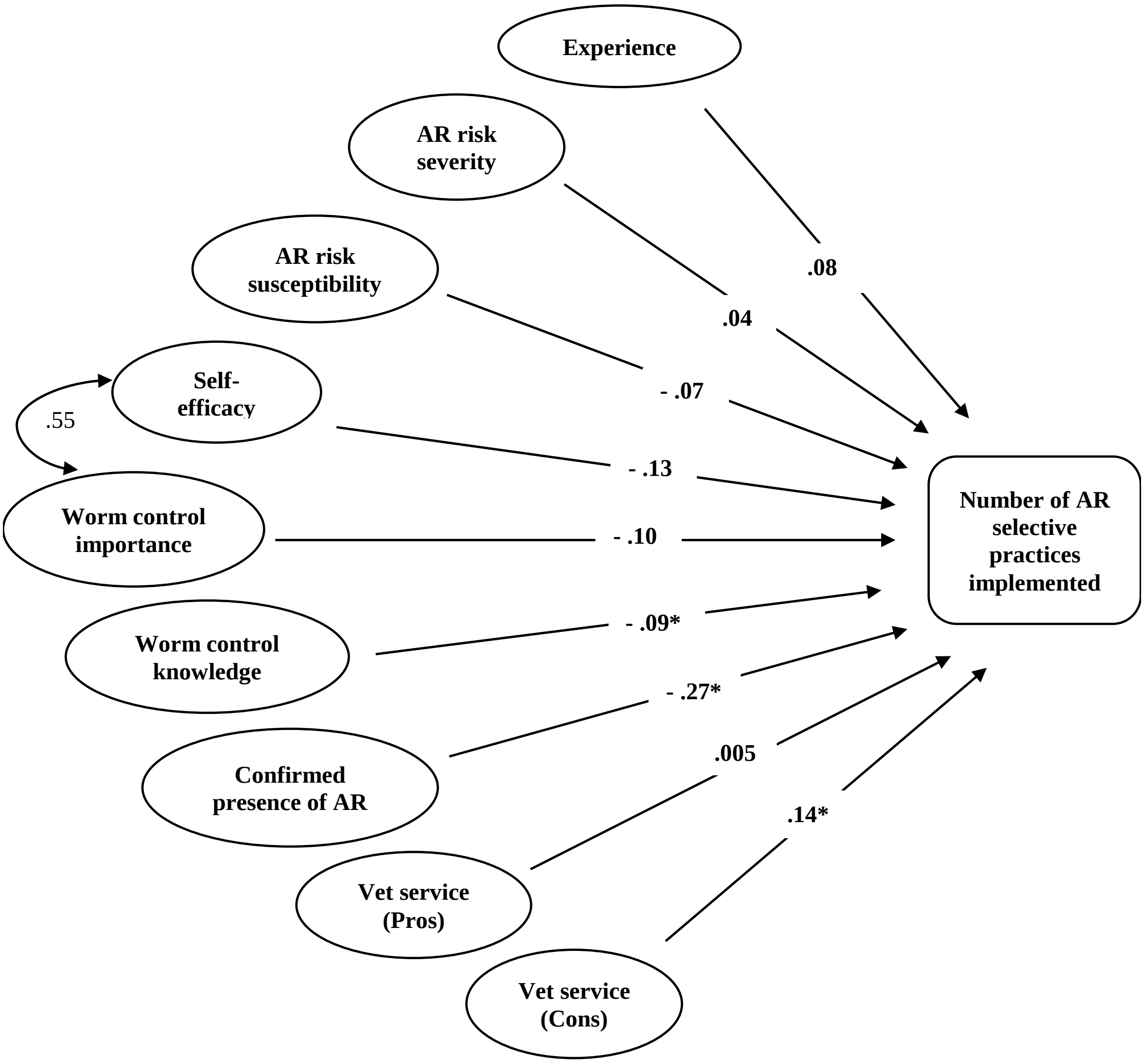


Fig. 3 Structural model representing latent influences on uptake of AR selective practices with numbers corresponding to standardised coefficient estimates between latent variables and the observed outcome variable (* P= <0.05)

DISCUSSION

The results presented highlight the perceived complex nature of parasite control by Scottish sheep farmers, which may have the greatest bearing on the successes of developing effective extension programmes. This inherent complexity can be expected due to the myriad of internal and external factors affecting its control, made only more challenging by the emergence of AR. Furthermore the advancements in parasitological research have identified a number of key selection pressures for the development of AR, which are likely to conflict with strategies to optimise productivity through parasite suppression (Morgan, 2013). The combination of such factors is likely to increase the perception of conflicting advice, with disparity between individual advisors' opinions, knowledge and experiences with regard to appropriate parasite control strategies.

Focus groups proved valuable in eliciting qualitative data on the range of factors influencing farmer's roundworm control decision making processes. The findings of the focus groups formed an integral part of the questionnaire design by highlighting specific aspects of best practice endorsement that are most challenging to sheep farmers such as complexity, practicality and cost-effectiveness. One such example of a questionnaire item developed from the farmer discussions included 'Different advisors provide conflicting roundworm control advice'. The use of ARS provided a valuable tool for engaging participants and stimulating discussion. It is thought that this improved engagement may be due to a number of features which ARS provides, such as anonymity of responses and instantaneous feedback from other participants (Sparks, 2011). Ultimately this allows all views to be recognised and as a result may help to make participants feel more actively involved in the discussion process (Kay & LeSage, 2009).

The preliminary use of SEM has identified a number of significant influences on the uptake of SCOPS and AR selective practices. The confirmation of AR is shown to be an important driver towards the uptake of sustainable parasite control practices and an equally important deterrent for the use of AR selective practices. This demonstrates a decisive mechanism for prompting farmers to assess their treatment efficacies and modify their parasite control strategies appropriately based on the knowledge of which nematode species are resistant to a particular class of anthelmintic. The results also show that the level of farming experience is influential in the adoption of SCOPS practices. This suggests that more experienced farmers are less likely to employ such measures, perhaps due to a greater reliance on their own sense of judgement and experience (Garforth et al., 2013). This concept of self-identity in relation to the importance of farmers' own abilities to identify problems based on stockmanship skills was frequently mentioned in the discussions and poses a likely barrier towards more experienced farmers seeking external guidance (Thompson, 2008).

Farmers' level of roundworm knowledge was shown to have a low negative impact on the adoption of AR selective practices in comparison to the uptake of SCOPS practices. This may suggest that farmers' roundworm control knowledge has a greater influence on the adoption of SCOPS practices, whereas other factors such as awareness of AR status may feature more heavily when deciding against using AR selective practices. Similar findings have also been reported by Kuiper et al. (2005) who found farmer knowledge on mastitis control did not explain the lack of preventive and curative practices employed compared to other factors such as sanctions, disease problems, incentives and internal normative beliefs. Furthermore, similar models investigating the influences of farmers' biosecurity identified knowledge having a comparable effect on the adoption of biosecurity practices (Toma et al., 2014).

Ultimately the use of qualitative and quantitative methods has highlighted a number of areas which could be targeted in future extension programmes. The need to ensure guidance is more comprehensible and rational to farmers' requirements, is one such step that will help to improve their overall acceptance. Additional emphasis and incentives for farmers to test their treatment efficacies may also be the type of 'cues to action' required to change farmers' perceptions of AR and motivate towards more sustainable parasite control approaches. Only through gaining such insights from the sheep farming community can we find ways to address the issues which may impede the adoption of best practice advice, in order to encourage the responsible use and safeguarding of anthelmintic treatments.

ACKNOWLEDGEMENTS

The authors would like to thank all the farmers involved for their active participation in the focus groups and telephone surveys, in addition to the Moredun regional advisors for their help in organising the focus group meetings. Furthermore, thanks to Fiona Vande Velde and Jiayi Liu for their assistance with the preliminary SEM analysis. This study is funded by the RESAS branch of the Scottish Government.

REFERENCES

Abbott, K.A., Taylor M.A., and Stubbings L.A. (2012). Sustainable Control of Parasites in Sheep (SCOPS) Sustainable Worm Control Strategies for sheep: A technical manual for veterinary surgeons and advisors. Accessed at: http://www.scops.org.uk/content/SCOPS-Technical-Manual-4th-Edition-updated-September-2013.pdf

Barbour, R. (2007). Doing Focus Groups. SAGE Publications, London. 174 p

Bartley, D.J., Jackson F., Jackson E. and Sargison N. (2004). Characterisation of two triple resistant field isolates of Teladorsagia from Scottish lowland sheep farms. Vet. Parasitol. 123, 189-199

Bartley, D.J. (2008). Prevalence, characterisation and management of anthelmintic resistance in gastro-intestinal nematodes of Scottish sheep. Unpublished PhD thesis. University of Edinburgh

Charlier, J., Velde F.V., van der Voort M., Van Meensel J., Lauwers L., Cauberghe V., Vercruysse J. and Claerebout E. (2015). ECONOHEALTH: Placing helminth infections of livestock in an economic and social context. Vet. Parasitol. 212, 62-67

Coles, G.C. (1997). Nematode control practices and anthelmintic resistance on British sheep farms. Vet. Rec. 141, 91-93

Garforth, C.J.,. Bailey A.P and Tranter R.B. (2013). Farmers' attitudes to disease risk management in England: a comparative analysis of sheep and pig farmers. Prev. Vet. Med. 110, 456-466

Hair, J.F., Tatham, R.L., Anderson, R.E., Black, W. (2006). Multivariate Data Analysis, 6th edition. Prentice Hall, 816p

Kay, R.H., and LeSage, A. (2009). Examining the benefits and challenges of using audience response systems: A review of the literature. Comput. Educ. 53, 819-827

Kuiper, D., Jansen, J. Renes, R.J. Leeuwis C. and van der Zwaag H. (2005). Social factors related to mastitis control practices: The role of dairy farmers' knowledge, attitude, values, behaviour and networks. Mastitis in Dairy Production 576-582

McMahon, C., McCoy M., Ellison S.E., Barley J.P., Edgar H.W., Hanna R.E., Malone F.E., Brennan G.P. and Fairweather I. (2013). Anthelmintic resistance in Northern Ireland (III): uptake of 'SCOPS' (Sustainable Control of Parasites in Sheep) recommendations by sheep farmers. Vet. Parasitol. 193, 179-184

Morgan, E.R., Hosking B.C., Burston S., Carder K.M., Hyslop A.C., Pritchard L.J., Whitmarsh A. K. and Coles G. C. (2012). A survey of helminth control practices on sheep farms in Great Britain and Ireland. Vet. J. 192, 390-397

Morgan, E.R. (2013). The 125-year life cycle of parasite control. Vet. Rec. 173, 89-91

National Statistics. (2015). Results from the June 2015 Scottish Agricultural Census. Environment and Marine Agriculture, The Scottish Government

Rosseel, Y. (2012). Lavaan: an R package for structural equation modeling. J. Stat.Softw. 48, 1–36

Sargison, N.D., Scott P.R and Jackson, F. (2002). Teladorsagiosis in young lambs and extended postparturient susceptibility in moxidectin-treated ewes grazing heavily contaminated pastures. Vet. Rec. 151, 353-355

Sargison, N.D., Jackson F., Bartley D.J., Wilson D.J., Stenhouse L.J. and Penny C.D. (2007). Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. Vet. Parasitol. 145, 65-76

Sparks, W. (2011). "Clicking, Learning, Telling: Audience Response System Use in Remote Locations." Learning solutions magazine, 8th August

Thompson, L.-J. (2008). Extending Complex Agricultural Research in Australia: The case of integrated parasite management in sheep. Doctor of Philosophy, University of New England, Armidale, Australia

Toma, L., Stott A.W., Heffernan C., Ringrose S. and Gunn G.J. (2013). Determinants of biosecurity behaviour of British cattle and sheep farmers-a behavioural economics analysis. Prev. Vet. Med. 108, 321-333

Velde, F.V., Claerebout, E., Cauberghe, V., Hudders, L., Van Loo, H., Vercruysse, J., & Charlier, J. (2015). Diagnosis before treatment: Identifying dairy farmers’ determinants for the adoption of sustainable practices in gastrointestinal nematode control. Vet. Parasitol. 212, 308-317

Wauters, E. & Rojo-Gimeno, C. (2014). Socio-psychological veterinary epidemiology. A new discipline for an old problem. Proceedings for the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) Conference, Dublin, Ireland, 26th to 28th March

ASSOCIATIONS BETWEEN FARMER OPINION, EMOTIONS AND PERSONALITY TO UPTAKE OF BEST PRACTICE BY SHEEP FARMERS: THE EXAMPLE OF FOOTROT

H. O'KANE, E. FERGUSON, J. KALER* AND L. GREEN

SUMMARY

A questionnaire on lameness prevalence, management, and farmer-orientated themes was sent to 4000 sheep farmers to explore impact of personality, emotions and beliefs on farmer management of lameness. Principal component analysis and latent class analysis were used to group behaviours and farmers. The response rate was 32%; the geometric mean prevalence of lameness (GMPL) was 3.7% (95% CI 3.5%–3.9%). There were three latent classes; LC1 ("best practice"), LC2 ("slow to act") and LC3 ("slow to act, delayed culling"), with GMPL 3.0%, 3.6% and 4.1% respectively. Farmers who reported the production cycle a barrier to treating lame sheep were more likely to be in LC2 than LC1. Farmers with negative emotions were in LC2, farmers with knowledge of preventing footrot were in LC2 or LC3 rather than LC1. Behaviours, emotions and knowledge were associated with differences in farmer management of footrot and prevalence of lameness in sheep.

INTRODUCTION

There is increasing interest in understanding farmer behaviour with respect to their management of livestock. Whilst farmers are likely to be interested in making profits (Gasson et al., 1993) and increasing production (Ploeg, 1993), farmers are not simply rational profit maximisers and are likely to have emotional reactions to their animals that also influence decision-making (Bigras-Poulin et al., 1985). Thus rural sociologists have applied theories like the theory of reasoned action (TRA) (Fishbein & Ajzen, 1975) and its extension, the theory of planned behaviour (TPB) (Ajzen, 1991) to understand farmers' decisions and explain variability in the health and welfare of the animals in their care (Hemsworth et al., 1989; Jansen et al., 2009; Kauppinen et al., 2012). Whilst useful, such models still focus on rational processes around attitudes and beliefs. In this paper we extend this to look at a wider spectrum of emotional factors such as the type and extent of farmers' emotional responses to animal disease, the Big-5 personality factors (Goldberg, 1993), all of which have strong effects on a broad range of human decision-making across a wide range of domains; such as relationships, health, employment and medical decision- making (Hunter & Hunter, 1984; Ferguson, 2013; Molloy et al., 2014). There is now a substantive body of evidence to support the central and

---

* Jasmeet Kaler, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK. Email: jasmeet.kaler@nottingham.ac.uk

crucial role of emotional experience (both positive and negative) and emotional processing in decision- making (Damasio et al., 1996; Dolan, 2002; Ferguson et al., 2009). Theoretical models show that traits act to influence how process indices operate to influence final decisions (Ferguson et al., 2011; Ferguson, 2013).

Lameness is a major welfare concern for both sheep farmers and veterinarians (Fitzpatrick et al., 2006; Goddard et al., 2006; Morgan-Davies et al., 2006). It is estimated to cost the UK sheep industry between £24 million (Nieuwhof & Bishop, 2005) and £80 million per year (Wassink et al., 2010) and in 2004 the global period prevalence of lameness in England was 10.6% (Kaler & Green, 2008), with more than 90% of lameness attributed to footrot.

Footrot is an infectious disease caused by Dichelobacter nodosus (Witcomb et al., 2014). It has two clinical presentations, inflammation of the interdigital skin of the foot (interdigital dermatitis) and separation of the hoof horn from the underlying tissue (severe footrot) (Winter et al., 2008). From the 1950s – 1990s, prevention of footrot focused on whole flock managements such as quarantine of new and diseased sheep, routine foot trimming and foot bathing and vaccination (Morgan, 1987). More recently, research has indicated that ‘best practice’, or prompt treatment of individual lame sheep with parenteral and topical antibiotics without foot trimming, reduces the duration of disease in affected individuals (Kaler et al., 2010a; Kaler et al., 2010b; Wassink et al., 2010). Rapid treatment also reduces recurrence (Kaler et al., 2010b) and protects flock mates (Green et al., 2007) and so reduces the incidence and prevalence of lameness. Whole flock managements of quarantine (Wassink et al., 2004; Winter et al., 2015) and vaccination (Winter et al., 2015) are also associated with a lower prevalence of footrot. In contrast, routine foot trimming and foot bathing are associated with a higher prevalence of footrot (Wassink et al., 2005; Green et al., 2007; Kaler & Green, 2008; King, 2013; Winter et al., 2015).

The objectives of the current study were to test the hypothesis that farmer personality, emotions and attitudes to footrot are associated with different approaches to management of footrot and the prevalence of lameness.

MATERIALS AND METHODS

Four-thousand English lowland flocks with at least 200 ewes were randomly selected by Defra (Department of Environments, Food and Rural Affairs) from the 2010 agricultural census and from the England Animal Health Development Board (AHDB) Beef & Lamb Better Returns programme (http://www.ahdb.org.uk).

Questionnaire design

A 16-page self-administered postal questionnaire was designed to capture data for the period May 2012 to April 2013 on farmer recognition of lameness and foot lesions, period prevalence of lameness, management of footrot (including both interdigital dermatitis (ID) and severe footrot (SFR) and farm and flock descriptors. The responses to this section of the questionnaire have been previously analysed and reported (Winter et al., 2015). A second section was designed to capture data on 3 themes based on models of emotion and traits: Theme 1 (barriers to treating footrot e.g. “I have difficulty identifying and finding a mildly lame sheep once the flock is gathered”), theme 2 (opinions about footrot e.g. “When a sheep has this disease it will lose weight”), theme 3 (knowledge about the aetiology of footrot e.g. “This disease is caused by infection in the pasture”) and also contained ten paired personality characteristics to measure the Big-5, using the ten-item-personality-inventory (TIPI) (Gosling et al., 2003). For themed sections 1- 3 farmers were asked to respond to statements using a 5 – point Likert-type scale where 1 = strongly disagree and 5 = strongly agree.

Data analysis

Farmers were excluded from all analyses if details of flock size or average prevalence of lameness in ewes was not provided. The geometric mean (GM) year period prevalence of lameness and median flock size were estimated. Frequency distributions of responses to each statement in the five themes were obtained. The Likert scores were treated as continuous variables. Items were reverse scored where necessary (Table 1) so that on all variables high scores equated to increased quantity of the measures (e.g. higher sadness).

Latent class analysis of farmer behaviour relating to management of lameness and footrot in their flock: Latent class analysis (LCA) was used in M*Plus* 7 (Muthén & Muthén, 1998-2012) to determine the number of subgroups of farmers on the basis of nine variables; 1) treat sheep within three days of seeing them lame, 2) number of sheep lame in a group before treatment, 3) trim the feet of lambs (highly correlated to ewes) with footrot, 4) ability to name interdigital dermatitis and footrot, 5) always treat footrot with parenteral antibiotic, 6) and foot spray, 7) identify sheep for culling because of lameness by memory, 8) number of episodes of lameness before culling and 9) vaccinate ewes against footrot. The geometric mean period prevalence of lameness and 95% CI was calculated for each latent class.

Principal component analysis of farmer knowledge, opinions, attitudes and emotions towards footrot and sheep: Exploratory principal component analyses (PCA) were conducted on statements in each of the themes 1-3 in the questionnaire (Fig. 1). A simple final solution was selected using a combination of the K1 rule, Scree plots, parallel analysis and theoretical and conceptual coherence (Ferguson & Cox, 1993). Cronbach's coefficients alpha (α) and mean inter-item correlations (i:i) were calculated for each component to assess the internal reliability of each scale, with α = 0.6-0.8 and i:i = 0.2-0.4 representing a scale with sufficient internal consistency (Ferguson & Cox, 1993). The unit scores for each component obtained by the PCA were calculated by taking the arithmetic mean of the item scores for that component. Farmers who did not respond to all items for a given component were classed as having a missing value for that component.

Modelling the relationship between management of footrot or prevalence of lameness and farmer opinions, attitudes, emotions and personality: Multinomial logistic regression modelling was conducted in STATA SE13 (Stata Corp, USA). The model took the form as described in Eq. (1) and (2):

$$\text{Logit}(\pi_{1k}/p_{i0k}) = \beta_{0k} + \sum \beta_0 x + e_K \tag{1}$$

$$\text{Logit}(\pi_{2k}/p_{i0k}) = \beta_{1k} + \sum \beta_1 x + e_K \tag{2}$$

where $\text{logit}(\pi_1/p_{i0})$ = the probability of latent class (LC)2 versus LC1 and $\text{logit}(\pi_2/p_{i0})$ = the probability of LC3 versus LC1, $\beta 0_k$ and β_{1k} are constants for LC2 and LC3, $\beta_0 x$ and $\beta_1 x$ are the series of coefficients of exploratory variables X for LC2 and LC3, and e_K is the residual variance fixed to a binomial distribution.

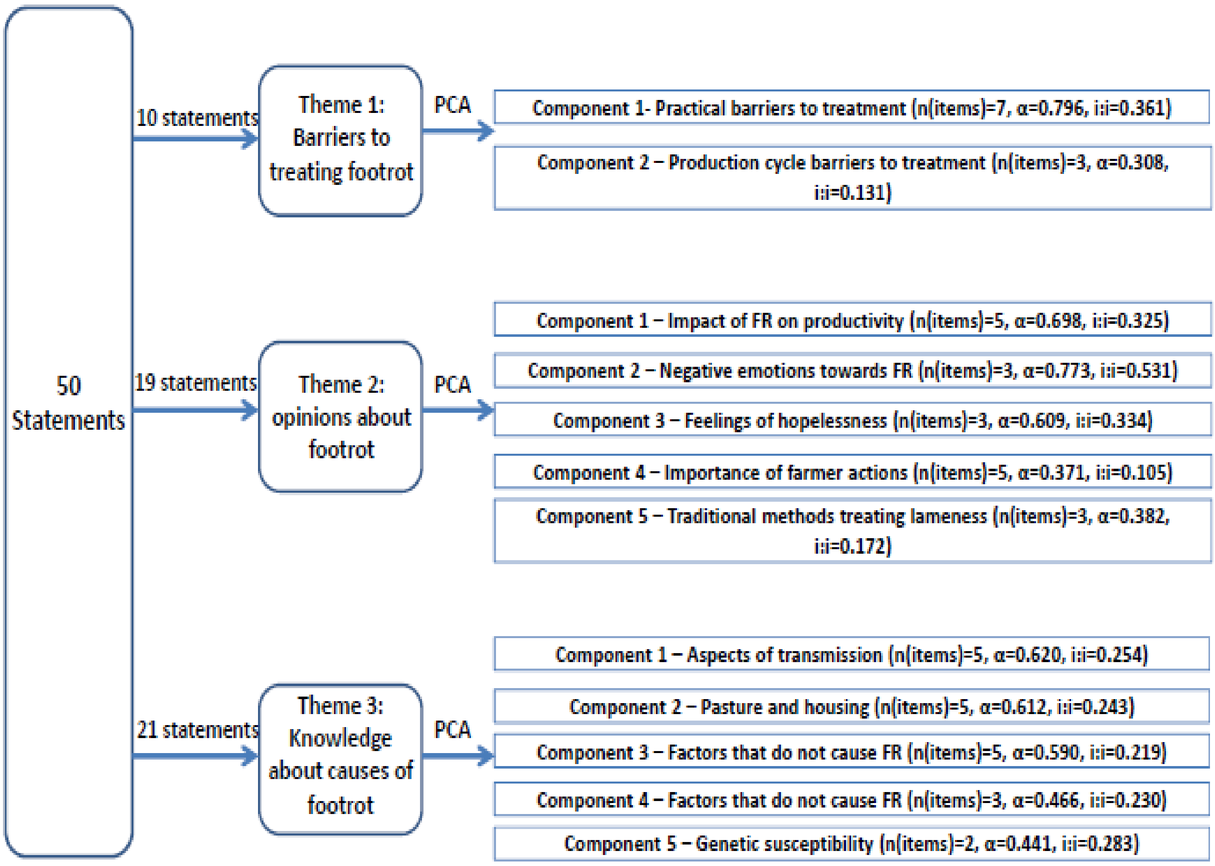


Fig. 1 Flow diagram of the application of Principle Component Analysis (PCA) on 1294 sheep farmer responses to questionnaire statements by theme, the components derived from each PCA and measures of scale reliability for each component (Cronbach's alpha (α) and mean inter-item correlation (i:i))

Separate univariable models were built for each exploratory variable derived from the PCAs and TIPI; variables with a P value ≤0.2 were identified for inclusion within a multivariable model. A multivariable model was built using forward stepwise selection (Dohoo et al., 2003). All remaining variables were retested in the multivariable model to check for residual confounding. Explanatory variables with p-values ≤0.05 using Wald's statistic, were left in the final model. The model fits were explored using log likelihood values and information criteria using fit statistics for the model.

RESULTS

The useable response proportion was 1294 (32%), although not all farmers answered all questions (for further information, see full paper). The number of ewes per flock ranged from 2-6000 (median 340) and 97% of farmers reported having lame sheep. The geometric mean year period prevalence of lameness in ewes was 3.7% (95% CI 3.5% – 3.9%).

Latent class analysis of farmer behaviours for treatment of footrot

Based on the goodness of fit statistics the three-class model was selected (Fig. 2). Respondents in LC1 were typical of "best practice" (as per recommendations in recent research), farmers in LC2 were "slow to act", farmers in LC3 were "slow to act, delayed culling". The conditional probability of each management being practiced by each LC group is presented in Fig. 2. There were 11%, 32% and 57% of respondents in the "best practice", "slow to act" and "slow to act, delayed culling" classes respectively with geometric mean prevalence of lameness of 3.0%, 3.6% and 4.1% respectively.

Principal component analysis of farmer knowledge, opinions, attitudes and emotions towards footrot, sheep and people

The 50 statements within 3 themed sections of the questionnaire were reduced to twelve components (Fig. 1).

Multinomial multivariable analyses of latent class membership and attitudes, beliefs and personality

Compared with farmers doing "best practice", farmers were more likely to be in the "slow to act" class if they reported that the production cycle was a barrier to treating footrot (RRR 1.36; Table 1). Farmers were more likely to be in the "slow to act, delayed culling" group (RRR 1.39) than in the "best practice" group if they had negative emotions towards footrot, that is, footrot in their flock made them feel sad and / or angry. Farmers were more likely to be in the

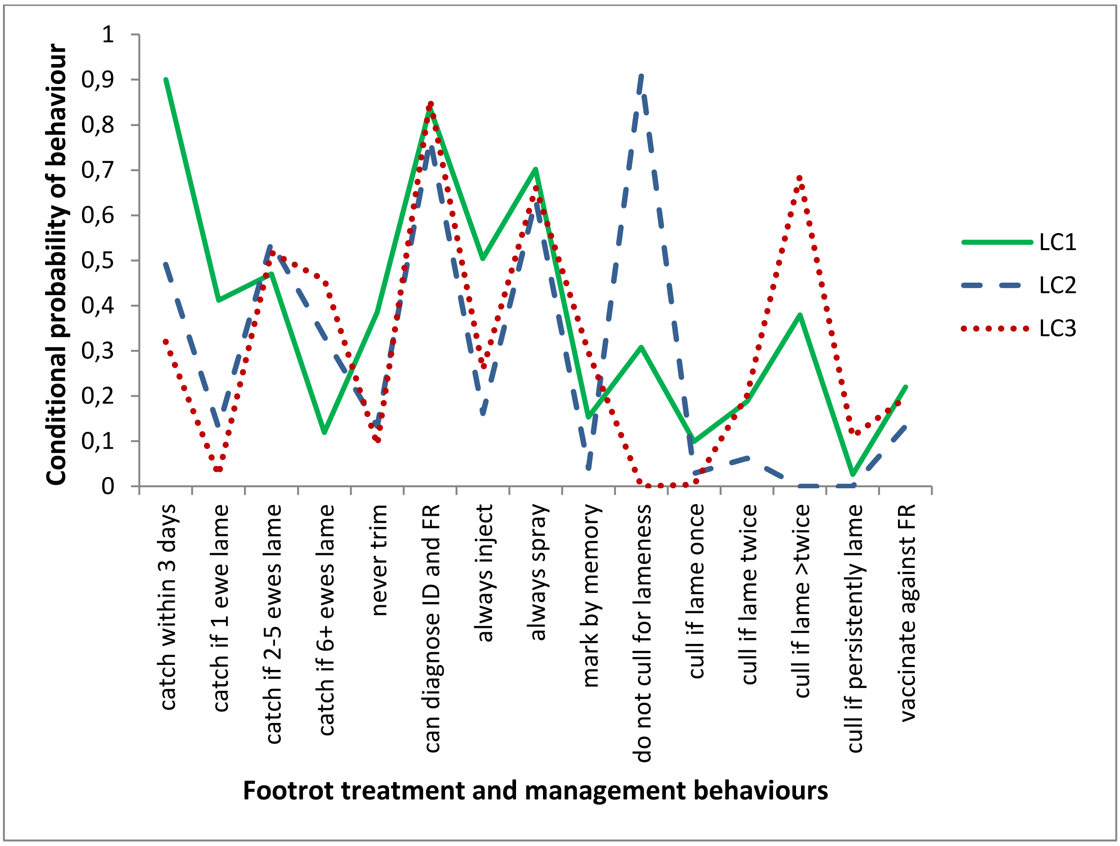


Fig. 2 Conditional probabilities* for the occurrence of behaviours to treat and manage footrot by 1294 English sheep farmers in a three-class model. *Probability that a farmer allocated to the latent class will perform that behaviour

"slow to act, delayed culling" (RRR 2.17) or "slow to act" (RRR 2.94) groups compared with the "best practice" group if they used traditional methods to treat lameness, seeing foot trimming as effective and antibiotics as ineffective. Knowledge about transmission of footrot (e.g. footrot is caused by other sheep with footrot in the flock) reduced the risk of being in the "slow to act" group (RRR 0.64) compared with the "best practice" group.

Table 1. Multivariable multinomial regression model for attitudes, emotions and personalities by latent class membership for 1294 English sheep farmers

Theme and component	Latent class	RRR[a]	95% CI
Theme 1: Barriers to treating footrot			
Component 1: Production cycle barriers to treatment	LC2	1.36	1.04 – 1.78
	LC3	1.25	0.94 – 1.65
Theme 2: Opinions about footrot			
Component 2: Negative emotions towards footrot	LC2	1.17	0.93 – 1.48
	LC3	1.39	1.09 – 1.77
Component 5: Traditional methods of treating lameness	LC2	2.94	2.17 – 4.00
	LC3	2.17	1.59 – 3.03
Theme 3: Knowledge about the causes			
Component 1: Aspects of transmission	LC2	0.64	0.41 – 0.99
	LC3	1.07	0.68 – 1.69

[a] Relative risk ratio

DISCUSSION

This is the first study to investigate farmer personality, emotions, attitudes and beliefs towards a livestock disease. A second innovation is the use of LCA to group farmers with different behavioural profiles with respect to the management of lame sheep. From the conditional probabilities and the significantly different period prevalence of lameness by LC ($X^2 = 14.16$, df=2, $P<0.01$) we can identify that 11% of farmers were in a "best practice" class with the remaining farmers in two classes representing non-compliance. While it may have been possible a-priori to identify a "best practice" group, different non-compliant groups could not have been identified without latent class analysis. The LCA results provide further support for research indicating that prompt treatment (within three days of onset) of individual lame sheep using parenteral and topical antibiotic, not trimming the hoof (Kaler et al., 2010a; Kaler et al., 2010b; Wassink et al., 2010) as well as vaccination (Winter et al., 2015) are effective in the treatment and control of lameness and footrot in sheep.

Latent class membership was also associated with beliefs, knowledge and emotions which can be used to gain insight into farmer attitudes and behaviours towards adopting new practices for the treatment and management of footrot. In terms of planned behaviour, barriers that led some farmers to avoid treating lame sheep at specific times of the production cycle (pregnancy and tupping for adults and finishing (fattening) period for lambs) also identified farmers less likely to use best practice and so less likely treat sheep promptly at any time of the year. This might reflect a general overall inappropriate attitude towards rapid management of lame animals that may act as a barrier to adopting best practice in the management of footrot.

Farmers who expressed negative emotions (feelings of frustration, anger, misery) towards footrot were more likely to be in the “slow to act, delayed culling” class with the highest mean prevalence of lameness. This validates the distinction between the two classes of non-compliance, because LC2 did not have such farmers and also shows that emotions are a crucial component for a certain class of farmers’ decision-making processes. Negative emotional reactions were also linked to a higher prevalence of lameness (results not shown). Such negative feelings are likely to result in inaction and resignation. As such, problems with lameness prevalence could escalate and such farmers enter a cycle of self-fulfilling behaviour; with farmers practicing behaviours associated with higher prevalence of lameness being more likely to have negative feelings leading to inaction and so on.

Farmers who understood “aspects of transmission” and the negative impact of “traditional methods of treating lameness” (the latter promotes maintenance and spread of footrot) were more likely to be in the “best practice” class compared with the less compliant classes. There was no significant association between the five personality domains and latent class membership. Latent class membership in the current study constituted multiple behaviours; there is a possibility that differences in personality exist for single behaviours, however, this needs to be explored in further analysis.

Overall our findings have a number of implications for interventions targeting farmer behaviour. First, it cannot be assumed that non-compliance is homogeneous, hence different interventions might be required for different sub-groups of non-compliant participants. Second, what types of interventions are likely to be effective? With more behaviour specific types of intervention we could target emotions that are associated with the target behaviour. Adapted cognitive behavioural therapy and guided self-help approaches to treat emotional problems for non-clinical contexts can be effective (Gellatly et al., 2007; Pleva & Wade, 2006) as can mindfulness (Cavanagh et al., 2013). The key barrier here might be acceptability of such interventions by farmers. However, the strong indication from our results of the key role of emotional factors warrants further investigation. Finally, there is misunderstanding about footrot itself. While educational materials, on their own, have not been effective in changing behaviour among some farmers, embedding these within framed messages may well be effective e.g. for the “slow to act” farmers who perceive barriers but without the negative emotional pull (Kahneman & Tversky, 1979).

There are some considerations with the hypotheses generated above; firstly, farmers in this study might be different from the target population of English farmers because they were willing to participate in this study. Secondly, the study relied on self-reported information which is subject to many types of bias. We are confident in the self-reported estimates of prevalence; sheep farmers have previously been found to estimate lameness quite accurately (King & Green, 2011). Thirdly, some of the components retained in the multinomial regression model did not meet our pre-set criteria (α and i:i) for internal consistency. Given that in most cases this was likely to be a result of the limited numbers of items in those components and that the items on each component demonstrated cognitive coherence, we consider these components were suitable measures. Fourthly, this study describes the relationship between farmer behaviour (latent class) and predictors of behaviour (attitudes and emotions) concurrently and so it does not determine a causal effect. Further research is needed in understanding the role of emotional factors on farmer behaviour and subsequent levels of lameness.

ACKNOWLEDGEMENTS

This research was funded by Defra as part of AW0512. Amy Kilbride contributed to the preparation and administration of the questionnaire. Joanne Winter assisted with tidying and checking the data. Laura Green and Jasmeet Kaler are funded by BBSRC (BB/M012980/1 and BB/M012964/1). We acknowledge Defra and AHDB Beef & Lamb for access to sheep farmers and, most importantly, we thank the sheep farmers for completing our questionnaire.

REFERENCES

Ajzen, I.(1991). The theory of planned behavior. Organ. Behav. Hum. Dec. 50, 179-211

Bigras-Poulin, M., Meek, A.H., Martin, S.W. and McMillan, I. (1985). Attitudes, management practices, and herd performance — a study of Ontario dairy farm managers. II. Associations. Prev. Vet. Med. 3, 241-250

Cavanagh, K., Strauss, C., Cicconi, F., Griffiths, N., and Wyper, A. (2013). A randomized controlled trial of a brief online midfulnes-based intervention. Behav. Res. Ther., 51, 573-578

Damasio, A.R., Everitt, B. and Bishop, D. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex [and discussion]. Philos. T. Roy. Soc. B. 351, 1413-1420

Dohoo, I., Martin, W. and Stryhn, H. (2003). Veterinary Epidemiologic Research. AVC Inc., Charlottetown, Canada

Dolan, R.J. (2002). Emotion, cognition, and behavior. Science 298, 1191-1194

Ferguson, E. (2013). Personality is of central concern to understand health: towards a theoretical model for health psychology. Health Psychol. Rev. 7, S32-S70

Ferguson, E. and Cox, T. (1993). Exploratory Factor Analysis: A Users’ Guide. Int. J. Select. Assess. 1, 84-94

Ferguson, E., Heckman, J.J. and Corr, P. (2011). Personality and economics: Overview and proposed framework. Pers. Indiv. Differ. 51, 201-209

Ferguson, E., Bibby, P.A., Rosamond, S., O'Grady, C., Parcell, A., Amos, C., McCutcheon, C. and O'Carroll, R. (2009). Alexithymia, cumulative feedback, and differential response patterns on the Iowa Gambling Task. J. Pers. 77, 883-902

Fishbein, M. and Ajzen, I. (1975). Belief; attitude, intention, and behavior: An Introduction to theory and research. Addison-Wesley, Reading, USA

Fitzpatrick, J., Scott, M. and Nolan, A. (2006). Assessment of pain and welfare in sheep. Small Ruminant Res. 62, 55-61

Gasson, R., Crow, G., Errington, A., Hutson, J., Marsden, T. and Winter, M. (1993). The farm family business. J. Agric. Econ. 47, 172-190

Gellatly, J., Bower, P., Hennessy, S., Richards, D., Gilbody, S and Lovell, K. (2007). What makes self-help interventions effective in the management of depressive symptoms? Meta-analysis and meta-regression. Psychol. Med. 37, 1217-1228

Goddard, P., Waterhouse, T., Dwyer, C. and Stott, A. (2006). The perception of the welfare of sheep in extensive systems. Small Ruminant Res. 62, 215-225

Goldberg, L.R. (1993). The structure of phenotypic personality traits. Am. Psychol. 48, 26

Gosling, S.D., Rentfrow, P.J. and Swann, W.B. (2003). A very brief measure of the Big-Five personality domains. J. Res. Pers. 37, 504-528

Green, L.E., Wassink, G.J., Grogono-Thomas, R., Moore, L.J. and Medley, G.F. (2007). Looking after the individual to reduce disease in the flock: A binomial mixed effects model investigating the impact of individual sheep management of footrot and interdigital dermatitis in a prospective longitudinal study on one farm. Prev. Vet. Med. 78, 172-178

Hemsworth, P.H., Barnett, J.L., Coleman, G.J. and Hansen, C. (1989). A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. Appl. Anim. Behav. Sci. 23, 301-314

Hunter, J.E. and Hunter, R.F. (1984). Validity and utility of alternative predictors of job performance. Psychol. Bull. 96, 72

Jansen, J., van den Borne, B.H.P., Renes, R.J., van Schaik, G., Lam, T.J.G.M. and Leeuwis, C. (2009). Explaining mastitis incidence in Dutch dairy farming: The influence of farmers' attitudes and behaviour. Prev. Vet. Med. 92, 210-223

Kahneman, D. and Tversky, A. (1979). Prospect Theory: An analysis of decision under risk. Econometrica, 47, 263-291

Kaler, J. and Green, L.E. (2008). Naming and recognition of six foot lesions of sheep using written and pictorial information: A study of 809 English sheep farmers. Prev. Vet. Med. 83, 52-64

Kaler, J., Daniels, S.L.S., Wright, J.L. and Green, L. (2010a). A randomised factorial design clinical trial to investigate the impact of parenteral long long acting oxytetracycline, foot trimming and flunixine meglumine on time to recovery from lameness and foot lesions in sheep lame with footrot. J. Vet. Intern. Med. 24, 420-425

Kaler, J., Daniels, S.L.S., Wright, J.L. and Green, L.E. (2010b). Randomized Clinical Trial of Long-Acting Oxytetracycline, Foot Trimming, and Flunixine Meglumine on Time to Recovery in Sheep with Footrot. J. Vet. Intern. Med. 24, 420-425

Kauppinen, T., Vesala, K.M. and Valros, A. (2012). Farmer attitude toward improvement of animal welfare is correlated with piglet production parameters. Livest. Sci. 143, 142-150

King, E.M. (2013). Lameness in English lowland sheep flocks: farmers' perspectives and behaviour. PhD thesis, University of Warwick

King, E.M. and Green, L.E. (2011). Assessment of farmer recognition and reporting of lameness in adults in 35 lowland sheep flocks in England. Anim. Welfare 20, 321-328

Molloy, G.J., O'Carroll, R.E. and Ferguson, E. (2014). Conscientiousness and Medication Adherence: A Meta-analysis. Ann. Behav. Med. 47, 92-101

Morgan-Davies, C., Waterhouse, A., Milne, C.E. and Stott, A.W. (2006). Farmers' opinions on welfare, health and production practices in extensive hill sheep flocks in Great Britain. Livest. Sci. 104, 268-277

Morgan, K. (1987). Footrot. In Practice 9, 124-129

Muthén, L.K. and Muthén, B.O. (1998-2012). Mplus User's Guide. Muthén & Muthén, Los Angeles, USA

Nieuwhof, G.J. and Bishop, S.C. (2005). Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. Anim. Sci. 81, 23-29

O'Connor, B. (2000). SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. Behav. Res. Meth. Ins. C. 32, 396-402

Pleva J and Wade, TD. (2006). Guided self-help versus pure self-help fir perfectionism: A randomized controlled trial. Behav. Res. Ther., 45, 849-861

Ploeg, J.D.V.D. (1993). Rural sociology and the new agrarian question: A perspective from the Netherlands. Sociol. Ruralis 33, 240-260

Wassink, G., Moore, L., Grogono-Thomas, R. and Green, L. (2005). Footrot and interdigital dermatitis in sheep: farmers' practices, opinions and attitudes. Vet. Rec. 157, 761-765

Wassink, G.J., Grogono-Thomas, R., Moore, L.J. and Green, L.E. (2004). Risk factors associated with the prevalence of interdigital dermatitis in sheep from 1999 to 2000. Vet. Rec. 154, 551-555

Wassink, G.J., King, E.M., Grogono-Thomas, R., Brown, J.C., Moore, L.J. and Green, L.E. (2010). A within farm clinical trial to compare two treatments (parenteral antibacterials and hoof trimming) for sheep lame with footrot. Prev. Vet. Med. 96, 93-103

Winter, A.C. (2008). Lameness in sheep, Small Rumin. Res. 76, 149–153

Winter, J.R., Kaler, J., Ferguson, E., Kilbride, A.L. and Green, L.E. (2015). Changes in prevalence of, and risk factors for, lameness in random samples of English sheep flocks: 2004–2013. Prev. Vet. Med. 122, 121-128

Witcomb, L.A., Green, L.E., Kaler, J., Ul-Hassan, A., Calvo-Bado, L.A., Medley, G.F., Grogono-Thomas, R. and Wellington, E.M. (2014). A longitudinal study of the role of Dichelobacter nodosus and Fusobacterium necrophorum load in initiation and severity of footrot in sheep. Prev. Vet. Med. 115, 48-55

PERCEPTIONS AND PRACTICES

UNDERSTANDING VETERINARIANS’ PRESCRIBING DECISIONS ON ANTIBIOTIC DRY COW THERAPY: A BAYESIAN APPROACH

H.M. HIGGINS[*], J. MOUNCEY, I. NANJIANI AND A.J. COOK

SUMMARY

Numerous factors may influence veterinarians’ prescribing decisions. This paper focuses on the efficacy of teat sealants compared to antibiotics, although many other factors were evaluated in the study. Evidence-based practice relies heavily on up-dating beliefs following the evaluation of new data. Yet people do not always respond logically when shown new evidence, especially if it contradicts their pre-existing beliefs. This study demonstrates a practical method to quantify veterinarians' beliefs regarding the efficacy of an antibiotic versus a teat sealant, pre- and post-exposure to new evidence. It assessed how logically veterinarians updated their beliefs by comparing how they changed to a gold standard: predictions from Bayes theorem. Results suggest that some veterinarians are not convinced of the efficacy of teat sealants, which has implications for responsible antimicrobial prescribing. The results also suggest that some veterinarians may need additional training to help them update their beliefs logically when challenged with new evidence.

INTRODUCTION

Evidence-based practice relies heavily on changing clinicians’ current beliefs by presenting them with new data. A statistical framework that is ideally suited and increasingly being used in evidence-based medicine is the Bayesian approach. This approach is conceptually straightforward. There are always two sources of information, the new data (e.g. from a recent experiment, such as a clinical trial) and the prior information. Prior information is any pre-existing information of relevance to the parameter of interest that has not arisen from the new experiment. The prior information must be expressed in a quantitative format as a probability distribution (called the prior). The information originating from the new data is summarised by a likelihood function. To conduct the analysis, Bayes theorem is used to combine the prior with the new data and produce a posterior probability distribution. Bayes theorem expresses how the prior information should, logically, be updated in light of the new evidence. Hence the posterior distribution encapsulates everything that is now known about the parameter, having updated the prior with the new data. If the prior information is weak (i.e. contains considerable uncertainty), and the new data comparatively strong, the posterior will be dominated by the new data, and vice versa.

By always including prior information formally in the statistical analysis itself, Bayesian statistics quantitatively places the new data in the context of pre-existing knowledge and

---

[*] Helen M. Higgins, School of Veterinary Sciences, Leahurst Campus, University of Liverpool, UK. CH64 7TE. Email: h.higgins@liverpool.ac.uk

addresses the question: how should the data change what we currently believe? It is, therefore, a formalisation of ‘learning from experience’ and hence evidence-based practice. In contrast, traditional statistical frameworks ignore prior information and hence the reader is left to quantify for themselves how the new data should be combined with prior knowledge to arrive at a final answer.

There are several possible choices for the prior information, including data from previously conducted experiments and ‘off-the-shelf’ theoretical distributions aimed at representing different prior perspectives, for example, a ‘reasonable cautious sceptic’ (Spiegelhalter et al., 1994). Another possibility is to base the prior information on pre-existing clinical knowledge (so-called ‘clinical priors’). In this case, the clinician’s current belief needs to be captured in a numerical format as a probability distribution. The technique used to do this is called probabilistic elicitation. Different priors may legitimately give different posterior distributions and hence different answers.

Currently, NHS data monitoring committees use a Bayesian analysis to aid their decisions over when to terminate a trial. Clinical priors obtained by eliciting doctors’ beliefs are combined with the accruing trial data. When the posterior distribution associated with the most sceptical clinical prior supports the new treatment, the trial may be trial stopped on the grounds that the new data will be sufficiently strong to convince the clinical community. The assumption is, of course, that doctors will actually update their beliefs logically. There is literature on the intuitiveness of Bayesian logic in several non-clinical contexts (O'Hagan et al, 2006; Kynn, 2008). Experimental psychologists’ in the 1960’s questioned clinicians’ abilities to reason logically due to heuristics. These are quick mental strategies that people may employ instinctively to make judgements when faced with uncertainty. They can be effective but may lead to severe bias and error (Tversky & Kahneman, 1974; Cooke, 1991; O'Hagan et al., 2006). There is also psychological, economic and behavioural literature indicating that people may react negatively (emotionally, cognitively and behaviourally), when their normative beliefs are challenged (Brehm, 1966; Politi et al., 2007; Ferguson & Lawrence, 2013). Such negative reactions are likely to contravene Bayesian logic.

In contrast, more recent work suggests that people’s judgements are very close to Bayesian estimates for certain tasks (Baker et al., 2006; Griffiths & Tenenbaum, 2006, Westover et al., 2011) and especially when information is represented in a way to facilitate Bayesian reasoning (Hoffrage et al., 2000; Gigerenzer, 2011). However, the existing literature has predominately involved undergraduate students and lay tasks, and has exclusively focused on discrete, rather than continuous, parameters. Given the increased emphasis on evidence-based medicine and the increased use of Bayesian methods in clinical care, it is of real practical interest to know how clinicians’ update their beliefs compared to Bayes Theorem when presented with new data; especially for continuous clinical parameters, such as cure rates. There is, however, a paucity of specific literature. A key aim of this study, therefore, was to demonstrate a simple practical method that can be used to quantify how logically veterinarians’ update their clinical beliefs compared to Bayes theorem for a clinical parameter.

The clinical context concerned dry cow therapy. In the UK, blanket antibiotic dry cow therapy (BDCT) is a commonly used strategy to aid mastitis control. It involves the infusion of an intra-mammary antibiotic in all quarters of all cows at dry-off, irrespective of infection status. The aim is to cure any pre-existing intra-mammary infections (IMI) and prevent new IMI over the dry period. An alternative strategy is selective dry cow therapy (SDCT) whereby cows with a low probability of an IMI receive an internal teat sealant (ITS) instead of antibiotics to prevent new IMI. Using SDCT instead of BDCT can considerably reduce antibiotic use. A key clinical question underpinning the use of SDCT is whether clinicians believe that ITS is as effective as an antibiotic (or better) at preventing new IMI in uninfected cows. Therefore, we aimed to quantify veterinarians' current beliefs for the efficacy of an antibiotics versus ITS, pre- and post-exposure to new data. Central to Bayesian philosophy is the question: how should new data change what we currently believe? Practically, and in comparison, we wanted to quantitatively measure: how does new data change what clinicians believe?

MATERIALS AND METHODS

Purposive sampling was used to recruit 20 veterinarians with a wide range of clinical large animal experience from 6 practices in the South of England. Inclusion criteria were veterinarians providing healthcare to cattle during their normal working hours. Voluntary signed consent was obtained. Individual face-to-face interviews lasting 30 minutes were conducted by HMH between 1 December 2014 and 31 January 2015, at the participants' workplaces. A standard script was used for consistency. The script was piloted on three vets to ensure the method was tenable and revised accordingly; this pilot data is not included in the results.

Elicited parameter, θ

The context was cows with all four quarters uninfected at dry-off. The outcome of interest was the dry period new infection rate, defined as the percentage of uninfected quarters that acquire a new infection during the dry period. The two treatments considered at dry-off were: (i) a long acting intra-mammary suspension containing 0.250g cefalonium per syringe, administered correctly; and, (ii) an ITS, specifically a 4g intra-mammary suspension containing 65% bismuth subnitrate, administered correctly. To ensure everyone considered the same baseline, participants were told the infection rate with cefalonium was 30%. The elicited parameter (denoted θ) was the difference in the infection rate if an ITS was used instead of cefalonium, assuming all other factors remained constant: negative differences denoted a lower infection rate with ITS. The probability that an ITS treated, uninfected quarter would be infected at calving was denoted ψ, and could subsequently be simply calculated using Eq. (1):

$$\psi = \frac{30+\theta}{100}; \quad \psi \epsilon [0,1] \tag{1}$$

Method to capture beliefs as probability distributions

A variety of different methods have been reported to elicit beliefs probabilistically (Johnson et al., 2010a). This study used a version of the roulette method (also called chip and bins) for two reasons. First, it is a method that has been shown to be feasible, valid and reliable in a clinical setting (Johnson et al., 2010b). Second, our experience of eliciting beliefs (Higgins et al., 2012, Higgins et al 2013) has lead us to prefer this approach because it is a method that avoids the need to specify probabilities directly, and it is a practical task that allows the participant to produce a visual representation of their belief by themselves. Current best practice for elicitation was followed (O'Hagan et al., 2006; Johnson et al., 2010b). This included: (i) a face-to-face interview; (ii) providing a training exercise; (iii) use of a standardized script; (iv) a design that avoided heuristics (Garthwaite et al., 2005); (v) provision of feedback; (vi) opportunity to revise responses; and, (vii) use of simple graphical methods.

Following the general methodology of Johnson et al. (2010b), participants were asked to express their belief probabilistically by indicating the weight of their belief for θ using 10 chips each worth 0.1 probability, and placing them in discrete five per cent intervals (the bins) over the range of θ. Coins, specifically five pence pieces, were used for the chips. Adhesive putty (Blu-Tack®, Bostik) was used to make the coins adhere to, but be easily detached from, a laminated sheet; this allowed participants to easily revise their answers. For the training exercise participants were shown three generic examples each demonstrating a different belief, and the meaning explained. No context was provided in order to minimise bias.

Task Structure

Participants' current beliefs for θ were captured as probability distributions (denoted θ_1, and referred to as the prior belief / distribution). They were then shown the results (point estimate and 95% classical confidence interval) from a clinical trial. The trial data was synthetic and followed a binomial distribution, X ~ Binomial(n, p), where n is the number of uninfected quarters at dry-off, p is the probability of a quarter treated with ITS being infected at calving, and the number of infected quarters were realisations (x) on the random variable X. n was set to 1000, which resulted in 95% confidence intervals that were 5% wide, i.e. the width of one bin.

The data each veterinarian was shown was dependent on their prior distribution, as follows. The data had a point estimate centred in the 5% interval (bin) adjacent to the mode of their prior and favouring cefalonium. Thus, for example, if the mode of the prior was in the -(5 to 10)% interval (i.e. $\psi =$ 0.2-0.25), the adjacent interval to this was -(0 to 5%), (i.e. ψ = 0.25-0.3) and the point estimate of the data was in the middle of this, such that $x = 275$ infected quarters out of n=1000; $p = 0.275$). By varying the data according to each participant's prior belief, all participants had their prior beliefs challenged to the same extent. Classical 95% binomial confidence intervals (Wilson, 1927) were calculated using the 'binom' package in the software program R (R Development Core Team, 2014). The veterinarian's belief for θ was re-elicited as a probability distribution after seeing the new data (denoted θ_2).

Data Analysis

The raw data was entered into a Microsoft Excel spread sheet (Version 2010, Microsoft Corp). For the analysis, for each participant, θ_1 and θ_2 were transformed using Eq. 1 to ψ_1 and ψ_2, which are referred to, respectfully, as their prior belief and updated belief for the probability of ITS treated quarters becoming infected.

Since the raw data comprised discrete quantities of probability placed in fixed intervals, it was convenient to use the discrete form of Bayes theorem to calculate a posterior distribution for each veterinarian which described how their prior belief should, logically, have been updated with the data they were shown (Eq. 2):

$$P(\psi|x) = \frac{P(\psi)\, P(x|\psi)}{\sum_{i=1}^{20} P(\psi_i)\, P(x|\psi_i)} \qquad (2)$$

where $P(\psi|x)$ is the posterior distribution (subsequently referred to as the predicted belief), $P(\psi)$ is the prior probability of ITS treated uninfected quarters being infected at calving, x is the clinical trial data, $P(x|\psi)$ is the binomial likelihood function, and the summation in the denominator is over the following values for ψ $\{0.025, 0.075, 0.125, 0.175, \ldots, 1\}$, corresponding to the midpoint of the elicited 5% intervals.

RESULTS

Prior beliefs

Figure 1 presents summary statistics for the veterinarians' prior beliefs for ψ. In total, 17 veterinarians (out of 20) had the mode of their distribution <0.3 indicating that their "best guess" was that ITS was superior to cefalonium. The remaining 3 veterinarians had 0.3 for their mode, indicating equivalence between the two treatments. In total, 8 (out of 20) had their entire prior distribution ≤ 0.3, that is, they gave negligible probability that ITS was the inferior treatment. However, the majority (12 out of 20: 60%) assigned some probability that ITS was the inferior treatment. Overall, there was heterogeneity in clinical beliefs with respect to the efficacy of ITS compared to cephalonium, and as illustrated in Fig. 1, several pairs of veterinarians had non-over-lapping 100% credible intervals for their distributions.

Updated beliefs compared to Bayesian predictions

Figure 2 presents summary statistics for the prior, updated and predicted beliefs for ψ for the 20 veterinarians. In total, 4 out of the 20 vets updated their beliefs perfectly logically in light of the new data (both central location and variance). Furthermore, the majority (18/20) centred their updated beliefs correctly. However, the majority (16/20) did not adjust the variance of their updated distributions in keeping with Bayesian predictions. Specifically, 8 veterinarians updated distributions with variance less than their prior but greater than predicted, 6 updated distributions with variances equal to their prior, and 2 updated distributions with variances greater than their prior. Thus, many veterinarians did not draw as much confidence from the new data as could logically be expected.

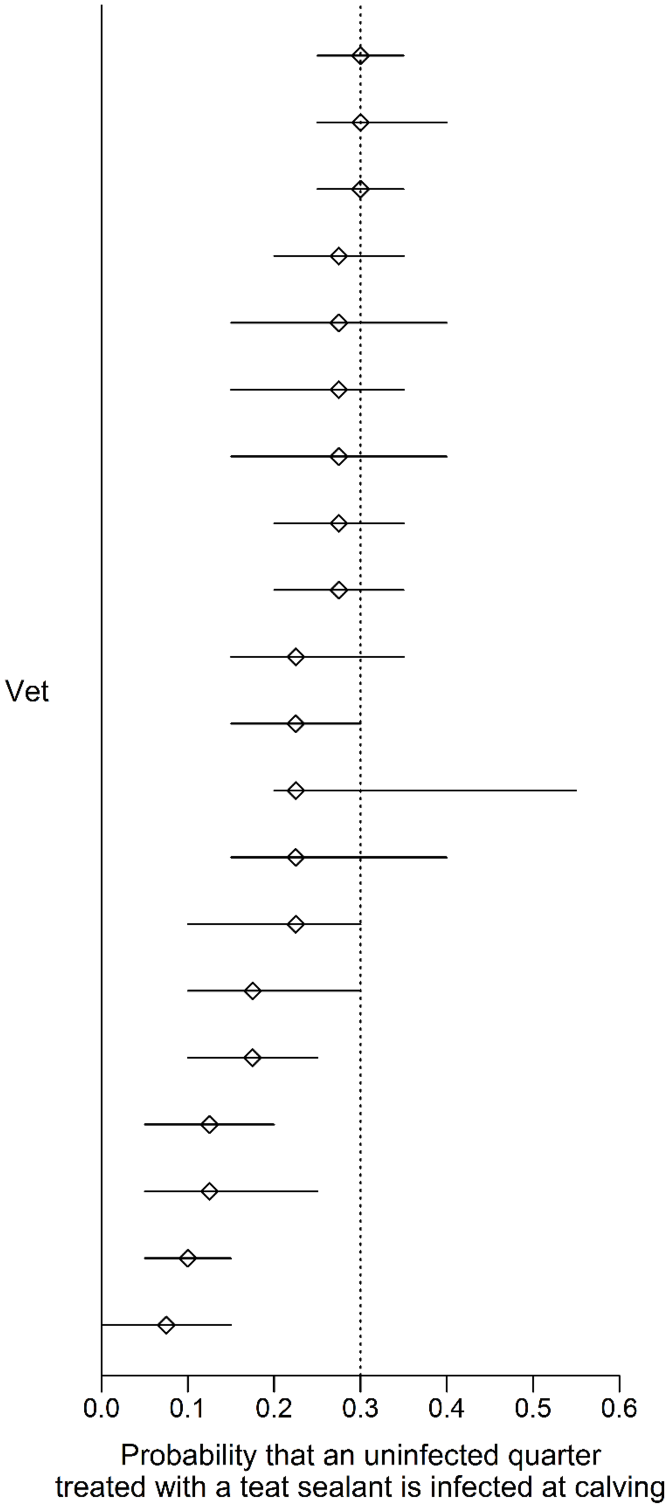


Fig. 1 Prior summary statistics (100% credible interval and mode) for 20 veterinarians for the probability of an infection at calving with a teat sealant given a 0.3 probability with cephalonium. Veterinarians are ordered vertically by their prior mode

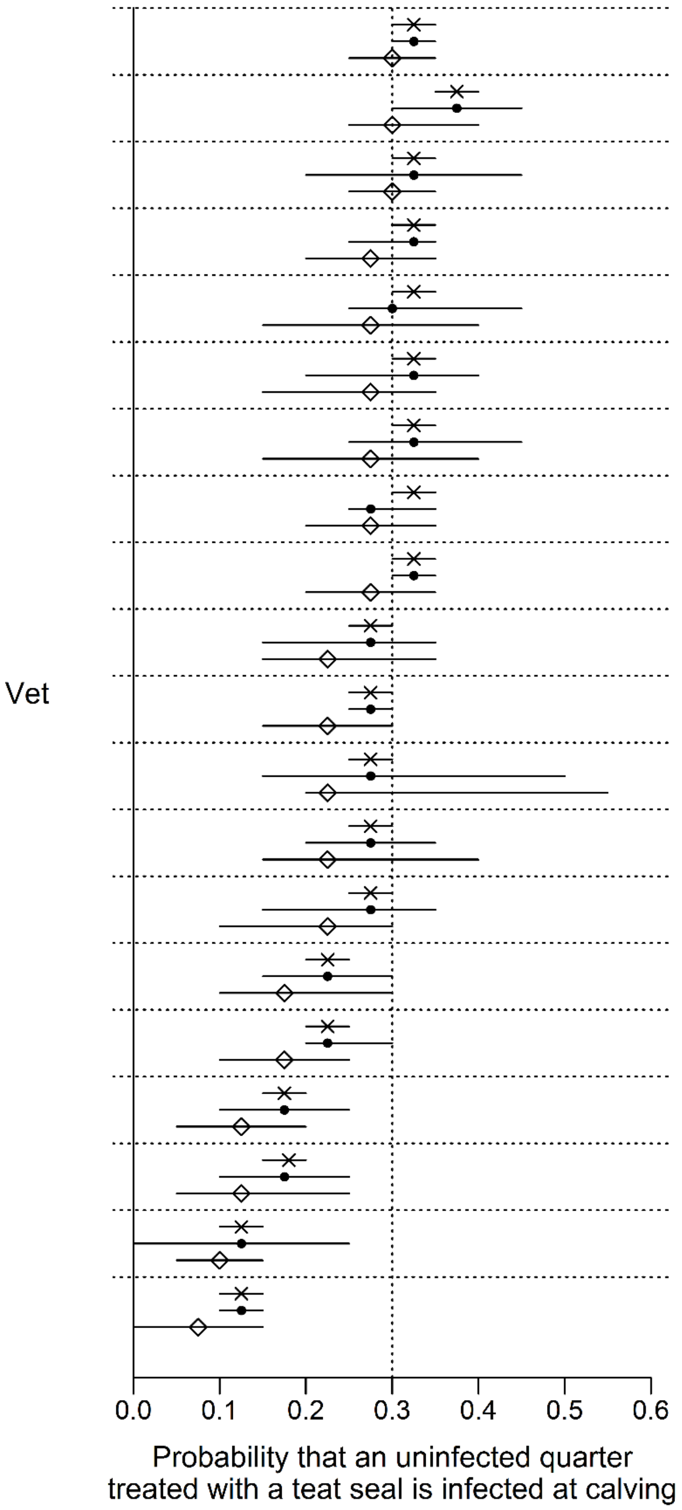


Fig. 2. Summary statistics (100% credible interval and mode) for the prior (diamond), updated (solid dot) and predicted (cross) distributions for 20 veterinarians regarding the probability of an infection at calving with ITS, given a 0.3 probability with cephalonium. Veterinarians are ordered vertically by their prior mode

DISCUSSION

Our results provide quantitative support for the notion that new data which differs from clinicians' priors can generate psychological uncertainty (Ellsberg, 1961; Politi et al., 2007). Thus, new data may result in clinicians having weaker beliefs upon which to base their clinical decisions than they did before. This 'psychological handicap', effectively a hurdle of doubt, is important for clinical researchers and data monitoring committees to bear in mind when assessing the strength of conviction that new data may bring to the clinical community.

In light of our findings, we suggest that as the results of a trial accumulate, the influence on current beliefs could be repeatedly elicited from clinicians, at the same time as off-the-shelf sceptical, enthusiastic, and reference priors are used, and all of these presented to data monitoring committees to help them better understand the impact of the trial results on the clinical community in reality, and to aid their decision-making task. Using the type of methodology employed here, this would not be arduous task, and could be done without the need for a facilitator.

The results also support the view that some clinicians may benefit from assistance to appropriately adjust their current beliefs in the light of new evidence. An interpretation section in clinical papers that presents a variety of prior beliefs and demonstrates how the trial result should influence them may be helpful. This would enable clinicians' to self-evaluate how they should adjust their clinical beliefs, and help them to make the best use of data; in turn this would facilitate the efficient uptake of new evidence into clinical practice. In our view, a Bayesian approach has, as yet, under-utilised potential to help clinicians' keep abreast of the current literature. The onus is on clinical researchers to make their results transparent and easily interpretable to all clinicians in the context of their current clinical beliefs, and a Bayesian framework is ideally suited to this; to this end, reporting guidelines have an important role to play (Kilkenny et al., 2010). As Ashby (2006) commented "the next frontier [for Bayesian statistics] is the medical literature".

The type of task described here could usefully and easily be used to help teach the logical updating of clinical beliefs and the concepts of Bayesian statistics to clinicians' as part of undergraduate curricula. There is a literature supporting the use of visual representation to aid clinical understanding associated with uncertainty (Gigerenzer & Edwards, 2003), but it should also be acknowledged that de-biasing heuristically biased beliefs can be difficult (Arkes, 1991).

It is possible that different results would be obtained using a different method to probabilistically elicit vets beliefs, thus repetition of this type of study is warranted. In addition, any method must be acceptable to clinicians' themselves and as simple as possible. Our perception was that the veterinarians in our sample found the methodology acceptable, and the training exercise is important.

The fact that this study used synthetic data may have given rise to some psychological implications, particularly that the results may not have been believed. Steps were taken to overcome this, however, including specifically emphasising at the outset that the task required participants to 'use their imagination and really believe' the trial results. Furthermore, our previous experience of using hypothetical data suggests that using hypothetical data is not psychologically problematic. Indeed, it is important to remind clinicians at the end of the interview that the data synthetic in order to avoid them transferring incorrect information to clients.

Using SDCT instead of BDCT can considerably reduce antibiotic use. The existing evidence suggests that in uninfected quarters at dry-off, using an ITS instead of an antibiotic is as effective at preventing new IMI. However, there are many practical and psychological barriers to implementing SDCT, which are of primary importance. The results suggest that some veterinarians' are not entirely convinced of the efficacy of teat sealants when administered correctly. This may play a role in the implementation of SDCT in practice, and while only part of the broader perspective, it has important implications for responsible antimicrobial prescribing.

ACKNOWLEDGEMENTS

Our thanks go to all the veterinarians who participated in this study. This research was funded by the University of Surrey.

REFERENCES

Arkes, H.R. (1991). Costs and benefits of judgment errors: Implications for debiasing. Psychol. Bull 110, 486-498

Ashby, D. (2006). Bayesian statistics in medicine: A 25 year review. Statist. Med., 25, 3589-3631

Baker, C. L., Tenenbaum, J. B. and Saxe, R. R. (2006). Bayesian models of human action understanding. Adv. Neur. In., 18, 99-106

Brehm, J. W. (1966). A theory of psychological reactance. New York: Academic Press

Cooke, R.M. (1991). Opinion and subjective probability. New York: Oxford University Press

Ellsberg, J. W. (1961). Risk, ambiguity, and the savage axioms. Q. J. Econ., 75, 643-669

Ferguson, E. and Lawrence, C. (2013). Normative and counter-normative stress messages and sypmtom reporting: Implications for health promotion and a methodological artifact for stress research. Br. J. Health Psychol., 18, 279-295

Garthwaite, P. H., Kadane, J. B. and O'Hagan, A. (2005). Statistical methods for eliciting prior distributions. J. Am. Statist. Ass., 100, 680-701

Gigerenzer, G. (2011). What are natural frequencies? Br. Med. J., 343, d6383

Gigerenzer, G. and Edwards, A. (2003). Simple tools for understanding risks: From innumeracy to insight. Br. Med. J., 327, 741-744

Griffiths, T.L. and Tenenbaum, J. B. (2006). Optimal predictions in everyday cognition. Psychol. Sci., 17, 767-773

Higgins, H.M., Dryden, I.L. and Green, M. J. (2012). A Bayesian elicitation of veterinary beliefs regarding systemic dry cow therapy: Variation and importance for clinical trial design. Prev. Vet. Med., 106, 87-96

Higgins, H.M., Green, L.E., Green, M.J. and Kaler, J. (2013). How does reviewing the evidence change veterinary surgeons’ beliefs regarding the treatment of ovine footrot? A quantitative and qualitative study. PloS One. 8 (5) e64175

Hoffrage, U., Lindsey, S., Hertwig, R. and Gigerenzer, G. (2000). Communicating statistical information. Science, 290, 2261-2262

Johnson, S. R., Tomlinson, G.A., Hawker, G. A., Granton, J. T., and Feldman, B. M. (2010a). Methods to elicit beliefs for Bayesian priors: A systematic review. J. Clin. Epidemiol 63, 355-369

Johnson, S.R., Tomlinson, G.A., Hawker, G.A., Granton, J. T., Grosbein, H. A. and Feldman, B. M. (2010b). A valid and reliable belief elicitation method for Bayesian priors. J. Clin. Epidemiol 63, 370-383

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. and Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS. Biol 8

Kynn, M. (2008). The 'heuristics and biases' bias in expert elicitation. J. R. Statist. Soc. A, 171, 239-264

O'Hagan, A., Buck, C.E., Daneshkhah, A., Eiser, J.R., Garthwaite, P.H., Jenkinson, D.J., Oakley, J.E. and Rakow, T. (2006). Uncertain judgements: Eliciting experts' probabilities. Chichester: Wiley

Politi, M. C., Han, P. K. J. and Col, N. F. (2007). Communicating the uncertainty of harms and benefits of medical interventions. Med. Decis. Making, 27, 681-695

R Development Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing

Spiegelhalter, D.J., Freedman, L.S. and Parmar, M.K. (1994). Bayesian approaches to randomized trials. J. R. Statist. Soc. A, 157, 357-416

Tversky, A. and Kahneman, D. (1974). Judgment under uncertainty: Heuristics and biases. Science, 185, 1124-1131

Westover, M. B., Westover, K. D. and Bianchi, M. T. (2011). Significance testing as perverse probabilistic reasoning. BMC Med., 9:20

Wilson, E. B. (1927). Probable inference, the law of succession, and statistical inference. J. Am. Statist. Ass. 22, 209-212

FACTORS INFLUENCING PIG FARMERS' AND VETERINARIANS' INTENTION TO REDUCE ANTIMICROBIAL USAGE IN SIX EUROPEAN COUNTRIES

L. COLLINEAU*, A. BACKHANS, S. LOESKEN, E. OKHOLM NIELSEN, M. POSTMA, C. BELLOC, J. DEWULF, U. EMANUELSON, E. GROSSE BEILAGE, M. SIEGRIST, M. SJÖLUND, K.D.C STÄRK AND V.H.M. VISSCHERS

SUMMARY

Veterinary antimicrobial (AM) usage should be reduced to mitigate the development of AM resistance. This study investigated factors that influence pig farmers' intention to reduce AM usage and veterinarians' reported reduction behaviour; these are a prerequisite for actual behaviour. A survey was conducted among 1,294 pig farmers and 334 veterinarians in Belgium, Denmark, France, Germany, Sweden and Switzerland to assess the perceived risks, benefits and need of AM usage, potential for AM usage reduction, farmers' self-efficacy (i.e. perceived own abilities to reduce AM usage), relation with farmers as clients and support from veterinarian. Farmers' self-efficacy and perceived feasibility of AM usage reduction were the main predictors of farmers' intention to reduce AM usage and veterinarians' reduction behaviour, respectively. To promote prudent AM usage, pig farmers need to learn how to control their AM usage by applying alternative measures, whereas veterinarians should strengthen their advisory role to support and educate farmers.

INTRODUCTION

Antimicrobial (AM) resistance has been recognized as an emerging threat for animal and public health (Vaarten, 2012; World Health Organization, 2014) and related, among others, to AM use in livestock (ECDC, EFSA, EMA, 2015) and especially in pig medicine. To tackle this threat, the Global action plan promotes the optimization of the use of AM in human and animal health (World Health Organization, 2015). This requires both pig farmers, who are in charge of their pig health management, and veterinarians who prescribe AM, to be actively involved.

So far, little is known about the factors that motivate and limit farmers' and veterinarians' intentions and behaviours in reducing AM usage in veterinary medicine. Recent research conducted on farmers' perceptions of AM usage in pig husbandry showed that there is little concern about potential harmful effects of on-farm antimicrobial use (Moreno, 2014; Visschers et al., 2015), while veterinarians seem to have higher awareness of the problem (Busani et al., 2004; Marvin et al., 2010). Yet, veterinarians were shown to be influenced by several

---

* Lucie Collineau, SAFOSO AG, Waldeggstrasse 1, CH 3097 Bern Liebefeld, Switzerland. Email: lucie.collineau@safoso.ch

determinants, e.g. risk avoidance or shortcomings in advisory skills when prescribing AM (Speksnijder et al., 2015).

The objective of this study was to identify the demographic and perception factors associated with the intention to reduce AM usage among pig farmers and reduction behaviours among veterinarians.

Following the theory of planned behaviour (TPB), farmers' intentions and veterinarians' self-reported behaviours regarding reducing AM usage were our dependent variables (Ajzen, 1991) and independent variables were grouped into three TPB components. First, the effects of farmers' and veterinarians' perceived risks of, benefits of and need for AM in pig farming, which relate to the attitude component in the TPB. Second, farmers' and veterinarians' beliefs about farmers' abilities to reduce AM usage and about the effects of AM reduction were explored. Beliefs about farmers' abilities relate to the perceived behavioural control component in the TPB and to the concept of self-efficacy (i.e. farmers' perception of their abilities or power to reduce AM usage; Bandura, 1977). Third, the effect of the relationship between farmers and veterinarians as well as veterinarians' experienced pressure to prescribe AM; these independent variables were used as a proxy for the subjective norms component in the TPB, i.e. beliefs about how significant others want the individual to behave (Ajzen, 1991).

MATERIALS AND METHODS

Procedure

Farmer survey: A survey was conducted between November 2013 and August 2014 among pig farmers using paper-and-pencil questionnaire in Denmark, France, Germany, Sweden and Switzerland, and an online survey in Belgium. In France, Denmark, Sweden and Switzerland, pig farmers' addresses were randomly sampled from national pig farmers' databases. Belgian farmers were recruited from a subscription list to a digital newsletter regularly issued by the University of Ghent. In Germany, a consultancy company contacted a random sample of pig farmers from their database by telephone; those who agreed to participate received an anonymous questionnaire. Inclusion criterion was the presence of more than 30 sows or 100 fattening pigs at the farm (when this information was available), and farmers of all farm types (e.g. farrow-to-finish, fattening and breeding farms) were recruited. Reminders were sent after four weeks to French farmers who had not returned the questionnaire, and to all Belgian and Swedish farmers, as it was impossible in the latter two countries to determine who had participated. A reminder could not be sent in Germany due to financial constraints. In Denmark and Switzerland, a reminder was not needed as more than 250 farmers had already responded after the first mailing.

Veterinarian survey: An online survey was developed and distributed to veterinarians with reported pig practice in all six countries. They were contacted through veterinarian organisations in Germany and Switzerland and through mailing lists from participating institutes in Belgium, Denmark and France. In Sweden, contacts from all veterinarians who had reported treatments on pigs (including treatments on pigs as companion animals) were obtained from the Board of Agriculture; 75 veterinarians received the questionnaire on paper since their e-mail address was unavailable. Random sampling was not feasible because the numbers of registered veterinarians were too low. A reminder to participate was sent to all members of the used mailing lists after two weeks.

Questionnaires

Farmer and veterinarian questionnaires were developed in collaboration within the MINAPIG Consortium (www.minapig.eu) using input from semi-structured interviews with 14 pig farmers and six pig veterinarians located in Germany and Switzerland, and building on experience acquired in a previous survey among 281 pig farmers from Belgium, France, Germany, Sweden and Switzerland (Visschers et al., 2015). Participants were assured that their responses would be treated anonymously and only used for scientific research.

Farmer questionnaire: Items of the 7-page paper-and-pencil questionnaire for the farmers were grouped into nine constructs. First, farmers had to estimate the amount of AM used on their farm in comparison to other pig farms in their country (Self-reported AM usage). This was assessed on a 5-point scale (from 1 = much less to 3 = about the same, and 5 = much more). Then, respondents were asked whether they had experienced five incidents related to AM resistance (Experience AM resistance), e.g. the need to increase AM doses because the doses used in the past were no longer effective.

The items of the following constructs were assessed on 6-point Likert scales, with higher values indicating stronger agreement with the item. The benefits of AM (Perceived benefits), e.g. the cost-efficiency of AM, and the level of risk that farmers associated with AM usage (Perceived risks), e.g. whether pig AM usage is dangerous for human health, were evaluated with four and two items, respectively. Three items described the need to use AM (Perceived need), e.g. the fact that only AM can control bacterial diseases in pigs. Farmers' intention to reduce AM usage on their farm (e.g. whether they were planning to reduce the number of AM treatments) and the Support from veterinarian (e.g. the farmer's trust in the veterinarian's advice) were both examined with four items. Two items explored the Efficacy of farmers in reducing AM usage (including the utility of and their abilities to reduce AM usage at their farm).

Farmers were also asked to estimate the percentage (between 0 and 100%) of the current amount of AM applied to their pigs that could be reduced in the next five years and would still be economically manageable. Demographic information was collected at the end of the questionnaire and included respondent's gender, year of birth, number of years working in pig farming, average numbers of pigs kept at the farm and the proportion of farmer's revenue coming from pig farming (i.e. farm specialisation).

Veterinarian questionnaire: Veterinarians were administrated a similar but shorter online questionnaire estimated to be able to be completed in 10 minutes. Eight constructs were explored with items answered on 6-point Likert scales, where higher values corresponded to higher agreement. Constructs focused on veterinarians' perceptions of AM benefits (Perceived benefits, three items), risks (Perceived risks, two items) and need (Perceived need, three items), using similar items as those asked to the farmers. Three items described the veterinarian Self-reported AM reduction behaviour, such as regularly advising farmers to use alternative measures to AM. Other constructs explored the perceived Feasibility of AM reduction (two items) i.e. the possibility to reduce AM usage assuming that farmers and veterinarians are willing to do it, and perceived Farmers' barriers (two items), i.e. their difficulties to change habits related to AM usage. Prescription pressure from farmers and Relation with farmers as perceived by veterinarians were both examined with two items.

Similarly to farmers, veterinarians were asked to estimate, using a slider between 0% and 100%, how much AM could be reduced in the pig sector in the next five years and still be economically manageable for farmers. Demographics data were also collected and included gender, year of birth, graduation year from veterinary school (i.e. experience), proportion of time devoted to pig medicine and types of additional courses and seminars (i.e. with different topics) attended after graduation.

Data analysis

The 6-point Likert scales were considered as quantitative continuous variables to allow further parametric statistical analyses. In each dataset, items were grouped into scales based on the results of a principle component analysis; only items that had a sufficient load on the predefined constructs (loads > |0.45|) and did not load on more than one factor were included. Scales were also checked for acceptable internal reliabilities (Cronbach's α >0.5). Then, the average of the respective items was calculated for each scale and respondent, and used in all further analyses. Statistical significance was set at $P < 0.05$.

In the farmer dataset, experience with AM resistance was recoded into a binary variable, with 0 = no experience with an AM resistance incident and 1 = experience with at least one of the listed AM resistance incidents. The specialisation variable was also recoded in a binary variable with 0 = receiving less than 50% and 1 = receiving more than 50% of revenue from pig farming. Similarly, in the veterinarian dataset, the time spent on pig practice variable was recoded with 0 = working less than 50% of the time on pig practice and 1 = more than 50% of the time.

An ANCOVA was applied separately to the farmer and veterinarian datasets, to explore whether the perceived potential for AM usage reduction in the next five years differed among farmers and veterinarians from different countries, including age, gender, total number of pigs (in the farmer dataset) and time spent in pig practice (in the veterinarian dataset) as covariates. A hierarchical linear regression analysis was then applied in each dataset to examine the factors associated with the farmers' reduction intentions and veterinarians' self-reported reduction behaviours. Independent variables were added in two steps: demographic variables, as well as Experience with AM resistance and Self-reported AM usage (in the farmer dataset) were included at step 1 and perception variables were added at step 2. Country of residence was included as five dummy variables with Denmark as reference category because its policies regarding AM usage were stricter compared to the other countries.

RESULTS

Samples

Farmers who reported no longer having pigs, and farmers and veterinarians with more than 75% missing responses were deleted from the respective datasets (n = 66 farmers and n = 7 veterinarians). After data cleaning, the sample of farmers contained 1,294 respondents, including 103 Belgian farmers (response rate: 25%), 262 Danish farmers (response rate: 44%), 175 French farmers (response rate: 23%), 114 German farmers, 392 Swedish farmers and 248 Swiss farmers (response rate: 40%). No response rate could be calculated in Germany or Sweden as the exact number of contacted farmers was not available. On average, the farmers were 49 years old (SD = 10), had 25 years of experience in pig farming (SD = 11) and most of them were specialised in pig production (66% of them received more than 50% of their income from pig farming, n = 836). Farmers from different countries differed in terms of age, years of experience, gender distributions, total number of pigs and specialisation. Because age and years of experience, as well as number of pigs and specialisation, were related, only age, gender and number of pigs were included as covariates in further analyses.

The veterinarian dataset included 334 persons: 50 from Belgium (response rate: 20%), 56 from Denmark (response rate: 50%), 68 from France (response rate: 57%), 54 from Germany (response rate: 21%), 65 from Sweden (response rate: 19%) and 41 from Switzerland (response rate: 39%). The veterinarians were on average 48 years old (SD = 11) with 21 years of experience (SD = 11) and 68% of them were specialised in pig medicine (devoting more than 50% of their time to pig practice, n = 228). Since graduation, they had followed on average 6.1 educational courses on different pig-related topics (SD = 2.3). The samples from the six countries again differed significantly in terms of gender distribution, age and time spent in pig practice, so that these variables were later included as covariates.

Farmers' and veterinarians' estimated AM reduction potential

Farmers estimated that, on average, 21.4% (SE = 0.59, median = 15) of the current amount of AM used on their farm could be reduced within the next five years and would still be economically manageable. Danish farmers estimated a significantly lower AM reduction potential than farmers from the other countries (Table 1a). French farmers reported a higher reduction potential compared to Danish, German, Swedish and Swiss farmers.

On average, veterinarians estimated that 28.1% (SE = 0.88, median = 22) of the current AM usage could be reduced in their country in the next five years and still be economically manageable for farmers. Danish veterinarians reported a significantly lower AM reduction potential than veterinarians from the other countries (Table 1b). Belgian and Swiss veterinarians estimated the highest AM reduction potential among the six countries.

Table 1. Mean percentage estimated AM reduction potential per country, among (a) farmers and (b) veterinarians

	a. Farmers			b. Veterinarians		
	Mean	SE	Median	Mean	SE	Median
Belgium	$25.48^{a,b}$	1.90	20	34.66^{a}	2.35	30
Denmark	12.15^{c}	1.24	10	15.34^{c}	2.19	10
France	29.60^{a}	1.44	20	24.17^{b}	2.06	20
Germany	21.83^{b}	1.77	20	$31.74^{a,b}$	2.20	28
Sweden	17.61^{b}	0.99	10	$29.17^{a,b}$	2.33	20
Switzerland	21.68^{b}	1.24	20	37.49^{a}	2.74	33
	F (5, 1233) = 20.00*** $\eta^2 = 0.08$			F (5, 311) = 12.22*** $\eta^2 = 0.16$		

SE: standard error of the mean. Within column means with different superscripts are significantly different ($P < 0.05$). ***$P < 0.001$. Adjusted means are presented after correcting for the covariates age, gender, total number of pigs (in the farmer dataset) and time spent in pig practice (in the veterinarian dataset).

Factors related to farmers' intention to reduce AM usage

At step 1, the hierarchical linear regression analysis on the intention to reduce AM usage in farmers revealed a significant model (F(11, 1203) =10.77, $P < 0.001$) that could explain 9% of the variance in the intention to reduce. The more AM resistance incidents farmers had experienced ($\beta = 0.14$, $P < 0.001$) and the higher their self-reported AM usage ($\beta = 0.16$, $P < 0.05$), the more they intended to reduce their AM usage. Intention to reduce was higher among

French, German and Swiss pig farmers compared with Danish pig farmers (β = 0.12, β = 0.09, and β = 0.09, respectively, $P < 0.05$). No other demographic variable was significantly associated with the farmers' intention to reduce AM usage at this step.

After including the perception variables at step 2, the model fit significantly improved, (Fchange(5, 1198) = 48.18, $P < 0.001$, ΔR2 = 0.15) and resulted in 24% of explained variance in the intention to reduce (Table 2a). Experience with AM resistance, self-reported AM usage and country (Sweden vs. Denmark) remained significantly related to the intention to reduce. Additionally, the farmers' perceived risks of AM, efficacy of farmers to reduce AM usage and the support from their veterinarians were significantly positively related to the intention to reduce. Perceived need for AM was significantly negatively related to the intention to reduce. The efficacy of farmers was the most important factor associated with the intention to reduce.

Factors related to veterinarians' self-reported reduction behaviour

At step 1, the demographic variables formed a significant model to predict the veterinarians' self-reported reduction behaviour (F(9, 310) = 5.86, $P < 0.001$) and explained 15% of the variance. The higher the reduction behaviour, the more types of additional courses veterinarians had followed (β = 0.28, $P < 0.001$). Being a veterinarian in France, Sweden or Switzerland was also associated with higher reduction behaviour (vs. in Denmark, β = 0.19, β = 0.21 and β = 0.16, respectively, $P < 0.05$). Self-reported reduction behaviour was lower when veterinarians spent more than 50% of their time in pig practice (vs. less than 50%, β = -0.19, $P < 0.001$).

The inclusion of the perception variables at step 2 significantly improved the model and its explained variance, Fchange(7, 303) = 6.42, $P < 0.001$, R2 = 0.26, ΔR2 = 0.11 (Table 2b). Two variables were still positively related to self-reported reduction behaviours: having followed more types of additional courses and being a veterinarian in France or Switzerland (vs. Denmark). In addition, higher self-reported reduction behaviours were associated with a higher perceived feasibility of AM reduction, better perceived relationships with farmers and lower perceived need for AM in pig farming. The feasibility of reducing AM usage was the most important predictor of veterinarians' reported reduction behaviours.

Table 2. Results of the regression analyses on (a) intention to reduce AM usage among farmers and (b) self-reported reduction behaviour among veterinarians

a. Farmers' intention to reduce AM usage				b. Veterinarians' self-reported reduction behaviour			
	B	SE	β		B	SE	β
Constant	1.53	0.34	***	Constant	3.20	0.49	***
Belgium[a]	0.18	0.14	0.04	Belgium[a]	0.19	0.16	0.08
France[a]	0.12	0.12	0.03	France[a]	0.34	0.15	0.17*
Germany[a]	0.19	0.13	0.04	Germany[a]	0.35	0.15	0.16*
Sweden[a]	-0.47	0.11	-0.17 ***	Sweden[a]	0.27	0.18	0.13
Switzerland[a]	0.01	0.11	0.00	Switzerland[a]	0.45	0.19	0.18*
Gender (1 = female)	0.13	0.10	0.04	Gender (1 = female)	0.15	0.10	0.09
Age	0.00	0.00	-0.01	Age	0.00	0.00	0.01
Total number of pigs	0.00	0.00	0.06	> 50% in pig practice[d]	-0.29	0.12	-0.16*
> 50% of revenues[b]	0.00	0.07	0.00	Additional educational courses	0.07	0.02	0.20***
Self-reported AM usage	0.33	0.04	0.22 ***	Prescription pressure	0.00	0.03	-0.01
Experience AM resistance[c]	0.23	0.07	0.09 ***	Feasibility of AM reduction	0.21	0.05	0.25***
Perceived benefits	-0.04	0.03	-0.03	Perceived benefits	-0.03	0.04	-0.04
Perceived risks	0.13	0.03	0.14 ***	Perceived risks	0.01	0.04	0.01
Perceived need	-0.17	0.03	-0.15 ***	Perceived need	-0.08	0.03	-0.13*
Support from veterinarian	0.12	0.03	0.10 ***	Relation with farmers	0.16	0.05	0.17**
Efficacy of farmers	0.27	0.03	0.26 ***	Farmers' barriers	-0.01	0.04	-0.01
Model statistics	F(16, 1198) = 23.91, P < 0.001, R^2 = 0.24			Model statistics	F(16, 303) = 6.51, P < 0.001, R^2 = 0.26		

B: unstandardized regression coefficient, SE: standard error around B, β: standardized regression coefficient.

[a] Dummy variable with Denmark as reference category. [b] Dummy variable with < 50% of revenues from pig farming as reference category. [c] Dummy variable with no experience with AM resistance incidents as reference category. [d] Dummy variable with 0-50% of time spent in pig practice as reference category.

*P < 0.05, **P < 0.01, ***P < 0.001

DISCUSSION

To our knowledge, this is the first study exploring the factors influencing intention to reduce AM usage among pig farmers and reduction behaviours among veterinarians across countries and in a comprehensive and quantitative manner. Some of the factors were common to both stakeholders: farmers with higher intention to reduce and veterinarians reporting more reduction behaviours had stronger beliefs that AM reduction is possible, i.e. they perceived higher farmers' efficacy and higher feasibility of reducing AM usage. Hence, the stronger the beliefs that farmers have about the effectiveness of strategies to reduce AM usage and the more positive experiences they have with these strategies, the more efficacy they believe they have in reducing AM and the more willing they are to reduce (Ajzen, 1991, Bandura, 1977). Farmers with high intention to reduce and veterinarians with strong reduction behaviours also perceived lower need for AM usage in pig farming; this could indicate that they are more knowledgeable of alternative measures to prevent or control pig diseases. The quality of the relationship between veterinarians and their farmers clients, as well as the support perceived by the farmers from their veterinarian were also positively correlated with farmers' intention to reduce and AM usage reduction behaviours among veterinarians. This confirms that a positive collaborative relationship is critical to move towards reducing AM usage.

In addition, farmers with higher risk perception for AM usage and farmers who had experienced previous incidents related to AM resistance had higher intention to reduce their AM usage. This reinforces the importance of increased farmer-awareness about possible public and animal health consequences of using AM to further promote the reduction of AM usage. Higher intention to reduce was also observed among farmers who self-reported higher AM usage at their farm. Informing farmers about the amount of AM used at the farm may thus increase farmers' intentions to reduce. This could be achieved using benchmarking systems, as implemented in Denmark since 2010 (Jensen et al., 2014) and more recently in the Netherlands, Belgium and Germany.

Swedish and Danish farmers reported a lower intention to reduce AM usage and lower efficacy over this behaviour than farmers from the other countries. It may be related to policies around AM usage that have been stricter and implemented for a longer time in these countries. Sweden was the first European country to ban the use of AM as growth promoters in 1986 (Wierup, 2001), and Denmark implemented in 2010 a "yellow card scheme" where AM usage in pigs is monitored at farm level and fines imposed on high AM users (Jensen et al., 2014). The overall lower AM usage in Denmark and Sweden (European Medicine Agency, 2015) could partly explain why farmers from these countries perceive little potential for further reductions. Also, Danish and Swedish veterinarians experienced less prescription pressure than veterinarians from the other countries. This may be related to the fact that in both countries, veterinarians are only allowed to prescribe AM but not to sell them.

Veterinarians were more optimistic than pig farmers about the potential AM usage reduction that could be achieved in pig farming, and believed this to be highly feasible. This could partly be explained by the different perspectives obtained from these stakeholders, as farmers were asked to evaluate their own farm, whereas veterinarians may have thought about the average pig farmer in their clientele or country. Farmers and veterinarians also have different roles in reducing AM usage. Veterinarians are the gatekeepers; they have to prescribe AM prudently and advise farmers about alternative measures that prevent and cure diseases. Farmers are the executors of these measures. They ultimately decide whether or not they stop a treatment or apply alternative measures, e.g. vaccination. They are also the ones who will directly experience the consequences of these measures, financially or in terms of time and effort. It was shown previously that their decision to follow their veterinarians' advice largely depend on the perceived costs and effectiveness of these measures (Alarcon et al., 2014, Garforth, 2015).

A maximum of 26% variance was explained by our models, which indicates that other factors not investigated here should also be considered to explain farmers' intention to reduce and veterinarians' reduction behaviour. These factors may include, for example, the epidemiological situation of major infectious diseases (e.g. occurrence of Porcine Reproductive and Respiratory Syndrome in the farm's area), the herd health status and the farm's financial situation. Also, intention to perform a certain behaviour does not necessary predict the actual behaviour (Sheeran, 2002, Webb & Sheeran, 2006). Similarly, self-reported behaviours have been found to be positively, but not perfectly related to actual behaviours (Kormos & Gifford, 2014). Therefore, the extent to which farmers' intention to reduce and veterinarians' reported behaviour predict their actual reduction of AM usage should be explored further. Another study limitation relates to the selection of participating farmers, who may have been more interested in the topic than farmers who did not participate. Consequently, participants may have been more positive regarding AM reduction (e.g. reporting higher intention to reduce and lower perceived need for AM) than the national populations of pig farmers and veterinarians.

To conclude, interventions that aim at stimulating prudent AM usage should aim to increase veterinarians' perceived feasibility and the efficacy of farmers to reduce their usage as these two factors had a central role in our findings. Farmers should, for example, be informed about measures they can take to reduce the need for AM on their farms, how they can implement them and how cost-effective they are (see also Postma et al., 2015). Additionally, farmers should be made aware of the level of AM usage on their farms via, for example, national herd-level AM usage monitoring programmes or benchmarking systems. Veterinarians strongly believed that it is feasible to reduce AM usage. However, this requires strong collaboration between veterinarians and farmers (Garforth, 2015, Speksnijder et al., 2015). Therefore, veterinarians need to have good supportive skills and communication competencies to assess each farmer's needs and provide them with the necessary skills and knowledge for more prudent AM usage (Speksnijder et al., 2015). Strategic action plans against AM resistance in the food chain could benefit from our findings while they are being developed and implemented by governments and industries across Europe and elsewhere.

ACKNOWLEDGEMENTS

Many thanks to the farmers and veterinarians who contributed to the study. This study was part of the European MINAPIG project (Evaluation of alternative strategies for raising pigs with minimal antimicrobial usage: Opportunities and constraints) funded by the ERA-NET programme EMIDA, and by the participating national funding agencies.

REFERENCES

Ajzen, I. (1991). The theory of planned behavior. Organ. Behav. Hum. Decis. Process. 50, 179-211

Alarcon, P., Wieland, B., Mateus, A.L.P. and Dewberry, C. (2014). Pig farmers' perceptions, attitudes, influences and management of information in the decision-making process for disease control. Prev. Vet. Med. 116, 223-242

Bandura, A. (1977). Self-efficacy: Toward a unifying theory of behavioral change. Psychol. Rev. 84, 191-215

Busani, L., Graziani, C., Binkin, N., Franco, A., Di Egidio, A. and Battisti, A. (2004). Survey of the knowledge, attitudes and practice of Italian beef and dairy cattle veterinarians concerning the use of antibiotics. Vet. Rec. 155, 733-738

European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Medicines Agency (EMA). (2015). First joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. EFSA Journal 13, 4006-4119. http://www.efsa.europa.eu/fr/efsajournal/pub/4006

European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption. (2015). Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013. EMA/387934/2015

Garforth, C. (2015). Livestock keepers' reasons for doing and not doing things which governments, vets and scientists would like them to do. Zoonoses Public Health 62, 29-38

Jensen, V.F., de Knegt L.V., Andersen V.D., Wingstrand A. (2014). Temporal relationship between decrease in antimicrobial prescription for Danish pigs and the “Yellow Card” legal intervention directed at reduction of antimicrobial use. Prev. Vet. Med. 117, 554-564

Kormos, C. and Gifford, R. (2014). The validity of self-report measures of proenvironmental behavior: A meta-analytic review. J. Environ. Psychol. 40, 359-371

Marvin, D.M., Dewey, C.E., Rajić, A., Poljak, Z. and Young, B. (2010). Knowledge of zoonoses among those affiliated with the Ontario swine industry: A questionnaire administered to selected producers, allied personnel, and veterinarians. Foodborne Pathog. Dis. 7, 159-166

Moreno, M. A. (2014). Opinions of Spanish pig producers on the role, the level and the risk to public health of antimicrobial use in pigs. Res. Vet. Sci. 97, 26-31

Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Grosse Beilage, E., Stärk, K.D.C. and Dewulf, J. (2016). The biosecurity status and its associations with production and management characteristics in farrow-to-finish pig herds. Animal, in press

Sheeran, P. (2002). Intention-behavior relations: A conceptual and empirical review. Eur. Rev. Soc. Psychol. 12, 1-36

Speksnijder, D.C., Jaarsma, A.D.C., van der Gugten, A.C., Verheij, T.J.M. and Wagenaar, J.A. (2015). Determinants associated with veterinary antimicrobial prescribing in farm animals in the Netherlands: A qualitative study. Zoonoses Public Health 62, 39-51

Vaarten, J. (2012). Clinical impact of antimicrobial resistance in animals. Rev. Sci. Tech. Int. Off. Epizoot. 31, 221–229

Visschers, V.H.M., Backhans, A., Collineau, L., Iten, D., Loesken, S., Postma, M., Belloc, C., Dewulf, J., Emanuelson, U., grosse Beilage, E., Siegrist, M., Sjölund, M. and Stärk, K.D.C.

(2015). Perceptions of antimicrobial usage, antimicrobial resistance and policy measures to reduce antimicrobial usage in convenient samples of Belgian, French, German, Swedish and Swiss pig farmers. Prev. Vet. Med. 119, 10-20

Webb, T.L. and Sheeran, P. (2006). Does changing behavioral intentions engender behavior change? A meta-analysis of the experimental evidence. Psychol Bull. 132, 249-268

Wierup, M. (2001). The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microb. Drug Resist. 7, 183–190

World Health Organization. (2014). Antimicrobial resistance: Global report on surveillance. Geneva: World Health Organization

World Health Organization. (2015). Global Action Plan on Antimicrobial Resistance. Geneva: World Health Organization

EXPLORING EXPERT OPINION ON THE PRACTICALITY AND EFFECTIVENESS OF BIOSECURITY MEASURES ON DAIRY FARMS USING BEST WORST CASE SCENARIO CHOICE ANALYSIS

O.K. SHORTALL[*] AND J. KALER

SUMMARY

Biosecurity – the exclusion and containment of disease causing pathogens – is important to the financial viability of the dairy sector as well as animal welfare. This study assesses expert views on the practicality and effectiveness of biosecurity measures on farms. It is the first study to apply the BWS method to this subject, to consult farmers as experts and to include practicality alongside effectiveness. In this study, keeping a closed herd and preventing nose-to-nose contact with neighbouring cattle were rated as the most effective measures, but were also among the least practical, suggesting more work needs to be done to address practicality constraints facing individual farmers. Scores for other measures, such as those related to isolating animals followed similar patterns. Maintaining regular contact with the vet was rated as the most practical measure, suggesting there is more scope for vets to help address farmers' individual biosecurity constraints.

INTRODUCTION

Biosecurity is the exclusion of disease causing agents from an area which one is trying to protect (Dargatz et al., 2002). Biosecurity is seen as very important to the dairy sector in the UK in order to ensure economic viability and improve animal welfare (Defra et al., 2004). However, there is little consensus about which biosecurity measures are seen to be the most effective at keeping disease out (Valeeva et al., 2011). The effectiveness of measures has been shown to be important to farmers: farmers do not want to take time and effort to implement measures which will not bring about identifiable benefits (Garforth et al., 2013). In addition, the practicality of measures is important to farmers: they can only carry out measures within their time and financial resource constraints (Kristensen and Jakobsen, 2011). There is a lack of consistency in biosecurity recommendations given to farmers (Moore et al., 2008) and studies have shown a lack of consistency in vets' beliefs about the effectiveness of disease control measures (Higgins et al., 2014). Thus more research is needed on the effectiveness and practicality of biosecurity measures. This paper reports the findings of a study aiming to address this gap, which elicited expert opinion on the practicality and effectiveness of

---

[*] Orla Shortall, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, LE12 5RD, UK. Email: orla.shortall@nottingham.ac.uk

biosecurity measures from expert vets, farmers, industry and government representatives, consultants and academics. The study is the first elicit expert opinion on both the practicality and effectiveness of biosecurity measures and to include farmers as an expert group.

Eliciting expert opinion is an important and well established method to use in relation to complex problems, where there is a lack of knowledge and a need for action (Martin et al., 2012). Two expert studies have been previously carried out assessing the effectiveness of different biosecurity measures in the dairy sector, asking experts to attribute risk reduction factors to different measures (Van Winden et al., 2005), and using a Likert scale to assess the importance of different measures (Sayers et al., 2014).

Likert scales and rating scales can involve "scale equivalence" where different people may interpret the scales differently (Adamsen et al., 2013) and scales do not always allow adequate discrimination between items (Louviere et al., 2013). There is also "acquiescence bias" which means people are more likely to respond positively to questions (Whitty et al., 2014). These deficiencies could be overcome through a method called best worst case scaling (BWS) (Finn & Louviere, 1992). BWS is a method of eliciting opinion which asks the respondent to choose their most and least preferred option according to a particular attribute (e.g. best and worst, or most practical and least practical) item from a set of items, usually four or five (Louviere et al., 2013). The rationale behind the method is that it avoids scale equivalence and low discrimination between items by asking respondents to consistently choose between items (Cohen & Orme, 2004). This method has been used in the agricultural sector to assess practicality and effectiveness. Cross et al. (2012) use the method to gather expert opinion on the effectiveness, and farmers' opinions on the practicality of measures to control E. coli O157 on cattle farms. Jones et al. (2013) use the method to assess the practicality and effectiveness of measures to mitigate greenhouse gas emissions on sheep farms.

The aim of this study was to use BWS to elicit views on both the effectiveness and practicality of biosecurity measures on dairy farms from expert groups of expert vets, farmers, academics, consultants, industry and government representatives.

MATERIALS AND METHODS

Expert opinion was gathered from expert vets, farmers, industry and government representatives, consultants and academics. Expert vets were selected through Royal College of Veterinary Surgeons lists of vets with the relevant qualifications. Expert farmers were recruited via industry recommendations and those fulfilling selection criteria. Other experts from academia, consultancy, government and industry were identified through a series of consultations and generation of a list that included key people having relevant expertise. All the experts were contacted by email and telephone and sent an online copy of the survey.

A list of 30 biosecurity measures to be used in the survey was developed, from a longer list of 72 measures initially identified from literature on biosecurity, through a series of consultations with a small group of experts on dairy herd health and biosecurity. The survey was piloted with 8 veterinary experts at the University of Nottingham School of Veterinary Medicine and Science and changes were made based on their suggestions. The list of 30 biosecurity measures used in the survey can be seen in Table 1. The survey consisted of 25 questions on practicality and 25 questions on effectiveness, with a choice between 5 items in each question. Respondents were asked to pick the most practical and least practical, or most effective and least effective measure out of a list of 5 biosecurity measures. Respondents were also asked questions on basic demographic information.

Table 1. Biosecurity measures used in the study

Item	Biosecurity measure
1	Monitor and record the herd health status through regular disease testing.
2	Establish a herd health protocol e.g. through using a herd health plan.
3	Maintain regular contact with your vet and discuss disease prevention and herd health status.
4	Isolate sick animals and carry out testing to monitor their disease status.
5	Vaccinate to control diseases already on the farm.
6	Implement rapid culling of persistently infected animals where it is appropriate for the disease.
7	Maintain a closed herd.
8	Verify the disease status of bought in animals by consulting with the seller farmer's vet.
9	Verify the disease status of bought in animals by asking the seller farmer.
10	Verify the disease status of bought in animals by viewing accredited test results.
11	Isolate new animals after purchasing for a minimum of three weeks.
12	Test new animals bought onto the farm to assess their disease status.
13	Avoid hiring bulls.
14	Prevent nose-to-nose contact with neighbouring animals by maintaining adequate fencing.
15	Do not graze animals on grass spread with slurry for at least 6 weeks.
16	Do not spread fields with imported slurry.
17	Prevent cattle having access to common waterways.
18	Isolate and test young stock re-entering the farm after being housed off-site.
19	Isolate animals returning from livestock shows.
20	Prevent wildlife accessing housing sheds and food supplies.
21	Maintain a pest control regime.
22	Minimise the number of visitors entering the farm.
23	Ensure that visitors' shoes, clothing and vehicles are clean when entering the farm.
24	Have a separate area on the farm for incoming vehicles and stock.
25	Avoid equipment sharing between farms.
26	Disinfect borrowed vehicles and equipment before and after use.
27	Ensure contractors use clean equipment on the farm.
28	Maintain good hygiene in the housing, yard, parlour and other farm areas.
29	Wash hands and disinfect clothing when going between herds on the farm.
30	Implement a programme of preventive vaccination for diseases not already on the farm.

The data were analysed within the framework Random Utility Theory (RUT) (Thurstone, 1927), which underlies most choice modelling. RUT assumes that a person's preference for item A over item B can be estimated based on how often they choose item A over item B. Effectiveness and practicality scores were calculated using Hierarchical Bayes (HB) analysis in the Sawtooth Software SSI Web 8.3.13 (Sawtooth Software Inc., Urem, UT) applying a multinomial logit model (MNL) (Louviere et al., 2013). The BWS method produces a fit statistic which assesses the consistency of each respondent's answers. This fit statistic is calculated based on the number of items per set and the number of times each item included in the survey. The fit statistic for all respondents in this survey exceeded the minimum suggested fit statistic (Jones et al., 2013).

RESULTS

In total the survey was sent to 86 expert vets. Of these, 28 completed the survey, giving a response rate of 33%. The survey was sent to 34 “other” experts, 16 of whom completed it, giving a response rate of 47%. A total of 62 expert farmers were contacted to take part in the study, 16 of whom completed the survey, giving a response rate of 26%.

Of the 16 farmers who completed the survey, 1 was based in Scotland, 1 in Wales, 4 in Central England, 4 in South East England, 2 in South West England, 1 in North East England and 3 in North West England. Eleven of the farmers classified their buying-in practices as closed and 5 classified their herd as open with biosecurity measures. Of the 28 expert vets who completed the survey, 2 were from Scotland, 2 from Wales, 2 from North East England, 8 from North West England, 2 from Central England, 2 from South East England and 10 from South West England. The median number of years spent practicing as a farm animal vet was 19 years with an interquartile range of 13-30 years. The median of the amount of time expert vets spent working time with dairy clients was 85%, with an interquartile range of 79-90%. Within the “other” expert group, 7 respondents filled in their occupational sector as industry, 3 as academia, 4 as government, 5 as consultancy and 1 as other, specified as dairy farming.

Figure 1 shows the practicality and effectiveness scores for each measure. The number to the right of each point on the graph indicates the number of the measure, which is shown in Table 1. The practicality and effectiveness scores were normalised so that the average score was 0 and the standard deviation was 1. Measures which scored above average for practicality appear to the right hand side of the Y axis and measures which scored above average for effectiveness appear above the X axis. Measures in the upper right quadrant are those which scored above average for both effectiveness and practicality, and measures in the lower left quadrant scored below average for both practicality and effectiveness. The most effective measure was measure 7, maintain a closed herd; followed by measure 14, prevent nose to nose contact with neighbouring animals by maintaining adequate fencing; measure 12, test new animals bought onto the farm to assess their disease status; and measure 6, implement rapid culling of persistently infected animals where it is appropriate for the disease. The most practical measure was measure 3, maintain regular contact with the veterinarian; followed by measure 16, do not spread fields with imported slurry; measure 13, avoid hiring bulls; and measure 2, establish a herd health protocol e.g. through using a herd health plan. The two highest scoring measures for effectiveness: measures 7 and 14 scored below average for practicality. Measures 11 and 18, isolate new animals for a minimum of 3 weeks and isolate animals returning to the farm after being housed elsewhere, respectively, also scored above average for effectiveness but below average for practicality. Nine measures scored above average for both practicality and effectiveness, including the most practical measure: measure 3 maintain regular contact with the veterinarian and discuss disease prevention and herd health status. Measures 20-29, relating to pest control, visitors, equipment and hygiene, all scored near or below average for effectiveness. Most also scored below or near average for practicality, with the exception of measure 28, maintain good hygiene on the farm which scored above average for practicality.

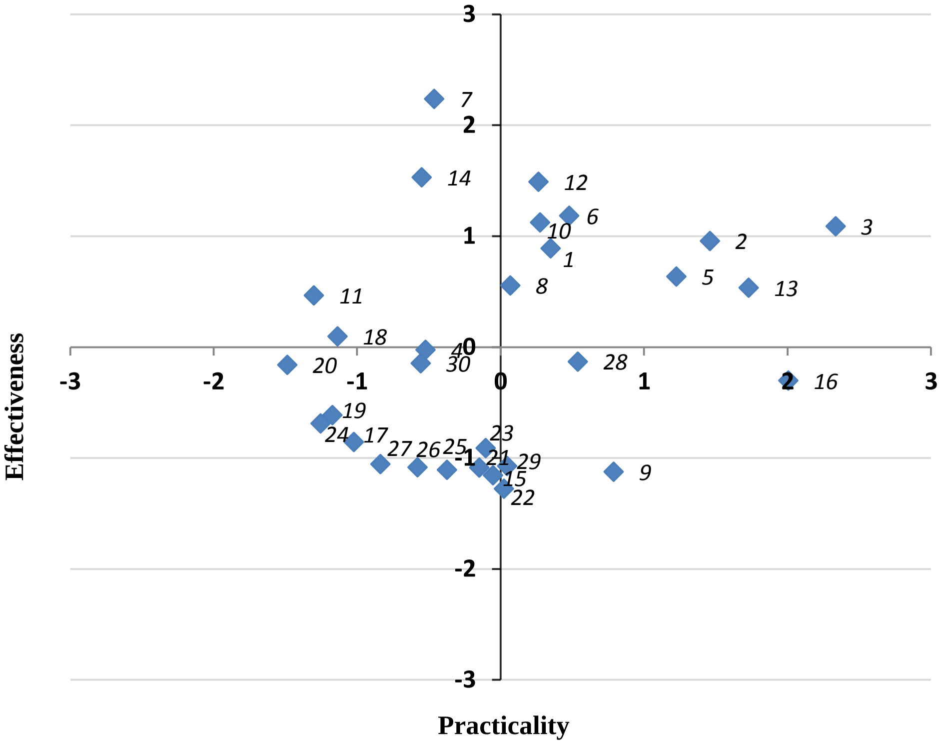


Fig.1 Zero centred scatter plot of effectiveness and practicality scores

DISCUSSION

The purpose of this study was to gather expert opinion on the relative practicality and effectiveness of different biosecurity measures. The findings of this study agree with previous studies on the effectiveness of biosecurity measures. Experts in this study rated keeping a closed herd as the most effective measure, which accords with the judgment of veterinary experts in the study by Sayer et al. (2014) who rated keeping a closed herd as the most important biosecurity measure, and experts in the study by van Winden et al. (2005) who rated keeping a closed herd as leading to the greatest disease risk reduction. Review studies on biosecurity have also stressed the importance of keeping a closed herd (Mee et al., 2012; Cooke & Brownlow, 2008). The measure rated as second most effective, measure 14, prevent nose-to-nose contact between neighbouring animals through maintaining adequate fencing was also rated highly by experts in the studies by van Winden et al. (2005) and Sayers et al. (2014). Gates et al. (2013) state that nose-to-nose contact is particularly important for dairy farms as they tend to fairly densely clustered.

These studies did not assess the practicality of these measures; however, in the current study these measures were rated below average for practicality. It is recognised that it is difficult for farmers to maintain a closed herd, and it likely to remain this way as farmers aim to expand production or need to replace stock from culling due to diseases such as bovine tuberculosis (Sibley, 2010). Similarly Sibley (2010) states that maintaining double fencing is an expensive and time consuming measure for farmers to take.

The finding that measure 3, maintain regular contact with the veterinarian, was rated as the most practical measure and the sixth most effective measure agrees with an extensive literature on the important of the vet-farmer relationship in ensuring effective biosecurity (Defra et al., 2004; Lowe, 2009; Orpin & Sibley, 2014). There have also been many suggestions in the literature that vets need to strengthen their role as herd health disease prevention consultants however, rather than their role in treating sick animals (Lowe, 2009; Enticott et al., 2011; Pritchard et al., 2015). Thus, while it may be seen as a practical measure, others have argued that there is work to be done in veterinary education and provision of veterinary services to improve the effectiveness and uptake of disease prevention services vets offer (Lowe, 2009). In addition, it has been pointed out that this measure on its own does not prevent disease but rather it needs to be followed up with action by vets and farmers (Atkinson, 2010).

Measures 20-29, which related to hygiene, pests, buildings, equipment and visitors mostly scored below average for practicality and effectiveness. This accords with the findings of van Winden et al. (2005) and Sayers et al. (2014) where experts rated the risk from visitors as relatively low and the risk from shared equipment as relatively low (van Winden et al., 2005). Mee et al. (2012) state that there are few studies published on the impact of visitors on farm biosecurity.

The interpretation of results is limited by the fact that the wording of the measures is by necessity open to interpretation. Respondents could interpret the measures in different ways: for instance people could have a different understanding of what counts as “adequate fencing” referred to in measure 14, which could have an impact on how they rate the practicality and effectiveness of this measure. The results of this study also provide relative, rather than absolute, scores for the practicality and effectiveness of biosecurity measures. This study explored expert views on the practicality and effectiveness of biosecurity measures on farms. It is the first study to use the BWS method on this subject, to consult farmers as experts and to include practicality alongside effectiveness. According to the findings of the current study keeping a closed herd and preventing nose-to-nose contact with neighbouring cattle were rated as the most effective measures, but were also among the least practical, suggesting more work needs to be done to address practicality constraints facing individual farmers, and to look for alternatives. Maintaining regular contact with the vet was rated as the most practical measure, suggesting there is more scope for vets to help address farmers’ individual biosecurity constraints.

ACKNOWLEDGEMENTS

We would like to acknowledge the funders of this work AHDB Dairy, University of Nottingham and CEVM. We would like to thank AHDB Dairy for their help in expert recruitment. We would like to thank Nottingham Dairy Herd Health Group for their help and useful discussions during the pilot testing phase. We would also like to thank all the respondents for giving up their time to take part in the study.

REFERENCES

Adamsen, J.M., Rundle-thiele, S. and Whitty, J.A. (2013). Best-worst scaling... Reflections on presentation, analysis, and lessons learnt from case 3 BWS experiments. Mark. Soc. Res. 21, 1–54

Atkinson, O. (2010). The role of the vet in knowledge transfer in the dairy industry. Trehane Trust, Taunton. 35p

Brennan, M.L. and Christley, R.M. (2012). Biosecurity on cattle farms: A study in north-west England. PLoS One 7, e28139

Cohen, S. and Orme, B. (2004). What's your preference? Mark. Res. 16, 32–37

Cooke, R. and Brownlow, A. (2008). Appendix 1: Biosecurity review. Defra, London

Cresswell, E., Brennan, M.L., Barkema, H.W. and Wapenaar, W. (2014). A questionnaire-based survey on the uptake and use of cattle vaccines in the UK. Vet. Rec. Open 1, e000042

Cross, P., Rigby, D. and Edwards-Jones, G. (2012). Eliciting expert opinion on the effectiveness and practicality of interventions in the farm and rural environment to reduce human exposure to Escherichia coli O157. Epidemiol. Infect. 140, 643–654

Dargatz, D. a., Garry, F.B. and Traub-Dargatz, J.L. (2002). An introduction to biosecurity of cattle operations. Vet. Clin. North Am. Food Anim. Pract. 18, 1–5

Defra, Scottish Executive and Welsh Assembly Government (2004). Animal Health and Welfare Strategy for Great Britain. Defra, London

Enticott, G., Donaldson, A., Lowe, P., Power, M., Proctor, A. and Wilkinson, K. (2011). The changing role of veterinary expertise in the food chain. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 366, 1955–1965

Finn, A., Louviere, J.J. (1992). Determining the Appropriate Response to Evidence of Public Concern: The Case of Food Safety. J. Public Policy Mark. 11, 12–25

Garforth, C.J. and Bailey, a. P., Tranter, R.B., (2013). Farmers' attitudes to disease risk management in England: A comparative analysis of sheep and pig farmers. Prev. Vet. Med. 110, 456–466

Gates, M.C., Woolhouse, M.E.J., Gunn, G.J. and Humphry, R.W. (2013). Relative associations of cattle movements, local spread, and biosecurity with bovine viral diarrhoea virus (BVDV) seropositivity in beef and dairy herds. Prev. Vet. Med. 112, 285–295

Higgins, H.M., Huxley, J.N., Wapenaar, W. and Green, M.J. (2014). Quantifying veterinarians' beliefs on disease control and exploring the effect of new evidence: A Bayesian approach. J. Dairy Sci. 97, 1–15

Jones, a. K., Jones, D.L., Edwards-Jones, G. and Cross, P. (2013). Informing decision making in agricultural greenhouse gas mitigation policy: A Best-Worst Scaling survey of expert and farmer opinion in the sheep industry. Environ. Sci. Policy 29, 46–56

Kristensen, E. and Jakobsen, E.B. (2011). Challenging the myth of the irrational dairy farmer; understanding decision-making related to herd health. N. Z. Vet. J. 59, 1–7

Louviere, J., Lings, I., Islam, T., Gudergan, S. and Flynn, T. (2013). An introduction to the application of (case 1) best-worst scaling in marketing research. Int. J. Res. Mark. 30, 292–303

Lowe, P., (2009). Unlocking potential: A report on veterinary expertise in food animal production. Vets and veterinary services steering group, London

Martin, T.G., Burgman, M. a., Fidler, F., Kuhnert, P.M., Low-Choy, S., Mcbride, M. and Mengersen, K. (2012). Eliciting Expert Knowledge in Conservation Science. Conserv. Biol. 26, 29–38.

Mee, J.F., Geraghty, T., O'Neill, R., More, S.J., (2012). Bioexclusion of diseases from dairy and beef farms: Risks of introducing infectious agents and risk reduction strategies. Vet. J. 194, 143–150

Moore, D. a, Merryman, M.M.L., Hartman, M.L. and Klingborg, D.J. (2008). Production Animal Species and Classes. J. Am. Vet. Med. Assoc. 233, 249–256

Orpin, P. and Sibley, D. (2014). Predict and prevent versus test and treat. Vet. Rec. 174, 403–406

Pritchard, K., Wapenaar, W. and Brennan, M.L. (2015). Cattle veterinarians' awareness and understanding of biosecurity. Vet. Rec. 176, 546-548

Sayers, R.G., Good, M. and Sayers, G.P. (2014). A survey of biosecurity-related practices, opinions and communications across dairy farm veterinarians and advisors. Vet. J. 200, 261–269

Sibley, R. (2010). Biosecurity in the dairy herd. In Pract. 32, 274–280

Thurstone, L.L. (1927). A law of comparative judgment. Psychol. Rev. 34, 273–286

Valeeva, N.I., van Asseldonk, M. a P.M. and Backus, G.B.C. (2011). Perceived risk and strategy efficacy as motivators of risk management strategy adoption to prevent animal diseases in pig farming. Prev. Vet. Med. 102, 284–295

Van Winden, S., Stevens, K., Guitian, J. and Mcgowan, M. (2005). Preliminary Findings of a Systematic Review and Expert Opinion Workshop on Biosecurity on Cattle Farms in the UK. Cattle Pract. 13, 135-140

Whitty, J. A., Walker, R., Golenko, X. and Ratcliffe, J. (2014). A think aloud study comparing the validity and acceptability of discrete choice and best worst scaling methods. PLoS One 9, e90635

ANTIMICROBIAL USAGE AND RESISTANCE

FARM-ECONOMIC ANALYSIS OF REDUCING ANTIMICROBIAL USE WHILST ADOPTING GOOD MANAGEMENT STRATEGIES ON FARROW-TO-FINISH PIG FARMS

C. ROJO-GIMENO*, M. POSTMA** J. DEWULF, H. HOGEVEEN, L. LAUWERS AND E. WAUTERS

SUMMARY

A quasi-experimental approach was used for assessing the farm-economic impact of substituting the use of antimicrobials by good management practices, namely biosecurity measures. To account for technological progress and to avoid selection bias, propensity score matching was used to estimate the difference-in-difference of the technical parameters between treatment farms (n=48) and control farms (n=69). The treated group consisted of Flemish farrow-to-finish pig farms that participated in a longitudinal study which aimed at enhancing general health management to reduce antimicrobial use. Results of the difference-in-difference of technical parameters, estimated costs of implemented measures, and price volatility of feed and meat were entered into a stochastic production economic model. Results showed that reducing antimicrobial use while improving general herd management did not only incur lower costs (median -€2.68/sow/year), but also the difference of enterprise profit after versus before the reduction of antimicrobial use increased by +€39.21/sow/year. These results can aid veterinarians to incite farmers to use biosecurity practices instead of antimicrobials.

INTRODUCTION

Pig farming employs high amounts of antimicrobials (European Medicines Agency, 2013; MARAN, 2014). A significant fraction of those are also administered in human medicine (Laxminarayan et al., 2013). Their extensive use in pig husbandry is linked to the selection and spread of resistant bacteria, which may be transferred interspecies through direct or indirect contact (Schwarz et al., 2001; Chantziaras et al., 2014). In 2012, Belgium was ranked 6th, out of 25 countries in the EU, in terms of the volume of sales of antimicrobials for food producing animals (European Medicines Agency, 2014), of which the majority are used in pig production (Filippitzi et al., 2014). Unfortunately, more recent reports of antimicrobial surveillance in Belgium have shown that, after a declining trend of the antimicrobial use in food production animals, their consumption again increased by 1.3% in 2014 with respect to 2013 (BelVet-SAC, 2014). This is despite the efforts of the Center of Expertise on Antimicrobial

---

* Cristina Rojo-Gimeno, Institute for Agriculture and Fisheries Research (ILVO), Social Sciences Unit, Burg. Van Gansberghelaan 115, box 2, Merelbeke, 9820, Belgium. Email: cristina.rojogimeno@ilvo.vlaanderen.be

** Shared first-authorship

Consumption and Resistance in Animals, whose guidelines are encouraged to be used by Belgian veterinarians to aid their judicious prescription of antimicrobial agents. These guidelines state, amongst others, that antibiotics cannot be used as substitutes for good hygiene, housing and appropriate feed. However, standard prophylactic antimicrobial treatments are often considered by farmers as an easier, cheaper and less labour-intensive way to prevent bad health conditions and to avoid repercussions on productivity and, indirectly, on the farm-financial situation, compared to therapeutic treatments (Callens et al., 2012), or investments in infrastructure or disinfection on the farm (Filippitzi et al., 2014).

Since the early 50's there are examples in literature which describe the relation between the use of antimicrobials and higher productivity. However, it was already acknowledged that the effect of the use of antimicrobials on productivity was lower when the farming conditions such as quality of the feed, hygienic conditions, level of ventilation in the barn were optimized (Coates et al., 1951; Hill et al., 1953). Studies post 2000 demonstrate that the effect of antibiotics on productivity are lower than those from the early trials. Current production conditions in Europe, and most of the developed countries, have substantially improved in the last decades, consequently it is questionable whether the effect of antimicrobials on productivity will remain high (Rushton et al., 2015).

Adopting general herd management strategies, such as biosecurity practices or specific vaccinations, is seen as a more sustainable alternative to prophylactic antimicrobials (Postma et al., 2015). As higher levels of biosecurity are associated with improved average daily weight gain, feed conversion ratio and a decreased consumption of antibiotics (Laanen et al., 2013), a positive farm economic impact is an important factor for considering the adoption of biosecurity strategies (Visschers et al., 2015). Veterinarians feel the need to give information about the economic impact of advised interventions (Gunn et al., 2008), and farmers tend to link their willingness to adopt biosecurity practices to estimated costs (Fraser et al., 2010). Farmers have shown interest in knowing the costs of biosecurity measures, as well as their benefits entailed (Laanen et al., 2014). Lack of such insight still represents a constraint, while such detailed information could foster awareness. To date, few studies have evaluated the economics of biosecurity compared to antibiotics. Siekkinen et al. (2012) estimated the direct costs associated with the use of preventive strategies in poultry farms. Two cross-sectional studies, which also accounted for the indirect economic impact due to changes in technical parameters, found that farrow-to-finish pig farms which exhibited a higher biosecurity and health status were correlated with improved technical parameters and a higher economic margin than farms with lowest biosecurity status (Corrégé et al., 2011; 2012). The methodological weakness of these studies are the lack of a control group and their cross sectional nature, which may have caused an overestimation of the effect. This could be controlled with a longitudinal study.

This study aims to use a quasi-experimental longitudinal approach for assessing the farm economic impact of substituting the use of antimicrobial by good management practices, in particular biosecurity strategies. The research focusses on a set of farrow-to-finish pig farms that performed the substitution in which the economic impact is assessed by matching them with a set of control farms.

MATERIALS AND METHODS

The overall approach was a quasi-experimental design (Harris et al., 2006), in which treated farms were matched, using propensity scores (Dehejia & Wahba, 2002) with control farms.

The latter were selected from the Flemish Farm Accountancy Data Network (FADN), an instrument for evaluating the income of agricultural holdings and the impacts of the Common Agricultural Policy. The treated farms received tailored advice regarding biosecurity, general management, vaccination and antimicrobial use. Technical parameters of pig production were recorded before and after the advice was supplied and implemented. To account for the technological progress of pig production and to reduce selection bias, propensity score matching was used. Accounting for technological progress in treated farms resulted in a difference in differences (DID) which compared the difference between after and before the intervention in the technical parameters for the treated group with the difference between after and before the intervention in the technical parameters for the control group. DID is the treatment effect that is attributable to the improvement in biosecurity, general management practices, vaccination and the change in antimicrobials usage. This DID served as input data in a farm production-economic input-output model whereby differences in enterprise profit are calculated. Direct economic effects of improved biosecurity, increased vaccination and reduced antimicrobial use were determined using a cost accounting analysis based on interviews with farmers and various databases for prices and purchase costs, and also entered into the farm production-economic model. To account for the heterogeneity in the pig farming population, the economic input-output model was simulated for 11 virtual but representative farms, which were constructed out of the full FADN-sample of farrow-to-finish pig farms in Flanders for the years 2010, 2011 and 2012.

Treated farms data collection

Out of 65 Flemish pig farms participating in the 'reduction of antimicrobials project', 50 were farrow-to-finish pig farms which were retained for the economic evaluation study. The treated farms were visited on 3 occasions between December 2010 and May 2014. On average 8 months elapsed between the 1st and 2nd visit (mean=8.59, SD=6.50) and between the 2nd and 3rd visit (mean=8.20, SD=2.51). During the 1st visit, data on specific aspects of health management like the used vaccination scheme, characteristics of anthelmintic therapy, or performed diagnostic tests were collected. Furthermore, antimicrobial use and biosecurity status were obtained. The biosecurity status of the farms was assessed using Biocheck.UGent. This is a risk-based weighted scoring system expressing via an objective evaluation the biosecurity status of a pig herd with a value from 0 to 100, with taking into account both internal and external biosecurity (Laanen et al., 2013). Data on antimicrobial use was translated into a treatment incidence with the help of the ABcheck.UGent calculation system (Postma et al., 2014). Data on technical performance was obtained through face-to-face surveys with the farmers, implying two technical parameters from the farrowing stage, litter size (LS) and farrowing index (FI) and two technical parameters from the finishing stage, average daily weight gain (ADWG) and mortality of the finishers (MF).

Based on the 1st visit information, a tailored advice plan was developed and disseminated to the farmers on the 2nd visit. This plan consisted of measures to improve internal and external biosecurity, general management, vaccination schemes and to reduce the use of antimicrobials. A follow-up of compliance to the given advice was conducted during 3rd visit. During this last visit, similar data as in the first visit were collected.

Propensity score matching of the control farms

Pig husbandry is a production system with rapid technological changes (Vrints & Deuninck, 2014) and therefore it cannot be guaranteed that interventions implemented are the only reason behind the change in the technical parameters. Moreover, the quasi-experimental approach may suffer from selection bias because treated farms were not randomly selected from the whole population of farrow-to-finish pig farms. To circumvent this problem, a methodology was introduced, propensity score matching with DID estimation, which is, to the authors knowledge, novel in the field of animal health economics. Put simply, this technique searches for control farms in a database with an as equal as possible probability to be in the treatment group and matches each treated farm with such a control farm. The outcome of the propensity score matching is the DID which accounts for the difference between the after-before difference in the treated group versus the control group.

Data on 117 farrow-to-finish pig farms were obtained from the Flemish FADN dataset for 2011 and 2012, of which 86 farms had records on both 2011 and 2012. Those served to extract a control group with similar baseline characteristics to the treated group after computing a propensity score, whereby the conditional probability of being treated conditional on observed baseline covariates was calculated (Rosenbaum & Rubin, 1983; Austin, 2011). Baseline characteristics collected equally on treated and on control farms were selected to match: (i) number of sows, as a proxy of size, (ii) farmer's years of experience, as a proxy of the farmer's ability and skills as a manager, (iii) building year of the oldest building of the farm, as a proxy of the degree of modernization, (iv) number of employees, as a proxy for size and management skills. The implicit assumption behind is that variables reflecting the size, the ability and skills of the farmer and the level of modernization influences their willingness to participate in the research project.

The analysis was conducted with the R (R development Core Team 2013) package matching (http://CRAN.R-project.org/package=Matching) in which a genetic matching and a one-to-one nearest neighbour matching without replacing was used. The mean DID of the technical parameters ADWG, FI, LS, and MF, and its Abadie-Imbens standard error was estimated.

Direct net costs of the interventions

The direct net costs of the tailored advice were assessed using a cost accounting analysis. Prices on commodities (e.g. boots, gloves, disinfectant products, etc.) were gathered from an online web shop often used by Belgian farmers (http://www.agrologic.be). Veterinary costs, including the analysis of samples, were obtained from Animal Health Care Flanders. The time spent performing certain proposed intervention tasks (such as changing boots between departments) was gathered from literature, consultation of experts, assumptions and common sense. Some purchased commodities were durable inputs, this means that they can be used over a period of years on the farm, and incurred fixed costs (e.g. boots, boards, brooms). Therefore, depreciation was accounted for with a straight line method (Rushton, 2009a).

Vaccination prices were obtained through a questionnaire sent to 2 veterinarians active in pig veterinary medicine. Information on antibiotic products used on farms was obtained by asking the farmer for their prophylactic and curative treatments. Further, invoices of the herd veterinarian, and/or invoices from feed mills on purchase of antibiotic products over the year preceding the visit were used. The prices (in €/g or €/ml) of used antibiotic were multiplied by the amount of g or ml of antimicrobial used by the animals treated to calculate the costs of the antibiotics in the 1st and the 3rd visit.

Input-output stochastic production economic model

Besides the direct costs, due to changes in technical performance, the interventions may also have had indirect economic consequences. These indirect effects were taken into account with a production-economic input-output model operationalized in Excel (Van Meensel et al., 2010), of which gross margin is the main outcome. Gross margin is defined as the farm revenues from the output production (i.e., the sale of finishers and piglets) minus the variable costs attributable to it (i.e., the purchase of feed and other inputs; Rushton, 2009b) and it is described in Eq. (1).

$$\text{Gross margin (GM)} = \text{Revenues} - \text{Variable costs} \qquad (1)$$

Several adaptations were added to this previously developed simulation model. First, since the adoption of some strategies incurred fixed costs due to the purchase of durable inputs which incurred depreciation and extra labour, as an opportunity costs, gross margin was an inadequate economic indicator for the goal of this study. Therefore, the enterprise profit (Rushton, 2009b), which is defined as the gross margin minus the fixed costs, was a more suitable indicator (Eq. (2)).

$$\text{Enterprise profit} = \text{GM- Total Fixed Costs} \qquad (2)$$

Data on the total fixed costs incurred by the 11 representative farms was not available. However, it was assumed that the total fixed costs, excluding the fixed costs associated to the implemented interventions, were equal before and after the intervention. This allowed to estimate the difference in enterprise profit after versus before the interventions (ΔEnterprise profit $_{\text{after- before}}$; Eq. (3)).

$$\Delta\text{Enterprise profit}_{\text{after- before}} = \text{GM}_{\text{after}} - \text{Fixed Costs Intervention} - \text{GM}_{\text{before}} \qquad (3)$$

The simulation model was a Monte-Carlo-based stochastic model within @Risk 6.0 (Palisade Corporation, California). The latter consist of a computerized mathematical technique which allows building models of possible results by substituting a variable with inherent uncertainty by a probabilistic distribution. It then calculates results for a number of iterations from a different set of random values from the probability functions.

Two types of stochasticity were inserted into the model. The first type reflects price volatility of the input: feed for sows (€/kg), for piglets (€/kg), and finishers (€/kg) and output prices: finishing pigs (€/kg) and sold piglets (€/piglet). Data on the volatility of the feed prices was extracted from the Belgian national statistics (Statistics Belgium). Likewise, historical prices of finishing pigs and piglets were obtained from a Belgian feed company for the years 2010, 2011, and 2012 (Anonymous, 2010; 2011; 2012). The second type of stochasticity accounted for was the uncertainty regarding the treatment effect on the technical parameters, and regarding the direct net costs of the treatment.

The final model was run with 1,000 iterations for each of the 11 representative Flemish farrow-to-finish pig farms. The mean, standard deviation and 95% confidence interval of the ΔEnterprise profit $_{\text{after- before}}$ were estimated in €/sow/year.

RESULTS

Descriptive statistics

From 50 treated farms, 48 remained in business during the whole study period. Treated farms had on average more sows (301) than control farms (175). In total, 69 of the 86 control farms in which data were collected on 2011 and 2012 were kept for further analysis because

17 farms were removed because they did not have records on the covariate used to match 'building year of the oldest building'.

Technical parameters: At baseline level, treated farms showed a slightly higher farrowing index, litter size and mortality of the finishers than control farms (Table 1). After the second visit, treated farms showed an improved litter size and mortality of the finishers (Table 1).

Table 1. Summary statistics of the average daily weight gain (ADWG), farrowing index (FI), litter size (LS), and mortality of the finishers (MF) for the 48 treated farms in visit 2, visit 3 and the 69 control farms in 2011 and 2012

	Treated				Control			
	Visit 2	Visit 3	Difference		2011	2012	Difference	
	Mean (SD)	Mean (SD)	Mean (SD)	P-value[a]	Mean (SD)	Mean (SD)	Mean (SD)	P-value[a]
ADWG	641.17 (85.92)	668.54 (78.86)	27.37 (76.64)	0.09	641.81 (63.65)	637.85 (66.53)	-3.96 (58.24)	0.57
FI	2.39 (0.07)	2.38 (0.08)	-0.01 (0.06)	0.26	2.18 (0.24)	2.18 (0.26)	0.00 (0.18)	0.90
LS	13.05 (1.15)	13.41 (1.28)	0.35 (0.50)	<0.01	11.71 (1.31)	11.78 (1.31)	0.07 (0.66)	0.40
MF	3.46 (2.40)	2.59 (1.74)	-0.87 (1.79)	0.01	2.45 (1.40)	2.47 (1.38)	0.02 (1.13)	0.88

[a] p value estimated with paired t-student comparison of the means

Propensity score analysis

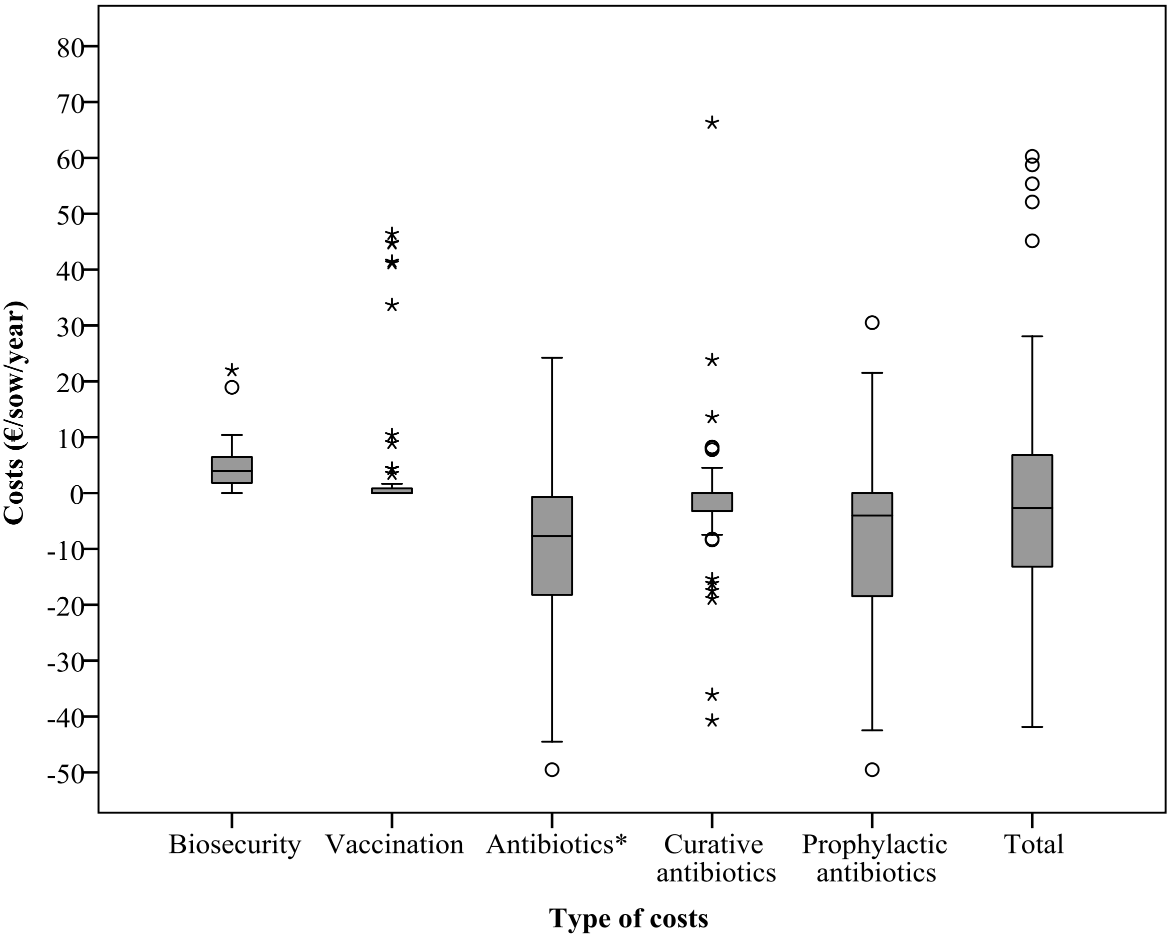
The mortality of the finishers was significantly lower on treated farms than on control farms after the intervention was implemented (mean -1.13%, SE: 0.02; Table 2).

Table 2. Summary statistics of technical parameters' difference in differences (DID) in % between visit 3 and visit 2 and between treated and control farms estimated with genetic propensity score matching

DID	Mean (SE)	P value
Average daily weight gain	5.88 (3.43)	0.09
Farrowing index	1.90 (2.10)	0.37
Litter size	0.90 (1.11)	0.40
Mortality of finishers	1.13 (0.02)	0.03

Direct net costs of the interventions

Figure 1 depicts the distribution of direct costs incurred by different adopted interventions. The median of the total direct net costs on the treated farms was reduced by -€2.68/sow/year between visits 2 and 3. The main reason was the reduction in antimicrobial use, especially on prophylactic treatments administered to piglets, leading to a median reduction in costs of -€7.68/sow/year. The latter were not exceeded by the median increase of the biosecurity costs (€3.96/sow/year) and vaccination costs (€0.00/sow/year).



*Antibiotics included the reduction in costs from curative and prophylactic antibiotic treatments

Fig. 1 Boxplot of the estimated change in direct costs (€/sow/year) incurred by the 48 treated farms between visit 2 and visit 3 as a result of the new implemented biosecurity strategies, new vaccinations, and change in antibiotic use for prophylactic and curative treatments

Enterprise profit

Without taking volatility of prices into account, farms presented on average +€107.47/sow/year higher difference of enterprise profit after intervention than before. When the price volatility was accounted for, the difference of the enterprise profit after versus before the antimicrobial use is lower than when volatility was not modelled, but it was still positive with +€39.21/sow/year (Table 3).

Table 3. Difference of the enterprise profit between after and before antimicrobials were reduced and general good management was implemented in €/sow/year for the simulation which did not account for volatility of the prices and the simulation which accounted for price volatility, simulated for 1,000 Monte Carlo iterations with an stochastic production input-output model for 11 Flemish farrow-to-finish pig farms

	ΔEnterprise profit $_{\text{after-before}}$ No volatility (€/sow/year)		ΔEnterprise profit $_{\text{after-before}}$ Volatility (€/sow/year)	
	Mean (SD)	95% CI	Mean (SD)	95% CI
Farm 1	153.44 (56.99)	38.00, 262.00	53.33 (61.10)	-76.13, 172.61
Farm 2	114.94 (46.83)	15.19, 200.58	35.52 (50.11)	-73.27, 126.75
Farm 3	62.38 (51.35)	-44.82, 164.41	38.15 (54.60)	-76.12, 140.25
Farm 4	98.21 (69.29)	-43.00, 237.00	69.49 (73.46)	-76.00, 212.00
Farm 5	74.91 (56.56)	-43.20, 187.26	44.05 (60.32)	-86.97, 157.71
Farm 6	108.23 (63.01)	-18.00, 233.00	66.92 (67.51)	-65.34, 196.31
Farm 7	96.18 (55.72)	-21.57, 201.94	38.97 (59.37)	-90.82, 146.23
Farm 8	217.53 (52.68)	108.06, 312.36	40.74 (58.41)	-79.69, 149.02
Farm 9	55.61 (42.78)	-38.06, 133.38	15.30 (45.06)	-85.94, 92.84
Farm 10	67.22 (40.57)	-23.80, 134.98	8.38 (41.92)	-94.31, 73.95
Farm 11	136.47 (45.97)	38.77, 217.30	20.47 (49.87)	-89.65, 109.76
Mean	107.74 (52.89)	-2.95, 207.66	39.21 (56.52)	-81.29, 143.40

DISCUSSION

In this study, the reduction of antimicrobial use, accompanied by improved biosecurity, optimized vaccination and general husbandry practices yielded a reduction in net direct costs between the 3rd and the 1st visit, which was mostly due to a reduction in the usage of prophylactic antibiotics for piglets. This implies that prophylactic treatments entail high costs. The additional costs incurred by the implementation of biosecurity strategies and the use of new vaccinations were lower than the eliminated costs associated with a reduction in antimicrobial use. Similar results were found by a recent randomized clinical trial which demonstrated that the efficacy of administering antimicrobial metaphylaxis in finishing pigs was limited to those with lowest starting weight, and even then the costs of the antimicrobials will surpass the benefits entailed due to improved productivity levels (Ramirez et al., 2015). The results of the current study suggested that, contrary to the farmers' overall perception (Moreno et al., 2014), antimicrobials were not necessarily cheaper than investing in adjusting management strategies of the farm.

The average Flemish farrow-to-finish pig farm exhibited better parameters in 2011 (ADWG=659.90 g/day, MF=3.30%, FI=2.20 farrowing/sow/year, LS=12.20 live born piglets/sow/year) and 2012 (ADGW=652.80 g/day, MF=2.90%, FI= 2.30 farrowing/sow/year, LS=12.40 living piglets/sow/year; Vrints & Deuninck, 2014) than the control farms, but worse than the treated farms (Table 1). This may have been caused by selection bias, in which participants, who are the forerunners in the reduction of antimicrobial usage, may have had higher production technical parameters and may have been more prone to participate in such a project. It was accounted for by computing a propensity score and the DID, which is intended to eliminate some of the selection bias, to estimate the attributable effect of the implemented interventions on the technical parameters. The results are in line with results of previous studies in which pig farms with higher biosecurity status were associated with better technical parameters (Corrégé et al., 2011; Laanen et al., 2013). To the authors' knowledge, the present study is one of the few in the field of animal health economics that conducted propensity score analysis. Whereas this statistical technique is extensively used in agricultural economics (e.g., Mendola, 2007), and is described for the use in veterinary epidemiology by Dohoo et al., (2009), only one article was found concerned with economics of animal health in which this methodology is performed to match a control group to a treated group (Key & Mcbride, 2014). In observational studies such as the present study, in which to conduct an experiment with random allocation of treatment is cumbersome, propensity score analysis demonstrated to be especially advantageous (LaLonde, 1986; Mendola et al., 2007).

At the time of the preparation of this manuscript, price evolutions were particularly adverse, with high prices for feed and low prices for finishers' meat, which negatively affected the net income of pig farmers. The situation has been more or less like this from 2007 till now (Anonymous, 2015). The results showed that the difference in enterprise profit was positive for both the model which accounted for volatility, which is more realistic, and for the model which did not account for volatility, which suggests that results are robust: even with volatile prices, the enterprise profit was +€2.45/finisher pig/year on average for the 11 representative farms (data not shown). Two cross-sectional studies in farrow-to-finish pig farms conducted in France found that farms which had the highest biosecurity status compared with those with the lowest were associated with an economic margin of €180/sow/year (Corrégé et al., 2011) and €200/sow/year (Corrégé et al., 2012). It was found that the difference of enterprise profit between after and before the intervention was of €39.21/sow/year which is smaller than the effect observed by the abovementioned studies. This may respond to their cross-sectional design and the lack of control farms, which may have led to an overestimation of the economic margin. Moreover, volatility of the inputs and output prices was not accounted for in these studies which may explain why the net benefit was higher than the results from the current model with price volatility (€39.21/sow/year) and more similar to the results of the model without volatility (€107.47/sow/year; Table 3).

CONCLUSIONS

When the economic context is adverse, it becomes even more important to be able to prove the profitability of any proposed shift of management to farmers. In this study it was demonstrated that reducing the antimicrobial use was not only possible without hampering productivity, but also the mean difference of enterprise profit after versus before the reduction of antimicrobial use was on average higher. The results of this study can be crucial to aid veterinarians and other stakeholders to incentive pig farmers to reduce their current use of antimicrobials and to shift to the use of more sustainable practices such as biosecurity measures or vaccinations.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Jef Van Meensel (Institute of Agriculture and Fisheries Research (ILVO), Social Sciences Unit) for his useful insights on the economic model and discussions. The authors also wish to thank Dakerlia Claeys (Institute of Agriculture and Fisheries Research (ILVO), Social Sciences Unit) for her help with the FADN data. Special thanks goes to Els De Fré (Veterinary support from the Large Animal Practice of the University of Ghent, Department of Obstetrics, Reproduction and Herd Health) for her help obtaining the prices of antimicrobials. The authors are pleased to acknowledge the two veterinarians active in pig veterinary medicine for replying to the questionnaires about the vaccination prices. The project 'reduction of antimicrobials project' was funded by Boerenbond and Certus. The authors are thankful for the collaboration of pig farmers who participated in this project as well as their veterinarians and herd advisors.

REFERENCES

Anonymous (2010). Marktprijzen 2010. http://www.vda-ooigem.be/library/2010_ Marktprijzen varkens.pdf?1329742665 (accessed 1.7.15)

Anonymous (2011). Marktprijzen 2011. http://www.vda-ooigem.be/library/2011_ Marktprijzen varkens.pdf?1329742681 (accessed 1.7.15)

Anonymous (2012). Marktprijzen 2012 Vleesvarkens Westvlees. http://www.vda-ooigem.be/library/Marktprijzen 2012/2012_Marktprijzen Westvlees.pdf?1434531802 (accessed 1.7.15)

Anonymous (2015). Actualisatie van de studie over de varkenskolom. http://economie.fgov.be/nl/binaries/Actualisatie_studie_varkenskolom_mei2015_tcm325-267698.pdf

Austin, P.C. (2011). An introduction to propensity score methods for reducing the effects of confounding in observational studies. Multivar. Behav. Res. 46, 399–424

Statistics Belgium. http://statbel.fgov.be/nl/statistieken/cijfers/ (accessed 1.7.2015)

BelVet-SAC, 2014. Belgian Veterinary Surveillance of Antimicrobial Consumption National consumption report. http://www.belvetsac.ugent.be/pages/home/BelvetSAC_report_2014%20finaal.pdf

Callens, B., Persoons, D., Maes, D., Laanen, M., Postma, M., Boyen, F., Haesebrouck, F., Butaye, P., Catry, B. and Dewulf, J. (2012). Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev. Vet. Med. 106, 53–62

Chantziaras, I., Boyen, F., Callens, B. and Dewulf, J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. J. Antimicrob. Chemother. 69, 827–834

Coates, M.E., Dickinson, C.D., Harrison, G.F., Kon, S.K., Cummins, S.H. and Cuthbertson, W.F.J. (1951). Mode of action of antibiotics in stimulating growth of chicks. Nature 168, 332-332

Corrégé, I., Berthelot, N., Badouard, B., Aubry, A. and Hemonic, A. (2011). Biosecurity, health control, farming conception and management factors : Impact on technical and economic performances. In: Proceedings of the 15th international congress of the international society for animal hygiene, 3-7 July 2011, Volume 2, Vienna, Austria, pp. 689–691

Corrégé, I., Fourchon, P., Le Brun, T. and Berthelot, N. (2012). Biosécurité et hygiène en élevage de porcs: état des lieux et impact sur les performances technico-économiques. Journées Recherche Porcine 44, 101–102

Dehejia, R.H. and Wahba, S. (2002). Propensity score-matching methods for nonexperimental causal studies. Rev. Econ. Stat. 84, 151–161

Dohoo, I. Martin, W. and Stryhn, H. (2009). Confounding: detection and control. In: Veterinary Epidemiologic Research. Ver Inc, Charlottetown, Canada, pp. 271–323

European Medicines Agency (2013). Sales of veterinary antimicrobial agents in 25 EU/EEA countries in 2011. Third ESVAC report. http://www.ema.europa.eu/docs/en_GB /document_library/Report/2013/10/WC500152311.pdf

European Medicines Agency (2014). Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2012. Fourth ESVAC report. http://www.ema.europa.eu/docs/en_GB/ document_library/Report/2014/10/WC500175671.pdf

Filippitzi, M.E., Callens, B., Pardon, B., Persoons, D. and Dewulf, J., 2014. Antimicrobial use in pigs, broilers and veal calves in Belgium. Vlaams Diergeneeskd. Tijdschr. 83, 215-224

Fraser, R.W., Williams, N.T., Powell, L.F. and Cook, A.J.C. (2010). Reducing *Campylobacter* and *Salmonella* infection: Two studies of the economic cost and attitude to adoption of on-farm biosecurity measures. Zoonoses Public Health. 57, 109–115

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

Harris, A., McGregor, J., Perencevich, E., Furuno, J., Zhu, J., Peterson, D. and Finkelstein, J. (2006). The use and interpreatation of quasi-experimental studies in medical informatics. J. Am. Med. Inf. Assoc. 13, 16–23

Hill, D.C., Branion H.D., Slinger, S.J. and Anderson, G.W. (1953). Influence of environment on the growth response of chicks to penicillin. Poultry Sci. 32, 462-466

Key, N. and Mcbride, W.D. (2014). Sub-therapeutic antibiotics and the efficiency of U.S. hog farms. Am. J. Agric. Econ. 96, 831–850

Laanen, M., Persoons, D., Ribbens, S., de Jong, E., Callens, B., Strubbe, M., Maes, D. and Dewulf, J. (2013). Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet. J. 198, 508–512

Laanen, M., Maes, D., Hendriksen, C., Gelaude, P., De Vliegher, S., Rosseel, Y. and Dewulf, J. (2014). Pig, cattle and poultry farmers with a known interest in research have comparable perspectives on disease prevention and on-farm biosecurity. Prev. Vet. Med. 115, 1–9

LaLonde, R. (1986). Evaluating the econometric evaluations of training programs with experimental data. Am. Econ. Rev. 76, 604-620

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K.M., Wertheim, H.F.L., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., Greko, C., So, A.D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A.Q., Qamar, F.N., Mir, F., Kariuki, S., Bhutta, Z.A., Coates, A., Bergstrom, R., Wright, G.D., Brown, E.D. and Cars, O. (2013). Antibiotic resistance-the need for global solutions. Lancet Infect. Dis. 13, 1057–1098

MARAN (2014). Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in The Netherlands in 2013. https://www.wageningenur.nl/upload_mm/7/8/9/52388c6c-858c-483c-b57d-227029fe778a_005738_Nethmap_2013%20def_web.pdf

Mendola, M. (2007). Agricultural technology adoption and poverty reduction: A propensity-score matching analysis for rural Bangladesh. Food Policy 32, 372–393

Moreno, M.A. (2014). Opinions of Spanish pig producers on the role, the level and the risk to public health of the use of antimicrobial use in pig. Res. Vet. Sci. 97, 26-31

Postma, M., Sjolund, M., Collineau, L., Losken, S., Stark, K.D.C. and Dewulf, J. (2014). Assigning defined daily doses animal: a European multi-country experience for antimicrobial products authorized for usage in pigs. J. Antimicrob. Chemoth. 70, 294–302

Postma, M., Stärk, K.D.C., Sjölund, M., Backhans, A., Beilage, E.G., Lösken, S., Belloc, C., Collineau, L., Iten, D., Visschers, V., Nielsen, E.O. and Dewulf, J. (2015). Alternatives to the use of antimicrobial agents in pig production: A multi-country expert-ranking of perceived effectiveness, feasibility and return on investment. Prev. Vet. Med. 118, 457–466

Ramirez, C.R., Harding, A.L. Forteguerri, E.B.R., Aldrige, B.M. and Lowe, J.F. (2015). Limited efficacy of antimicrobial metaphylaxis in finishing pigs: A randomized clinical trial. Prev. Vet. Med. 121 176-178

Rosenbaum, P.R. and Rubin, D.B. (1983). The central role of the propensity score in observational studies for causal effects. Biometrika 70, 41–55

Rushton, J. (2009a). Livestock production economics. In: The economics of animal health and production. CAB International, Wallingford, UK

Rushton, J. (2009b). Economic analysis tools. In: The economics of animal health and production. CAB International, Wallingford, UK

Rushton, J. (2015). Anti-microbial use in animals: how to assess the trade-offs. Zoonoses Public Health 62, 10-21

Schwarz, S., Kehrenberg, C. and Walsh, T.R. (2001). Use of antimicrobial agents in veterinary medicine and food animal production. Int. J. Antimicrob. Ag. 17, 431–437

Siekkinen, K.M., Heikkilä, J., Tammiranta, N. and Rosengren, H. (2012). Measuring the costs of biosecurity on poultry farms: a case study in broiler production in Finland. Acta Vet. Scand. 54, 12

Van Meensel, J., Kanora, A., Lauwers, L., Jourquin, J., Goossens, L. and Van Huylenbroeck, G. (2010). From research to farm: Ex ante evaluation of strategic deworming in pig fnishing. Vet. Med. (Praha). 55, 483–493

Visschers, V.H.M., Backhans, A., Collineau, L., Iten, D., Loesken, S., Postma, M., Belloc, C., Dewulf, J., Emanuelson, U., Beilage, E.G., Siegrist, M., Sjölund, M. and Stärk, K.D.C. (2015). Perceptions of antimicrobial usage, antimicrobial resistance and policy measures to reduce antimicrobial usage in convenient samples of Belgian, French, German, Swedish and Swiss pig farmers. Prev. Vet. Med. 119, 10–20

Vrints, G. and Deuninck, J. (2014). Technische en economische resultaten van de varkenshouderij op basis van het Landbouwmonitoringsnetwerk. Boekjaren 2011-2013. https://www.vlaanderen.be/nl/publicaties/detail/technische-en-economische-resultaten-van-de-varkenshouderij-op-basis-van-het-landbouwmonitoringsnetwerk-boekjaren-2011-2013

SPATIAL PATTERNS OF ANTIMICROBIAL RESISTANCE GENES IN DANISH PIG FARMS

A.C. BIRKEGÅRD*, A.K. ERSBØLL, T. HALASA, J. CLASEN, A. FOLKESSON, H. VIGRE AND N. TOFT

SUMMARY

Samples from 687 Danish pig farms were collected at five finisher slaughterhouses in February and March 2015. Faecal samples from five pigs per farm were collected randomly at the slaughter line and pooled into one sample per farm. DNA was extracted from the pooled samples and the level of seven antimicrobial resistance genes, ermB, ermF, sulI, sulII, tet(M), tet(O) and tet(W), was quantified by a high-throughput qPCR. It was evaluated whether the sample method resulted in a study population representative of Danish pig farms with finishers where it was found that the study population was biased towards farms having more finisher and a higher productivity. Spatial cluster analyses were performed in SaTScan®. The results showed significant spatial clusters for ermF, ermB, sulII and tet(W) whereas no significant clusters were found for sulI, tet(M) and tet(O).

INTRODUCTION

Antimicrobial resistance (AMR) is a growing concern due to the risk of treatable diseases becoming untreatable. Antimicrobial use is the most important risk factor for acquiring AMR. However, this is not the only risk factor. The existence of AMR genes harboured in pathogenic as well as non-pathogenic bacteria in porcine faeces poses a risk of transferral of AMR genes to human pathogens along the whole food chain from farm to fork, especially through faecal contamination of the carcass at slaughter.

Local patterns of antimicrobial usage in livestock should be expected in Denmark where antimicrobials are prescribed by veterinarians who primarily work locally, and as expected, spatial patterns of antimicrobial usage in Danish pig farms have been found (Fertner et al., 2015). Furthermore previous studies have found spatial patterns of different bacterial pathogens in livestock in Denmark (Bihrmann et al., 2012; Ersbøll & Nielsen, 2011; Vigre et al., 2005). Therefore it is not surprisingly that spatial patterns of AMR in indicator bacteria have been found (Abatih et al., 2009a). The present study was, in contrast to previous studies on spatial patterns of AMR, specifically designed to evaluate spatial randomness. Spatial patterns were assessed for a quantitative measure for selected AMR-genes in total community DNA from Danish pig farms.

---

* Anna Camilla Birkegård, Danish Technical University-Vet, Bülowsvej 27, 1870 Frederiksberg C, Denmark. Email: acbir@vet.dtu.dk

The objective of this cross-sectional study was to evaluate spatial patterns of AMR genes in faecal samples from Danish pig farms obtained in February and March 2015. A secondary objective was to evaluate whether the sample method resulted in a study population representative of Danish pig farms with finishers.

MATERIALS AND METHODS

Register data

All pig farms in Denmark are registered in the national database, Central Husbandry Register (CHR), with a unique identification number (CHR-number). Information in the CHR includes Cartesian coordinates given in "UTM EUREF89 zone 32" format, the number of pen places for three age groups defined as 1) sows, gilts and boars 2) pigs above 30 kg not included in age group 1 (in this study referred to as finishers) and 3) pigs from 7 to 30 kg. A pig farm with finishers was defined as a registered CHR-number with pen places for finishers.

Data from seven major Danish owned slaughterhouses in Denmark were used to design the study. In total, 1,531,600 finishers were slaughtered in Denmark in February 2015 (Statistics Denmark, 2015) of which 1,365,963 pigs were slaughtered at the slaughterhouses included in this study.

Data from the Pig Movement Database were used to calculate the number of pigs sent for slaughter per herd. The pig movement database contains information on the CHR-numbers of the receiving and sending farms, the number of pigs moved and the date of the movement.

Sampling

The level of AMR in pig farms is seasonal (Abatih et al., 2009b). Therefore, the sampling period had to be kept short. The strict quarantine rules of most Danish pig farms made it difficult to sample many farms in a short period of time. Therefore it was decided to sample at the slaughterhouse as done by others (van Den Bogaard et al., 2000; DANMAP, 2014).

Five of the seven major Danish owned slaughterhouses for finisher pigs in Denmark were selected as sampling sites. The reasons for leaving out two slaughterhouses were that one was primarily handling slaughter pigs from free range and organic farms and the other was located at the remote island Bornholm. The remaining five slaughterhouses were included to ensure that farms from the entire country, except Bornholm, could be sampled. By mapping the location of farms sending pigs to the slaughterhouses, it was seen that each slaughterhouse covers a certain region of the country.

The numbers of farms to sample per slaughterhouse were stratified based on the mean number of farms sending pigs for slaughter at the slaughterhouse for weeks 6-10 and weeks 46-50 in 2014. Given the resources available in the project, it was planned to have 500-800 farms with finishers in the study population. Sampling took place several days for each slaughterhouse and farms can deliver pigs in more than one batch per day. There was thus a chance of sampling the same farms more than once. It is therefore that a total of 800 farms was targeted to optimize the number of farms in the final study population.

Samples were taken at the slaughterhouses during weeks 6-10 in 2015. Per day, samples were taken from 20-67 farms per slaughterhouse and from one or two slaughterhouses. Farms were chosen at random when running the slaughter line where an identification number was noted for the farm. Samples were taken by slaughterhouse technicians who were introduced to the sampling methods at the first sampling day by the first author. As suggested by Clasen et al. (unpublished data) five pigs per farm were randomly sampled. Samples were taken at the slaughter line after removal of the gut from the carcass. A small amount of faeces was squeezed out of the rectum of the pig gut into an empty 12.5 mL sample glass. Sampling time was four to seven hours per day. Samples were kept at room temperature until all samples were collected for the day. They were then placed in a Styrofoam box with cooling elements and sent by mail to the laboratory where they arrived the next day. Few deliveries were delayed by one day, but the cooling element was still frozen at arrival.

Representative sample

It was evaluated whether the sample method resulted in a study population representative of Danish pig farms with finishers. Two target populations were defined to compare with the study population: 1) Farms registered in a data extraction of the CHR-database from the 19th of January 2015 with pen places for finishers and not registered as organic farms, free range farms or hobby farms. 2) Farms sending pigs to the slaughterhouses where the sampling took place, during the sampling period. From both target populations farms without coordinates and farms located on Bornholm were excluded. Using the available data, it was possible to compare the sampled farms with the non-sampled farms in the target populations according to farm size and productivity. Farm size was defined as the number of finishers registered in the CHR. Productivity was calculated as the cumulated 26 week delivery of pigs for slaughter traced back from the first day of slaughter in the pig movement database. This proxy is estimated to be the size of two batches of finishers and is used instead of 13 weeks deliverance to reduce the influence of seasonal variation. Non-parametric tests were used to compare the medians for both variables and they were plotted as a boxplot to visualise the difference.

Quantification of AMR gene levels

At arrival at the laboratory, samples were pooled into one sample per farm according to Clasen et al. (unpublished data). Briefly, a sample that fitted the eye of a 10µl inoculation loop from each of the 5 samples per farm was dissolved in 3.5 mL PBS. The pooled sampled was vortexed and 2 mL was stored at -20 °C. DNA was extracted using the Maxwell® 16 Blood DNA Purification Kit (Promega). DNA concentrations and purity were assessed by the 260/280 nm-ratio using the NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc.) and concentrations were diluted to 40 ng/µl. Quantification of seven AMR genes (*ermB*, *ermF*, *sulI*, *sulII*, *tet*(M), *tet*(O) and *tet*(W)) was done using the high-throughput qPCR chip "Gene Expression 192x24", GE192.24 (Fluidigm®) using two technical replicates as previously described by Clasen et al. (unpublished data). Efficiency of the primers was determined using standard curves and obtained results were normalized with 16S reference gene ribosomal DNA.

Data analysis

Raw quantification cycle (Cq) values generated by the qPCR were taken from the Fluidigm Real-Time PCR Analysis Software, version 42.1 (Fluidigm) and exported to R (R Core Team, 2015). The mean of the Cq values for technical replicates for each sample per gene was calculated. Cq values were corrected with the interplate calibrators included in all runs, along with an efficiency calibration (Ståhlberg et al., 2013) based on standard curves generated for each of the primer sets (unpublished data). The Cq-value reflects the number of PCR-cycles until a predefined threshold is reached. Higher Cq-values thus reflect a lower level of presence of the gene. Values above gene-specific limits of detection were coded as a non-detect. Genes were handled in three ways:

1) Genes without non-detects (*ermB*, *tet*(O), *tet*(W)), were handled on a continuous scale.

2) Genes with many non-detects (*sulI* and *sulII*), were evaluated dichotomously as present or absent. The gene was regarded as being present, if the qPCR assay resulted in a Cq-value even though it was above limit of detection.

3) Genes with few non-detects (*ermF* and *tet*(M)), were handled in two ways: dichotomously as described above and the samples with the gene present were handled on a continuous scale.

Relative Quantity (RQ)-values, which indicate the relative quantity of the gene compared with the total amount of bacterial DNA found in the sample, were calculated for samples and genes handled on a continuous scale using the Livak-Method as in Eq. (1) (Livak & Schmittgen, 2001):

$$RQ_{\text{primer set X}} = 2^{(-(\text{Cq, gene of interest} - \text{Cq reference gene}))} \tag{1}$$

RQ values for each gene were plotted using boxplots for each gene. Distributions of presence and absence of genes were plotted using bar plots. Additionally, farms were grouped in four groups per gene according to the quantiles of the RQ-values for each gene handled on a continuous scale.

Spatial cluster analyses

Data handling was done in R (R Core Team, 2015) before exporting to SaTScan version 9.4.1 (Kulldorff M. and Information Management Services Inc, 2015) for cluster analysis.

A Poisson model (Kulldorff, 1997) was used to evaluate the sampling plan for both target populations as described by Vieira et al. (2009) where sampled farms were defined as cases and non-sampled farms as controls. The relative risk for farms to be in the study population was calculated for each cluster.

For evaluation of spatial patterns of AMR genes, each gene was analysed separately and three different models predefined in SatScan® were used to run the scan statistics:

1) A multinomial model (Jung et al., 2010) on grouped AMR gene levels (ermB, ermF, tet(M), tet(O) and tet(W)). In the model, the expected and observed number of observation within each category for each search window was calculated and thus resulted in a relative risk given for each of the four categories of the genes.

2) A normal model (Kulldorff et al., 2009) on continuous RQ-values (ermB, ermF, tet(M), tet(O) and tet(W)). In the model the means inside and outside the cluster were calculated.

3) A Bernoulli model (Kulldorff, 1997) for genes with a dichotomous classification, (ermF, sulI, sulII and tet(M)), where samples with presence of the gene were defined as cases and samples where the gene was not found as controls. The relative risk for the gene to be present in the sample was calculated for each cluster.

For each model and gene, the cluster analysis was run with different parameter settings for the shape of the search window (elliptic or circular) and the maximum percentage of the population at risk to be included in clusters (1, 5, 12.5, 25 or 50% of the population). For the Poisson and normal models, high and low levels were searched simultaneously. Testing was performed using Monte Carlo simulations (999 permutations) and only secondary clusters that did not overlap with the most likely cluster were requested. A cluster was regarded as significant when the P-value was below 0.05.

RESULTS

Sampling

Stratification on number of farms per slaughterhouse resulted in 140, 300, 160, 120 and 80 farms targeted for sampling per slaughterhouse. Only results of the first sampling were for farms sampled more than once, resulting in 687 farms divided on 129, 253, 125, 104 and 76 farms per slaughterhouse.

Representative sample

There were 6,018 farms in target population 1 (124 farms were excluded due to location on Bornholm and 3 due to missing coordinates) of which 11% were sampled farms. 3,326 farms were in target population 2 (1 farm was excluded due to location on Bornholm and 2 due to missing coordinates) of which 21 % were sampled farms.

Sampled farms had a higher number of finishers and a higher productivity than non-sampled farms for both target populations (Fig. 1). Median number of finishers was 1,500 for sampled farms and 750 for non-sampled farms in target population 1 and 1000 for non-sampled farms in target population 2. Median number of pigs in the 26 weeks delivery was 2,458 for sampled farms and 795 for non-sampled farms in target population 1 and 1,534 for non-sampled farms in target population 2. Differences were significant for both variables and for both target populations, with P-values < 0.001.

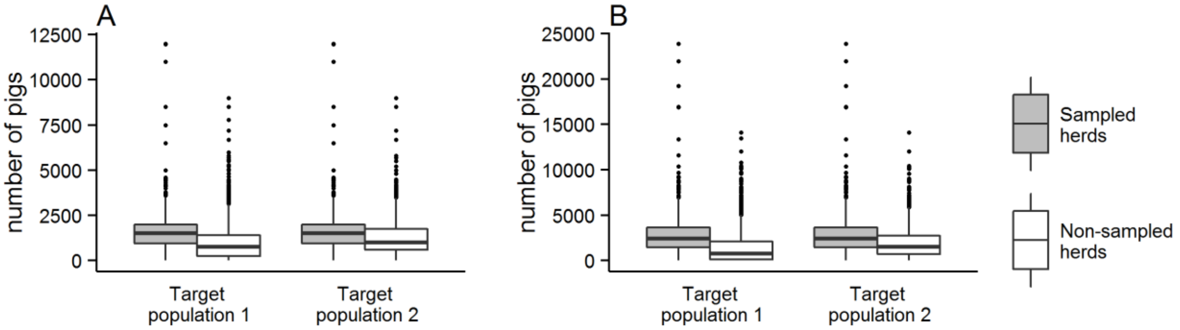


Fig. 1 Comparison of sampled and non-sampled farms in target population 1 and 2 for the number of finishers (A) and the number of pigs sent for slaughter in 26 weeks (B)

AMR levels

It can be seen in Fig. 2, that there is a much higher variation and level of *tet*(W) compared to the other genes. It also shows that the *sulII* gene was found more frequent than the *sulI* gene.

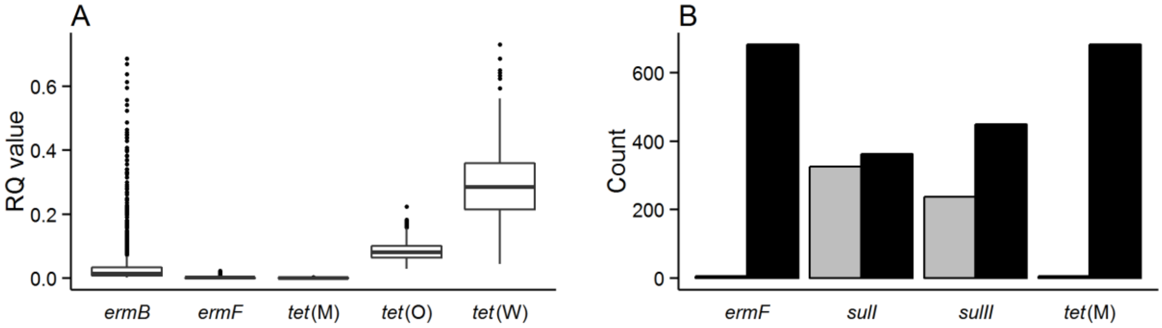


Fig. 2 A: Distribution of RQ-values for genes and samples handled on a continuous scale. B: Distribution of genes handled on a dichotomously scale, grey indicating absence of the gene and black presence of the gene

Spatial cluster analyses

Changes in parameter settings resulted in slightly different cluster locations and sizes. Clusters occurring with most different parameter settings or clusters that best enclosed all farms, also found by other parameter settings, were chosen and shown in Fig. 3 and 4.

Figure 3 shows the spatial distribution of sampled and non-sampled farms for target population 1 and 2. For both target populations, an under-sampled area in Jutland was found for the sampled farms in target population 1 (circle in C) and 2 (ellipse in D).

Figure 4 shows the results of the spatial cluster analysis of AMR genes. Two high risk clusters for *ermF* (black ellipse and circle in B), one high risk cluster for *sulII* (black ellipse in C) and one low risk cluster for *ermB* (grey ellipse in A), *ermF* (grey circle in B), and *tet*(W) (grey ellipse in D) were identified in the analysis. No spatial clusters were found for *sulI*, *tet*(M) and *tet*(O).

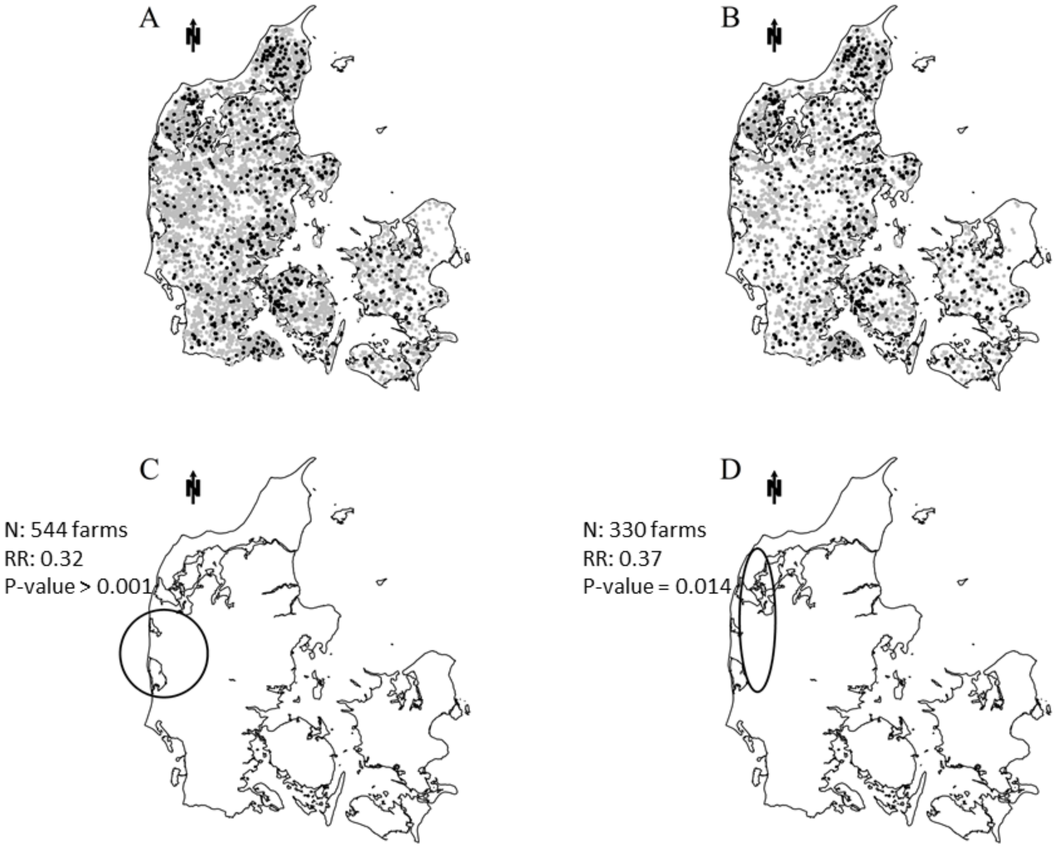


Fig. 3 Distribution of farms in the two target populations; A, B: point location of farms in target population 1 (A) and 2 (B). •: sampled farms, •: non-sampled farms. C,D results of the cluster analysis of the spatial distribution on the risk for farms being sampled among target population 1 (C) and 2 (D). RR: Relative risk, N: number of farms in the cluster

DISCUSSION

In the cluster analysis of AMR genes, two high risk clusters for *ermF* and one low risk cluster for *tet*(W), *ermF* and *ermB*, and one high risk cluster for *sulII* were identified, whereas no clusters for *sulI*, *tet*(O) and *tet*(M) were found. The cluster analysis found similar results with different parameter settings, indicating that the absence and present of clustering of these genes seems reliable. However, it is possible that the current sample size lacks the power to show significance of clustering for the three latter genes, if it truly exists.

It was found that sampled farms had a higher number of finishers than non-sampled farms and a higher productivity. It is not surprising that larger farms are over-represented in the study population. These farms are expected to deliver pigs more often and in larger batches, which leads to a higher chance of being sampled at the slaughterhouse. However, this could have biased the results. In this study, spatial scan statistics based on a Poisson model was used to identify under- and over-sampled areas with reference to the target population as suggested by Vieira et al. (2009). An under-sampled area in the western part of Jutland was found both when comparing the location of sampled farms with the target population of all Danish pig farms with finishers and with the target population of Danish pig farms that send pigs for slaughter at the slaughterhouses where the sampling took place. Therefore, it is less likely that it is due to the choice of slaughterhouses. Vieira et al. (2009) found under-sampled areas in the other side of the country when evaluating a sampling method stratified on slaughterhouse and differed from year to year, which shows that uneven sampling could be expected to arise by chance or unknown factors.

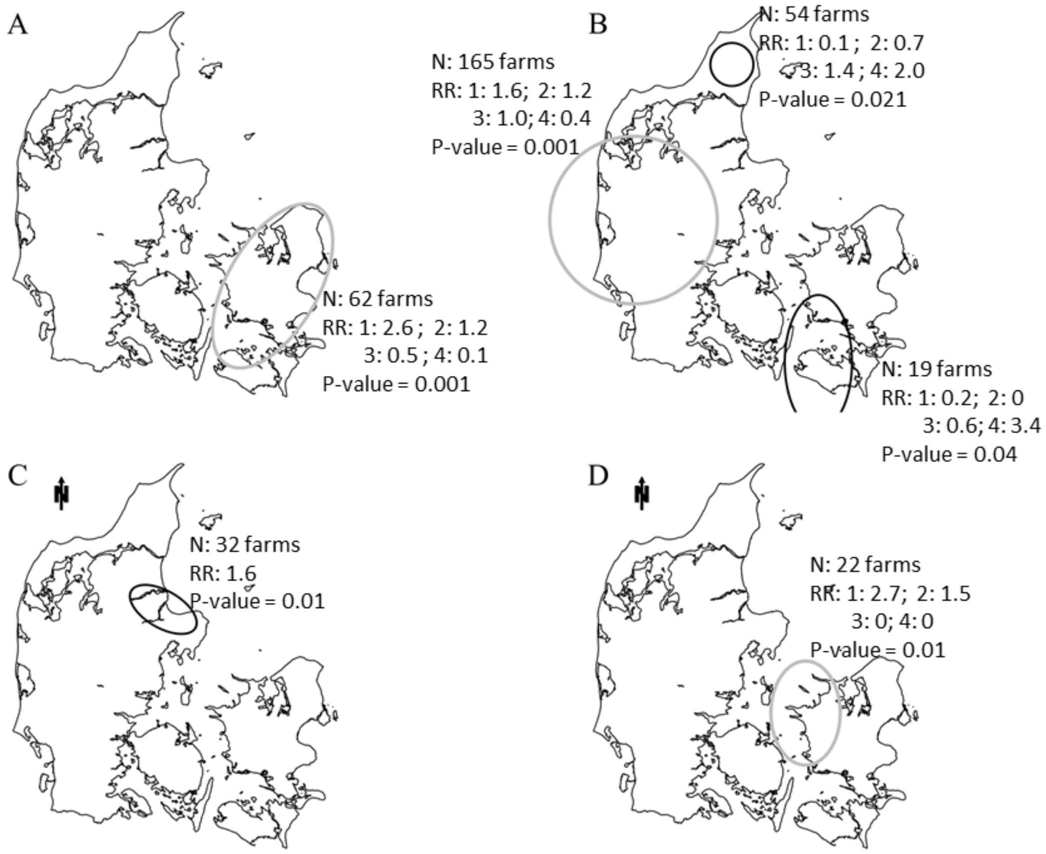


Fig. 4 Results of cluster analysis for AMR genes. Grey indicates a low risk cluster and black a high risk cluster A: ermB, B: ermF,; C: sulII; D: tet(W). RR: Relative Risk, N: number of farms in the cluster

To the knowledge of the authors, this is the first study to report spatial patterns of AMR genes of total community DNA from porcine faeces. However, it is not the first time that spatial patterns of AMR in Danish pig farms are found (Abatih et al., 2009a). Fertner et al (2015) found spatial clustering of antibiotic use in Danish pig farms for 2012 and 2013 but not in the same areas where there were found clusters in this study. They only looked for high risk clusters on overall usage patterns which can have masked local differences in the usage pattern of specific antimicrobial classes. Thus, it should not be ruled out, that the local differences in the usage patterns of antimicrobials could explain the spatial distribution of AMR genes found in the present study. It is interesting to note that for genes coding for resistance of the same antimicrobial class, significant spatial clusters were found for some for the genes, such as tet(W) and sulII but not for tet(M), tet(O) and sulI. Therefore it might be speculated that it is not only the local patterns of antimicrobials usage which explain the spatial clusters. Other factors, such as trade patterns and clustering of bacteria, might influence the presence and spread of AMR genes. Unpublished data showed that most Danish pig fattening farms buy their pigs from local farms.

In conclusion, there are spatial differences for AMR genes in Danish pig farms. Further studies are needed to elucidate the consequences and possible reasons for these spatial differences. The study also showed that the presented sampling method will slightly bias the study population towards larger farms.

ACKNOWLEDGEMENTS

This study was funded by the Danish Food and Veterinary administration. The authors would like to thank the employees of Danish Crown and Tican for cooperation, assistance with sampling and help of setting up the study, especially Marie B. Hansen, Vagner Bøge and Henrik Bækstrøm. The authors would also like to thank Jesper Larsen from the Danish Classification Inspection, for support with the meat inspection data, Margarida Arede for help with sampling, Joanna Zeitman Amenuvor for help with sample pooling, Karin Tarp and Sophia Rasmussen for their technical assistance. We would also like to thank Kerstin Skovgaard for advice on handling qPCR data.

REFERENCES

Abatih, E.N., Emborg, H.D., Jensen, V.F., Lo Fo Wong, D.M.A. and Ersbøll, A.K. (2009b). Regional, seasonal, and temporal variations in the prevalence of antimicrobial-resistant *Escherichia coli* isolated from pigs at slaughter in Denmark (1997-2005). Foodborne Pathog. Dis. 6, 305–319

Abatih, E.N., Ersbøll, A.K., Lo Fo Wong, D.M.A. and Emborg, H.D. (2009a). Space-time clustering of ampicillin resistant Escherichia coli isolated from Danish pigs at slaughter between 1997 and 2005. Prev. Vet. Med. 89, 90–101

Bihrmann, K., Nielsen, S.S., Toft, N. and Ersbøll, A.K. (2012). Spatial differences in occurrence of paratuberculosis in Danish dairy herds and in control programme participation. Prev. Vet. Med. 103, 112–119

DANMAP (2014). DANMAP 2013 - use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

Ersbøll, A.K. and Nielsen, L.R. (2011). Spatial patterns in surveillance data during control of *Salmonella Dublin* in bovine dairy herds in Jutland, Denmark 2003-2009. Spat. Spatiotemporal. Epidemiol. 2, 195–204

Fertner, M., Sanchez, J., Boklund, A., Stryhn, H., Dupont, N. and Toft, N. (2015). Persistent spatial clusters of prescribed antimicrobials among Danish pig farms - A register-based study. PLoS One 10, e0136834

Jung, I., Kulldorff, M. and Richard, O.J. (2010). A spatial scan statistic for multinomial data. Stat. Med. 29, 1910–1918

Kulldorff, M. (1997). A spatial scan statistic. Commun. Stat. Theory Methods 26, 1481–1496

Kulldorff, M., Huang, L. and Konty, K. (2009). A scan statistic for continuous data based on the normal probability model. Int. J. Health Geogr. 8, 58

Kulldorff M. and Information Management Services Inc (2015). SaTScanTM ver. 9.4.1: Software for the spatial and space-time scan statistics

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408

R Core Team (2015). R: A Language and Environment for Statistical Computing.

Statistics Denmark (2015). StatBank Denmark. accessed on 2015-10-06, http://www.statistikbanken.dk/statbank5a/SelectVarVal/Define.asp?Maintable=SVIN&PLanguage=1

Ståhlberg, A., Rusnakova, V., Forootan, A., Anderova, M. and Kubista, M. (2013). RT-qPCR work-flow for single-cell data analysis. Methods 59, 80–88

van den Bogaard, A.E.J.M., London, N. and Stobberingh, E.E. (2000). Antimicrobial resistance in pig faecal samples from the Netherlands (five abattoirs) and Sweden. J. Antimicrob. Chemother. 45, 663–671

Vieira, A.R., Houe, H., Wegener, H.C., Lo Fo Wong, D.M.A., Bødker, R. and Emborg, H.-D. (2009). Spatial scan statistics to assess sampling strategy of antimicrobial resistance monitoring program. Foodborne Pathog. Dis. 6, 15–21

Vigre, H., Baekbo, P., Jorsal, S.E., Bille-Hansen, V., Hassing, A.-G., Enøe, C. and Bøtner, A. (2005). Spatial and temporal patterns of pig herds diagnosed with Postweaning Multisystemic Wasting Syndrome (PMWS) during the first two years of its occurrence in Denmark. Vet. Microbiol. 110, 17–26

ANTIMICROBIAL USE AND BIOSECURITY PRACTICES IN PIG PRODUCTION IN FOUR EUROPEAN COUNTRIES, COMPARISON, ASSOCIATIONS AND OPPORTUNITIES

J. DEWULF[*], A. BACKHANS, L. COLLINEAU, S. LOESKEN, M. POSTMA, M. SJÖLUND, E. OKHOLM NIELSEN, C. BELLOC, U. EMANUELSON, E. GROSSE BEILAGE AND K.D.C. STÄRK

SUMMARY

To identify potential intervention factors that may help in the reduction of the need for antimicrobials in animal production, antimicrobial use in pig herds originating from 4 European countries and its association with animal health, production, biosecurity and other disease prevention measures was studied. In the 227 pig herds from Belgium, France, Germany and Sweden, large differences in antimicrobial use were observed within and between countries. As the biosecurity is concerned, large within country differences were observed whereas the between country differences were more limited. Antimicrobial usage in sows was found to be significantly associated with the antimicrobial usage from birth till slaughter in the growers, the use of anti-inflammatory products in weaners and the number of pathogens vaccinated against. Higher antimicrobial usage from birth till slaughter was associated with a shorter farrowing rhythm and a younger weaning age, whereas a better external biosecurity was related with a lower antimicrobial usage. A higher external biosecurity was also associated with more weaned piglets per sow per year. Farmers that managed to combine below average antimicrobial use and above national average technical performances were characterised by higher internal biosecurity and were generally located in a more favourable environment.

INTRODUCTION

The global development and spread of antimicrobial resistance is largely driven by the extensive use of antimicrobials both in human and animal medicine (ECDC, EFSA and EMA 2015). In a recent study, data on sales of antimicrobials for food-producing animals were compared to data on antimicrobial resistance, and a clear association between use and resistance was found (Chantziaras et al., 2013). The ESVAC (European Surveillance Veterinary Antimicrobial Consumption) reports have shown that there are vast differences between countries in amounts of antimicrobials sold for food-producing animals (ESVAC, 2013; Grave et al., 2014). Yet, these sales figures (expressed in kg active substance in relation to a certain measure of biomass produced) are only a crude measurement of actual consumption and do not provide insight into actual use and how use with regards to antimicrobial substance

---

[*] Jeroen Dewulf, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium. Email: Jeroen.Dewulf@ugent.be

is distributed between different animal species and age categories. Moreover they do not take into account the large differences in molecular weight between different antimicrobial compounds.

Within livestock production, pig husbandry has been identified as one of the main consumers of antimicrobials (Callens et al., 2012; Fillipitzi et al., 2014; MARAN, 2014). To allow for the development of plans for antimicrobial usage (AMU) reduction, first a detailed understanding of the current use and its associations with animal health and production characteristics is required. Limited knowledge is currently available about the main drivers for antimicrobial usage as well as farmers' perceptions, attitudes and behaviours regarding antimicrobial usage and resistance.

Secondly, to be able to reduce AMU, it is important to have healthier animals and therefore reduce the need for antimicrobial treatment. Many potential alternatives for antimicrobials have been suggested (Postma et al., 2015c). However, most are based upon clinical observations or rational deduction rather than quantitative observations making them prone to critics due to insufficient scientific bases of their efficacy for the replacement of antimicrobials. Therefore, a good insight in the associations between preventive measures, management factors, production parameters, biosecurity status and AMU is of great importance.

To inform evidence-based measures for reduced AMU, antimicrobial use in pig herds originating from 4 European countries (Belgium, France, Germany, Sweden) and its association with animal health, production, biosecurity and other disease prevention measures was studied to identify potential intervention factors that may help in the reduction of the need for antimicrobials in animal production.

MATERIALS AND METHODS

Data collection

Data were collected between December 2012 and December 2013 in a cross-sectional study in 227 farrow-to-finish pig herds with at least 100 sows and 500 finishing pigs in Belgium (n = 47), France (n = 60), Germany (n = 60) and Sweden (n = 60). In Belgium, farms were recruited among those subscribing to a professional newsletter regularly issued by the University of Ghent. In France, herds were randomly selected from the Northwestern region among those registered in a technical database maintained by the French Institute for pig and pork industry (IFIP). German herds were recruited via consultancy circles together with contacts provided by several veterinary practices in the three regions, Mecklenburg-Vorpommern, Niedersachsen and Nordrhein-Westfalen. Swedish herds were selected among those either affiliated with the Swedish Animal Health Service (SvDHV) with a herd veterinarian working for SvDHV, or were herds with previous contact with researchers at the National Veterinary Institute. Each herd was visited once to collect detailed information on antimicrobial consumption over the past year expressed as volume or mass by product, strength of product, administration route and age category (sows, piglets, weaners and finishers).

Quantification of antimicrobial usage

Antimicrobial usage was quantified using the ABcheck.UGent™ online tool developed by the Unit for Veterinary Epidemiology of the Faculty of Veterinary Medicine, University of Ghent (http://www.abcheck.ugent.be/). The ABcheck converts recorded antimicrobial use to active substance expressed as mg and then to treatment incidence (TI) based on Defined Daily Doses Animal (DDDA). The TI is given as the number of DDDAs per 1000 pig-days at risk which is equivalent to how many pigs per 1000 pigs that receive a dose of antimicrobials each day (Timmerman et al., 2006). The TI was calculated for each herd and age group in accordance with Timmerman et al. (2006) as follows:

$$TI = \frac{\text{Total amount of antimicrobials administered (mg)}}{\text{DDDA (mg/kg) x number of days at risk x kg animal at risk}}\ x\ 1000\ pigs\ at\ risk$$

Previously established consensus DDDAs, as described by Postma and co-workers (2015b), were used. Briefly, DDDAs were established by taking the mean of the recommended dose per kg live weight for all products authorised for pigs with the same active substance and the same administration route. The number of days at risk for each age category was equal to the rearing period for that category. The actual rearing periods for the different age categories of the individual herds were used in the calculations. For breeding pigs (boars, sows, gilts), the time period was set to 365 days. The assumed average weight at treatment used were: two kg for suckling pigs, seven kg for weaners, 35 kg for fatteners, 60 kg for gilts and 220 kg for adult pigs (boars and sows). They were subsequently multiplied with the actual number of pigs in the particular age groups within the herds to determine the kg animal at risk.

Quantification of biosecurity and production parameters

Technical parameters (e.g. number of weaned piglets per sow per year (WSY)) and herd management characteristics (e.g. farrowing rhythm) were collected from the herd management system if available or by interviewing the farmer. Information on the biosecurity status of the herd was quantified using the risk-based scoring system Biocheck.UGent™ (www.Biocheck.UGent.be; Laanen et al., 2013; Postma et al., 2015a).This assessment tool makes comparison of the biosecurity status of herds within and between countries possible by returning 109, mainly dichotomous and trichotomous, questions into a score from 0 until 100 for both external and internal biosecurity, where zero means absolute lack of any biosecurity measures and 100 means a full application of all in the system tested biosecurity measures. The Biocheck.UGent™ consists of 6 subcategories for internal biosecurity and 6 for external biosecurity.

Statistical analysis

After performing descriptive statistics for the relevant variables, all TI variables were LOG transformed (first a value of 1 was added to all observations) to overcome the problem of a skewed distribution. Differences in TIs between countries were tested by means of ANOVA. Scheffé’s method was used for post hoc comparison. To compare TIs for the different age categories, multivariable linear regression was performed including country as independent fixed variable to account for country differences. To study the potential associations, first all possible causal routes linking AMU, biosecurity status, herd characteristics and technical parameters (e.g. number of sows, WSY, average daily weight gain (ADG, g/day), mortalities (%)) were identified based on logical reasoning with the main focus on parameters influencing AMU or the ones being influenced by AMU. Subsequently, each of the identified possible associations was assessed using a regression model with the specific predictor always in combination with country as a second predictor variable to account for country specific characteristics. All associations that showed a univariable p-value of < 0.20 were retained for the multivariable analysis. The multivariable general linear model was constructed using the stepwise backward selection procedure, including testing of two-way interactions of significant main effects. Confounding effects were evaluated during the modelling process by checking changes in parameter estimates. The association was considered significant if $p< 0.05$ and a p-value between 0.05 and 0.10 was considered nearly significant and relevant to describe. Normal probability tests and plots were examined to check whether the assumptions of normality and homoscedasticity of residuals were fulfilled.

Finally, “top farmers” were identified that managed to combine both below national average usage of antimicrobials and above national average technical performances. In addition, farms that reported using prophylactic treatments with third and fourth generation cephalosporins or fluoroquinolons, considered as critically important antimicrobials, were excluded from the top farms group and allocated to the regular group. Differences in farm characteristics, biosecurity and symptoms scores between the top and the regular groups were assessed using non-parametric Mann-Whitney U tests with $P < 0.05$ as a significance threshold.

All statistical analyses were performed using SPSS statistics 22 (IBM Corporation, 2013, Armonk, New-York).

RESULTS

Treatment incidences expressed as the number of treatments per 1000 pig-days at risk for growing pigs over a standardised life span of 200 days (TI200) varied considerably between countries as shown in Fig. 1a. The lowest average TI200 was observed for Swedish (SE) herds with a TI of 22.7 (range; 1.6 – 116.0) followed by French (FR) herds with a TI of 108.8 (range; 0.0 – 539.0), Belgian (BE) herds with a TI of 142.9 (range; 0.0 – 531.1) and lastly German (DE) herds with a TI of 242.8 (range; 3.8 – 673.9). Belgian and French herds did not differ significantly from each other ($p < 0.1$) whereas significant differences were observed between the other countries. Also for breeding animals (Fig. 1b), Swedish herds had the lowest average TI followed by Belgian and French herds. German herds had a significantly higher ($p<0.05$) TI. In all countries, considerable variations between herds were observed (Fig. 1 a & b).

These results indicate that an average pig was treated 10 times more with antimicrobials in Germany than in Sweden. Antimicrobials were most often applied through feed or water except in Swedish herds where parenteral treatments were most frequent.

When TIs for growing pigs were split for different age categories, it was observed that weaned piglets received most treatments followed by suckling piglets. However, the reverse situation was observed for Swedish herds where most treatments were applied to suckling piglets. Overall, treatments of finishing pigs were low but also varied between herds and countries. When TIs were investigated for the different age categories (corrected for country effect), a significant ($p < 0.01$) association was found indicating that higher use in the preceding age category was positively associated with a higher usage in the following age category.

Expressed in TI’s, the most commonly used antimicrobials were aminopenicillins followed by polymyxins, macrolides and tetracyclines although this order did vary across countries. On country level, overall use of the critically important third and fourth generation cephalosporins constituted 10.8% (BE), 1.2% (FR), 1.8% (DE) and 0.0% (SE) of total use. For the critically important fluoroquinolones, the corresponding figures were 5.3% (BE), 0.4% (FR), 1.3% (DE) and 1.3% (SE). Overall, 71.2% of antimicrobials were applied orally and 28.8% parentally which included a few topical (TI = 10 in total) treatments. Differences were, however, observed between countries, where Swedish herds applied 13.1% of the antimicrobials orally whereas French herds applied 86.9% orally.

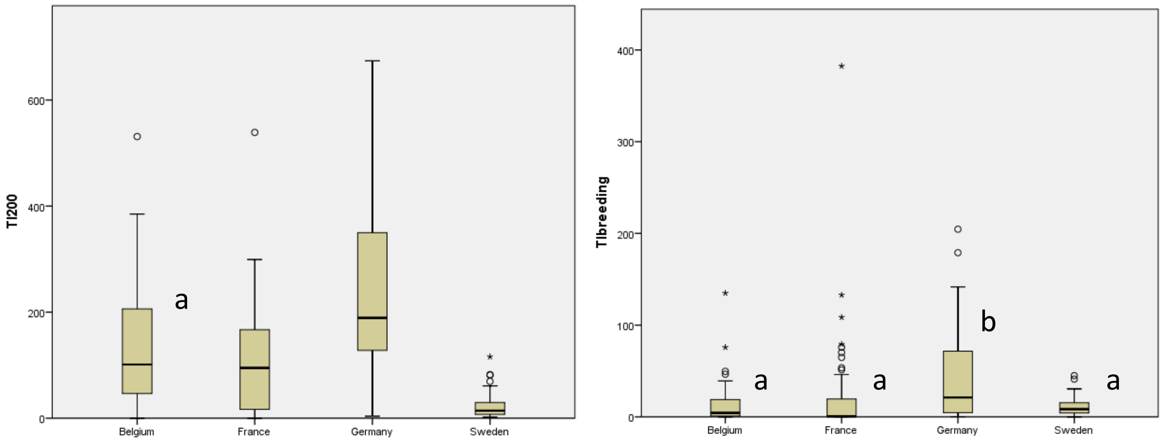


Fig. 1 Antimicrobial treatment incidences (TI), expressed as treatment incidence per 1000 pig-days at risk, for growing (a) and breeding (b) pigs in farrow-to-finish herds in Belgium (n=47), France (n=60), Germany (n=60) and Sweden (n=60). TI200 denotes the overall TI for growing pigs with a standardised lifespan of 200 days. Different letters denote significant differences between countries within the respective age categories

As biosecurity is concerned, Sweden had on average the highest total biosecurity status (63.7), followed by Germany (63.0), France (58.6) and Belgium (57.8) In all countries external biosecurity scored higher compared to internal biosecurity. A 3-week farrowing rhythm system was most commonly used (80/227 herds). The mean weaning age was highest in Sweden (35 days) and lowest in Belgium (23.5 days). The number of piglets weaned per sow per year was comparable in Belgium (27.2), France (26.5) and Germany (27.4), but lower in Sweden (23.2). In Belgium, France and Germany, the number of pathogens vaccinated against had a median of 7, while in Sweden this was 4. Out of 227 herds, 71 reported to use anti-inflammatory products in weaners, while 90 out of 227 used anti-coccidial products in suckling piglets.

Associations between antimicrobial usage and biosecurity and production characteristics that remained significant in the multivariable models are shown in the causal path in Fig. . The multivariable model revealed that a higher AMU in the breeders (LOG TI Breeders) was associated with a higher AMU in the growing pigs (LOG TI 200) as well as with the number of weaned piglets per sows per year ($p = 0.06$), meaning that farms with a higher AM in the breeding animals on average weaned slightly more piglets. For the AMU in the growing pigs the multivariable model showed associations with: 1) the weaning age ($p = 0.06$) indicating that herds with a higher weaning age showed a lower TI 200; 2) the farrowing rhythm suggesting lower AMU for 5-week or >5-week systems; 3) the external biosecurity status indicating that herds with a better biosecurity status had a lower TI 200 ($p < 0.01$); 4) the number of pathogens vaccinated against which is positively associated with the AMU ($p < 0.01$). It should be noted that parameters significant in the univariable analysis such as the internal biosecurity level, number of sows or employees, gender of the responsible person in the farrowing unit, the education level of the responsible person or the use of products like zinc oxide were not retained in the multivariable models. The level of AMU was furthermore not associated with production parameters such as ADG or mortality till weaning.

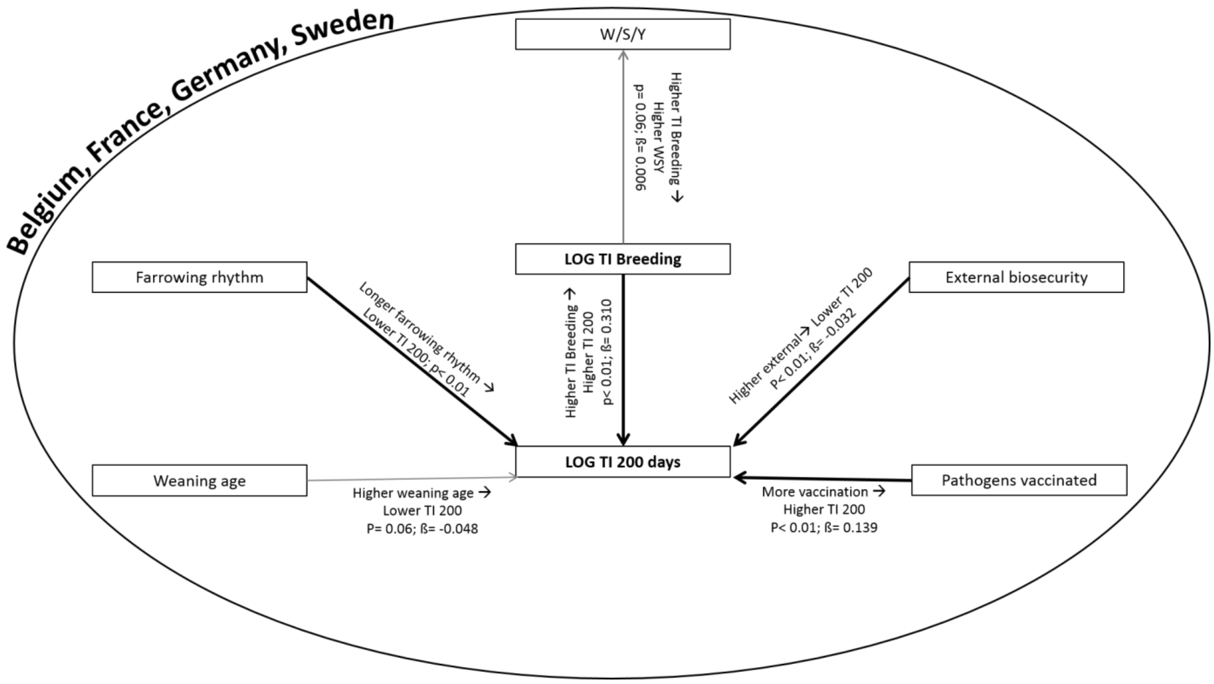


Fig. 2 Causal pathway with statistical significant associations in the multivariable models for TI 200 days and TI Breeding associated with significant production, management or biosecurity variables. TI= treatment incidence (antimicrobial usage quantification), WSY= number of weaned piglets per sow per year. Black lines represent the result of a multivariable linear regression analysis based on data from 4 EU countries. The light gray line indicates $0.05<p<0.10$. The p-values and β-values correspond to the multivariable model. All models were corrected for the country effect

The top farmers were characterised by an on average higher total and internal biosecurity status and were generally located in a more favourable environment (lower pig density and limited contact with wildlife). They also had fewer respiratory clinical symptoms in all age groups. However, the occurrence of gastro-intestinal symptoms in weaners in the top farms did not differ from the regular farms. In addition, twelve farms of the top group were located in unfavourable environment with high pig density, and three of them were affected both by Porcine reproductive and respiratory syndrome virus and Actinobacillus pleuropneumoniae.

DISCUSSION

Although it was attempted to select representative herds in each participating country, it should be noted that the participating herds could be members of the better end of the population since their inclusion was based on willingness to participate which may have caused some selection bias. Moreover, it should be stressed that the results were obtained based on a cross-sectional study which does not allow for claiming any causal associations. Through the causal web, however, it was attempted to describe and quantify the obtained associations and their interrelationships.

The highest average overall antimicrobial consumption in investigated herds was found among German herds and the lowest in Swedish herds when TIs for the entire growing period, TI200, were compared. This corresponds with European sales figures (ESVAC, 2015). In addition to the variation between countries, there were also large between-herd variations within the different countries. Besides management and biosecurity aspects, these differences might also partially be explained by treatment related factors such as route of administration (more parenteral treatments in Sweden) and the use of antimicrobials for prophylactic and metaphylactic treatments to entire batches of pigs.

Another very interesting finding was the positive association in AMU between the different age categories as well as the positive association between usage in the breeding animals and usage in pigs from birth to slaughter. This is in contrast to an often heard statement that a high AMU of young animals prevents disease problems in their further life. The observed associations may reflect a certain attitude/behaviour of the farmer towards regular usage of medicines (Visschers et al., 2015). It may also be caused by an overall higher disease pressure in these herds. A final potential explanation might be the deteriorating effect of AMU at young age on the bacterial gut flora resulting in higher disease susceptibility and treatment needs in later stages of their lives (Callens et al., 2014).

A number of relevant associations were identified. The first is the positive relation between the number of weaned piglets per sow per year and the amount of antimicrobial use in the breeders. Although this might be seen as a production stimulating effect of antimicrobial treatments in sows, it is more likely that the association should be reversed and that high productive sows are more sensitive to diseases and require more AM treatments, in which the higher productivity leads to a higher TI in the sows. The positive association between the number of pathogens vaccinated against and TI200 might be due to a high disease pressure on these herds which has not (yet) been brought under control through vaccination or other management practices, or might be an indication of a certain attitude of the farmer and/or his/her veterinarian, i.e. using/prescribing a lot of veterinary medicinal products as an insurance against disease (Visschers et al., 2015; Speksnijder et al., 2015). The association between weaning age and TI 200 suggests that a higher weaning age results in more robust animals who have a reduced necessity for antimicrobials. This is in agreement with the idea that stronger animals, for example when weaned at a later age, are also more likely to have better immunological coping abilities against possible (pathogenic) threats. One explanation for the lower TI 200 in longer farrowing rhythms might be that a longer period between two batches guarantees a better separation between age groups, resulting in less risk of transmission of pathogens between them. A last important finding was the association between the level of external biosecurity and the TI 200 ($p< 0.01$). This is in line with previous studies that have shown comparable associations (Laanen et al., 2013).

No significant positive associations were detected between a higher AMU and better production results such as ADG or mortality. This is in line with results of others who did not find such a relationship either (Chauvin et al., 2005; van der Fels-Klerx et al., 2011). It means that farms with high technical performances can have low to high antimicrobial usage levels. This is also confirmed by the “top farmers” analysis were it was found that a substantial part of the farmers did manage to combine low AMU and high technical performances. These results suggest that it is possible to control the impact of infectious diseases using other approaches than high antimicrobial usage (e.g. biosecurity), even in farms with challenging environmental and health conditions.

All above described results suggest that - besides the studied associations - it is very likely that the level of antimicrobial use is also strongly influenced by the attitudes of both farmers and veterinarians as described and discussed by Collineau et al. (2016).

In conclusion, the study documented high within and between country variation both in antimicrobial usage, biosecurity and management practices indicating substantial room for improvement. Moreover, some cross-country associations were identified suggesting potential interventions such as using a longer farrowing rhythm, weaning of the piglets at an older age and improvement of the biosecurity status. At the same time, an improved biosecurity is expected to result in improved technical performance.

ACKNOWLEDGEMENTS

The MINAPIG consortium would like to thank all participating farmers, their employees and herd veterinarians for their cooperation. The authors would also like to thank all members of the MINAPIG consortium for their help in developing the protocol for this study.

REFERENCES

Callens, B., Faes, C., Maes, D., Catry, B., Boyen, F., Francoys, D., de Jong, E., Haesebrouck, F. and Dewulf, J. (2014). Presence of antimicrobial resistance and antimicrobial use in sows are risk factors for antimicrobial resistance in their offspring. Microb. Drug Resist. 21, 50-58

Callens, B., Persoons, D., Maes, D., Laanen, M., Postma, M., Boyen, F., Haesebrouck, F., Butaye, P., Catry, B. and Dewulf, J. (2012). Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev. Vet. Med. 106, 53-62

Chauvin, C., Bouvarel, I., Beloeil, P.-A., Orand, J.-P., Guillemot, D. and Sanders, P. (2005). A pharmaco-epidemiological analysis of factors associated with antimicrobial consumption level in Turkey broiler flocks. Vet. Res. 36, 199–211

Chantziaras, I., Boyen, F., Callens, B. and Dewulf, J. (2014). Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. J. Antimicrob. Chemother. 69, 827-834

Collineau, L., Backhans, A., Loesken, S., Okholm Nielsen, E., Postma, M., Belloc, C., Dewulf, J., Emanuelson, U., Grosse Beilage, E., Siegrist, M., Sjölund, M., Stärk, K.D.C. and Visschers, V.H.M. (2016). Factors influencing pig farmers' and veterinarians' intention to reduce antimicrobial usage in six European countries. In: Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Copenhagen, Denmark

ECDC, EFSA and EMA (2015). ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. EFSA J. 13, 4006

ESVAC (2015). Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption

Filippitzi, M., Callens, B., Pardon, B., Persoons, D. and Dewulf, J. (2014). Antimicrobial use in pigs, broilers and veal calves. Vlaams Diergeneeskd. Tijdschr. 83, 215-224

Grave, K., Torren-Edo, J., Muller, A., Greko, C., Moulin, G. and Mackay, D. (2014). Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries. J. Antimicrob. Chemother. 69, 2284-2291

Laanen, M., Persoons, D., Ribbens, S., de Jong, E., Callen, B., Strubbe, M., Maes, D. and Dewulf, J. (2013). Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet. J. 198, 508-512

MARAN (2014). Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2013. http://wageningenur.nl/upload_mm/d/c/3/9e6f26a2-4a19-4042-9fb0-e32921d8bdee_NethMap-MARAN2014.pdf

Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Grosse Beilage, E., Stärk, K.D.C. and Dewulf, J. (2015a). The biosecurity status and its associations with production and management characteristics in farrow-to-finish pig herds. Animal, in press

Postma, M., Sjölund, M., Collineau, L., Lösken, S., Stärk, K.D.C. and Dewulf, J. (2015b). Assigning defined daily doses animal: A European multi-country experience for antimicrobial products authorized for usage in pigs. J. Antimicrob. Chemother. 70:294-302

Postma, M., Stärk, K.D.C., Sjölund, M., Backhans, A., Grosse Beilage, E., Lösken, S., Belloc, C., Collineau, L., Iten, D., Visschers, V., Nielsen, E.O. and Dewulf, J. (2015c). Alternatives to the use of antimicrobial agents in pig production: A multi-country expert-ranking of perceived effectiveness, feasibility and return on investment. Prev. Vet. Med. 118, 457-466

Speksnijder, D.C., Jaarsma, A.D.C., van der Gugten, A.C., Verheij, T.J.M. and Wagenaar, J.A. (2015). Determinants associated with veterinary antimicrobial prescribing in farm animals in the Netherlands: A qualitative study. Zoonoses Public Health 62, 39-51

Timmerman, T., Dewulf, J., Catry, B., Feyen, B., Opsomer, G., de Kruif, A. and Maes, D. (2006). Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. Prev. Vet. Med. 74, 251-263

van der Fels-Klerx, H.J., Puister-Jansen, L. F., van Asselt, E. D. and Burgers, S.L.G.E. (2011). Farm factors associated with the use of antibiotics in pig production. J. Anim. Sci. 89, 1922–1929

Visschers, V.H.M., Backhans, A., Collineau, L., Iten, D., Loesken, S., Postma, M., Belloc, C., Dewulf, J., Emanuelson, U., Grosse Beilage, E., Siegrist, M., Sjölund, M. and Stärk, K.D.C. (2015). Perceptions of antimicrobial usage, antimicrobial resistance and policy measures to reduce antimicrobial usage in convenient samples of Belgian, French, German, Swedish and Swiss pig farmers. Prev. Vet. Med. 119, 10-20

EMERGING INFECTIOUS DISEASES

SEASONAL VARIATION IN DEMAND OF ANIMAL PRODUCTS AS DRIVER OF DISEASE DYNAMICS: THE CASE OF AVIAN INFLUENZA IN VIETNAM

A. DELABOUGLISE*, D.T. PHAN, M. CHOISY, N. ANTOINE-MOUSSIAUX, D.T. VU,
M. PEYRE, D.U. PFEIFFER, G. FOURNIÉ

SUMMARY

The study aims to explore the climatic and anthropogenic factors that may explain the seasonality of HPAI H5N1 outbreaks in poultry in Vietnam. Wavelet coherence analysis was used to assess the effect of seasonal variations of several weather variables on occurrence of H5N1 highly pathogenic avian influenza (HPAI) in the domestic poultry population of Vietnam. This effect was not found to be coherent across the 3 main climatic zones of the country. However, half of the recorded outbreaks occurred during the month following the annual Lunar New Year festival, which takes place in January-February. According to a living standards survey conducted in 2004, the daily poultry meat consumption increases 4- to 9-fold during this period. To meet this rise in demand, the broiler poultry population and intensity of poultry movements are expected to peak during the months prior to this festival. This is likely to contribute to the seasonality of H5N1 HPAI.

INTRODUCTION

Factors influencing the temporal dynamics of communicable diseases, and, particularly, the seasonality of influenza viruses in humans are still subject to much discussion (Lipsitch & Viboud, 2009). So far the debate has mainly focused on the relative influence of ecological drivers (Tamerius et al., 2010). It has been suggested that low temperature and low relative humidity promote virus transmission (Lowen et al., 2007) and that reduced duration of exposure to sunlight affects the immune response of hosts to influenza infection (Cannell et al., 2006). Rainy seasons have been found to be significantly associated with peaks in influenza cases in tropical areas (Tamerius et al., 2013), and absolute humidity has been promoted as a parsimonious explanatory factor of influenza seasonality by experimental and epidemiological studies (Shaman & Kohn, 2009, Thai et al., 2015). Nevertheless, anthropogenic factors cannot be dismissed. For example, school closure during holidays reduces influenza transmission in children (Cauchemez et al., 2008).

Density and movements of hosts are likely to have an even greater effect on transmission of zoonotic influenza viruses in animals, and from animals to humans. Examples of such pathogens are influenza A subtypes H5N1 and H7N9 viruses, present in the domestic poultry populations of several countries of South and Southeast Asia, whose ongoing risk of emergence

---

* Alexis Delabouglise, Royal Veterinary College, Veterinary Epidemiology, Economy and Public Health Group, Hawkshead Lane, Hatfield, Hertfordshire, AL97TA, United Kingdom. Email: adelabouglise3@rvc.ac.uk

as pandemic viruses highly lethal for humans constitutes one of the major threats to public health (WHO, 2014). Population densities and intensity of movements of domestic animals are highly fluctuating in response to a continuously changing demand and supply of animal products (Rosen et al., 1993). Due to their shorter generation intervals, rapid growth and efficient feed conversion rates (Eurostat, 2014), poultry populations are faster to adapt to changes in demand than other domestic animal species, such as cattle or pigs. Until now this economic aspect of the HPAI problem has only received very limited consideration, mainly because of a lack of relevant data. Contrary to climatic variables, which are regularly recorded in meteorological stations, animal production is not closely monitored, especially in developing countries, where it remains mostly confined to the informal sector (Steinfeld et al., 2010). Further interdisciplinary work is needed to carefully address the relative contribution of economic and environmental factors to influenza epidemics, in order to properly design and evaluate surveillance and mitigation strategies (Gilbert & Pfeiffer, 2012).

In this study, the relationship between poultry production dynamics and the occurrence of highly pathogenic avian influenza (HPAI) H5N1 in Vietnam is investigated. Using Vietnam as case study country provides three advantages for examining this research question. There is climatic diversity across the country, from a subtropical climate in the North to a tropical climate in the South (Thai et al., 2015). The associated ecological diversity does not correlate with the ethnic diversity, where the dominant Kinh ethnic group encompasses 89% of the Vietnamese population (General Statistics Office of Vietnam, 2004) and is widely distributed across the whole country. People of this ethnic group share similar cultural features, including food consumption, religious customs and holidays. Finally, H5N1 avian influenza is endemic in the domestic poultry population since its first occurrence in 2003, affecting southern as well as northern areas of Vietnam (Pfeiffer et al., 2007). The occurrence of the disease is highly seasonal, with peaks occurring during the winter season in all regions (Minh et al., 2009). This seasonal pattern is still poorly understood.

A mixed investigational approach is presented here, combining an ecological and an economic study to understand the influence of different climate variables and changes in animal product consumption on the temporal distribution of cases of HPAI virus subtype H5N1 occurring in the domestic poultry population of Vietnam.

MATERIALS AND METHODS

Data sources

Monthly meteorological data records from January 2005 to December 2010 were supplied by the Vietnamese Institute of Meteorology, Hydrology and Environment. These records come from 67 climatic stations encompassing all provinces of Vietnam. Recorded variables were: average relative humidity (%), average absolute humidity (mmHg), cumulative rainfall (mm), total number of hours of sunshine, average, maximum and minimum temperature (°C).

Records of notified outbreaks of HPAI subtype H5N1 from January 2005 to September 2015 were provided by the Department of Animal Health of Vietnam. Records included dates of outbreak detection (or date of reception of samples at the laboratory), and names of provinces, districts and communes were outbreaks were detected.

Data obtained from the Vietnam Households Living Standards Survey (VHLSS), conducted in 2004, were supplied by the General Statistics Office of Vietnam. This survey was based on a sample of Vietnamese households covering all provinces of the country. It included questions on demographic characteristics of households as well as their sources of incomes and expenditures. Estimates of the quantity of poultry meat and eggs bought and home-produced and the estimated monetary value of these purchases and productions were recorded. The survey sample was stratified by province and by type of area (urban and rural), and clustered. Communes were randomly selected in each stratum; then 3 households per commune were randomly sampled.

Identification of climatic zones

The first step was the identification of geographical zones based on differences in the temporal variation of climate variables. Time series data from meteorological stations where all 7 climate variables were recorded from 2005 until 2010 were used for this purpose.

All 7 variables were scaled, so that their variances equalled 1 and their means equalled 0. We computed the Euclidean distance between wavelet transforms (Zhang et al., 2006) of scaled time series of each variable on the different meteorological stations using the R package *TSclust* (Montero & Vilar, 2014). Distances of each station to all others were summed to obtain dissimilarity scores.

Principal component analysis was performed on the obtained dissimilarity scores. To group stations into a pre-defined number of clusters, *k-means* clustering was performed on the values predicted by the principal component transformation, using 25 initial random sets. The stations belonging to the different clusters were mapped, as were the identified provinces belonging to each of the areas covered by the clusters of stations. These groups of provinces became the defined climatic zones.

Ecological analysis based on wavelet coherence

Wavelet coherence (Cazelles et al., 2008) between time series of monthly counts of HPAI H5N1 outbreaks and the medians of 5 climatic variables (average temperature, absolute humidity, relative humidity, sunshine duration, rainfall) was computed in each of the 3 climatic zones, using the R package biwavelet (Gouhier, 2014). Statistical significance of coherence levels between time series was computed based on a comparison of the obtained coherence with Monte Carlo simulations of 2000 control time series having the same first order auto-regressive coefficient as the series of observations.

Econometric analysis based on poultry consumption

Change in poultry consumption: The national livelihood standards survey includes two separate parts on food consumption; one including the total quantity of food specifically purchased or home produced by interviewed households during holidays for the purpose of specific celebrations, and another part dedicated to food consumed during the remaining parts of the year. A minimum and a maximum estimate of duration (in days) of each of these celebrations associated with an increased poultry consumption by Vietnamese households was provided by an expert of poultry production from the National University of Agriculture of Hanoi. It was considered that poultry consumption of households of minority ethnic groups remains unchanged during these holidays, as these groups have their own traditional festivities at different times of the year.

Based on these data, the ratio of average quantity of poultry meat consumed on a daily basis by Vietnamese households during and outside celebrations was estimated. The variance of the ratio was estimated using first-order Taylor series approximation. Weighting and clustering effects were accounted for in the calculation of the variances and sample weights were accounted for in the calculation of the ratio (Wears, 2002).

Factors affecting changes in poultry consumption: A two-level linear regression model was used to isolate the effect of several demographic variables on the absolute difference in daily poultry consumption between the celebrations of interest and the remaining parts of the year, using the R package lme4 (Bates et al., 2014). As the sample of households was stratified by province and type of area (rural and urban), these two categorical variables were included as independent variables in the model. Other independent variables included were: quantity of poultry consumed during the rest of the year by the household, age, gender and number of years of schooling of the head the household, size of household, income and proportion of infants, children and elderly members in the household. Ethnic minorities were not included in the analysis.

Distribution of outbreaks: Based on the previous results, yearly events characterised by a particularly strong increase in poultry consumption were targeted. HPAI H5N1 outbreaks notified in defined time intervals preceding and following these events were counted. Only the period 2008-2014 was considered, as the period 2005-2007 showed important waves of outbreaks which are likely to be linked with specific control strategies (Minh et al., 2009, Pfeiffer et al., 2007).

Statistical analysis and visualisation

All analyses and graphical and spatial representations were produced using R version 3.2.0 software (R core team, 2014).

RESULTS

Identification of the climatic regions

Results of principal component analysis of the 7 variables on dissimilarity scores of meteorological stations are displayed in Fig. 1. The two first components explain more than 73% of the overall variance of dissimilarity scores. The first component is mainly composed of absolute monthly humidity and monthly temperature, and the second of cumulative monthly rainfall, monthly cumulative hours of sunshine and monthly average relative humidity. Two stations have extreme values; they are both located at high altitude (>1500m) while all the others are located at less than 800m altitude above sea level. The other stations could be visually separated into 3 groups. It was therefore chosen to separate the stations into 4 clusters using the k-means clustering method.

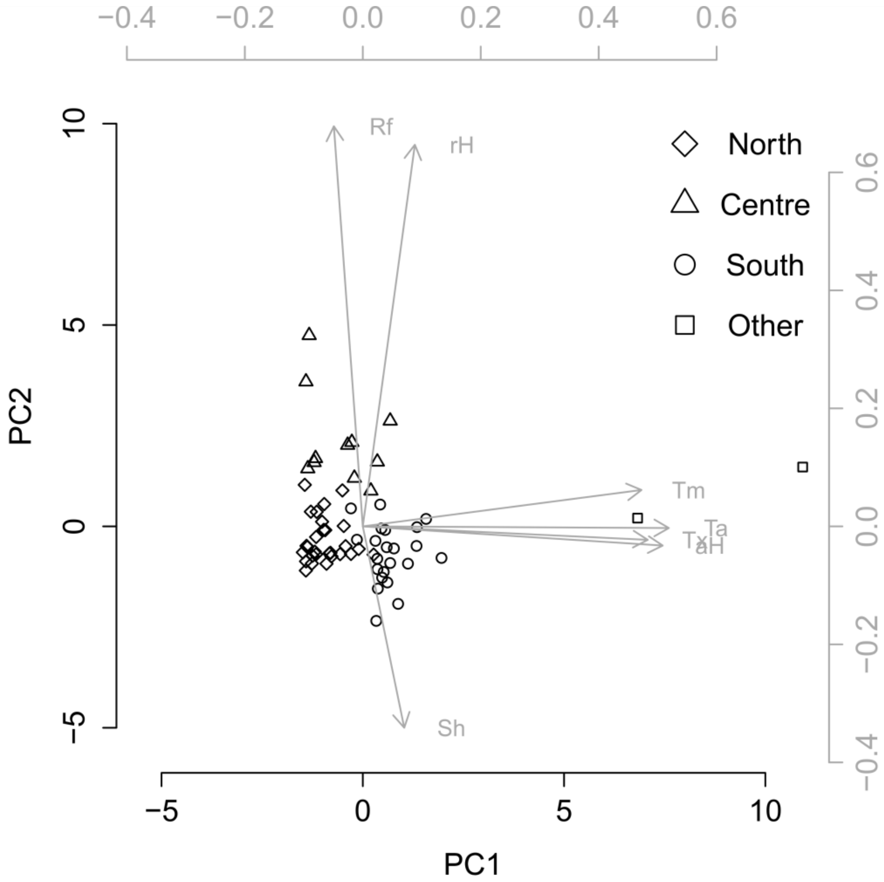


Fig. 1 Results of the principal component analysis of the 7 climatic variables (aH: absolute humidity; rH: relative humidity; Sh: hours of sunshine; Ta: average temperature; Tm: minimal temperature; Tx: maximal temperature). Dots correspond to meteorological stations with symbols indicating the different clusters

The geographical locations of stations belonging to each cluster, the climatic zones and the incidence of H5N1 outbreaks recorded in the domestic poultry population are displayed in Fig. 2. Three main climatic zones are distinguished by the clusters: North, Centre and South, all with high numbers of HPAI H5N1 outbreaks. All subsequent analyses was therefore conducted in each of these 3 zones.

Ecological analysis based on wavelet coherence

In the northern climatic zone, no coherence was detected during a one year period between wavelet transforms of monthly counts of HPAI H5N1 outbreaks and wavelet transforms of any climatic variables. In the central climatic zone, a coherence between one-year period wavelet transforms is noticeable, from 2007 until 2010. This coherence is statistically significant for wavelet transforms of median relative humidity, cumulative rainfall and cumulative hours of sunshine. In the southern climatic zone, coherence between one-year period wavelet transforms is observable at from 2005 until 2010. This coherence is statistically significant for wavelet transforms of median absolute humidity, cumulative rainfall, cumulative hours of sunshine and average temperature. Phase shifts between wavelet transforms in the 3 climatic zones are always different except for cumulative hours of sunshine (Table 1).

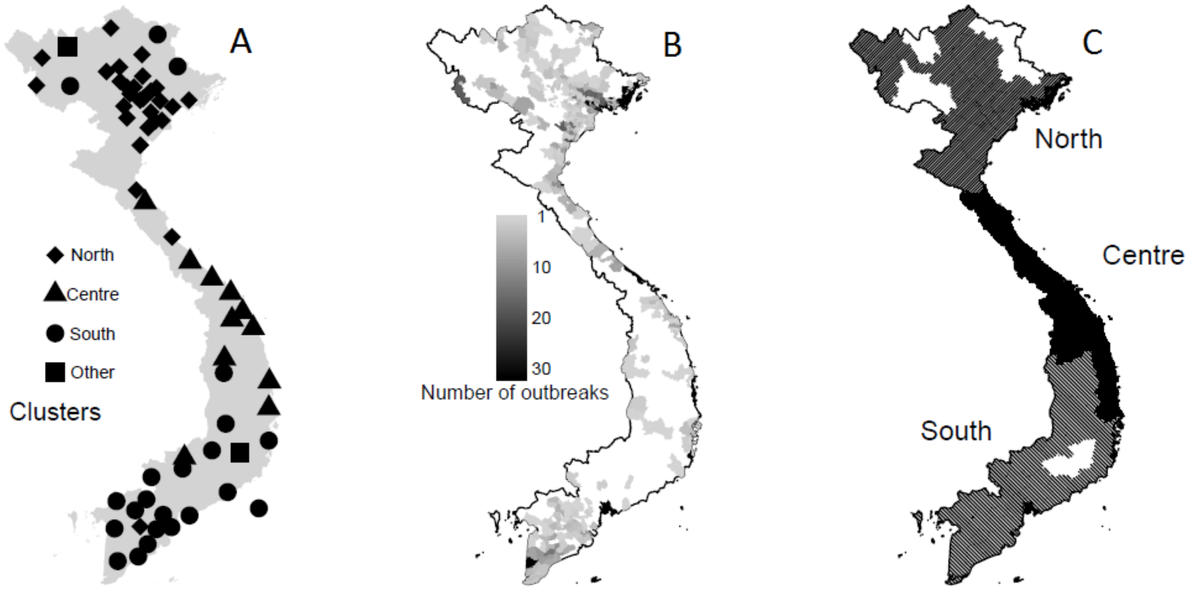


Fig. 2 Division of Vietnam into climatic zones, resulting from *k-means* clustering using climatic variables. A: location of meteorological stations belonging to each cluster. B: distribution of number of notified HPAI H5N1 outbreaks in domestic poultry per district. C: the 3 climatic zones

Table 1. Results of wavelet coherence analysis comparing monthly counts of HPAI H5N1 outbreaks with monthly climatic variables (aH: absolute humidity; rH: relative humidity; Sh: hours of sunshine; Ta: average temperature) in the 4 climatic regions. Absence of statistically significant coherence is indicated by (-). Where significant coherence was found, the trigonometric phase difference is given

Climatic zone	aH	rH	Rf	Sh	Ta
North	-	-	-	-	-
Centre	-	-1.3	2.8	+1.1	-
South	+2.7	-	-2.4	+1.0	+2.7

Econometric analysis based on poultry consumption

Events associated with increased poultry consumption: According to expert knowledge, poultry consumption mainly increases during the Lunar New Year holidays (*Tết Nguyên Đán*): traditional meals cooked during the festivities and worship of ancestors' spirits result in an increased demand for native or cross-bred chicken meat. This increased poultry consumption is likely to occur during at least six days: one celebration on the eve of Lunar New Year (*Rước ông bà*), followed by celebrations during the first four days of the year (*Tân Niên*) and one celebration on the day trade activities restart (*Khai Trường Đầu Năm*). But festivities can extend well beyond, depending on households. Other holidays characterised by increased poultry consumption are: King Hung day (*Ngày Giỗ tổ Hùng Vương*), the fifth day of the fifth lunar month (only for duck meat) (*Mùng 5 tháng 5) and the day of the dead (Cúng cô hồn*).

Depending on households, the reunification day or the independence day might also be an occasion for celebration. In total, it was estimated that a minimum of 10 days (including 6 days during the Lunar New Year period) and a maximum of 22 days (including 16 days during the Lunar New Year period) were characterised by increased poultry consumption.

The 2004 VHLSS survey targeted a total of 9189 households, of which 9180 indicated their poultry meat consumption. Ratios between the average quantity of poultry meat consumed during holidays and the average quantity consumed during remaining parts of the year are displayed in Table 2. The total quantity of meat consumed increased 4.3 to 9.6 fold. The increase in home produced meat consumption is higher than the increase in purchased meat consumption.

Table 2. Ratio between the estimated average quantity of poultry meat consumed during holidays and the quantity of meat consumed during the remaining parts of the year for Vietnamese households

		Min		Max	
		Ratio	Standard error	Ratio	Standard error
Source of poultry meat	Purchased	3.8	0.15	8.6	0.33
	Home produced	4.9	0.21	11.0	0.47
Total per climatic zone	North	4.9	0.16	10.9	0.36
	Centre	6.9	0.39	15.8	0.90
	South	3.3	0.15	7.4	0.34
TOTAL		4.3	0.11	9.6	0.24

The distribution of the ratios by province is displayed in Fig. 3. In accordance with the results displayed in Table 1, the increase in poultry consumption during holidays is higher in the central climatic zone (6.9-15.8), lower in the North (4.9-10.9) and even lower in the South (3.3-7.4).

Factors affecting difference of daily poultry consumption during holidays: Large, wealthy and well educated households living in rural areas tend to have a higher increase in their poultry consumption during selected holiday periods compared with other households ($p<0.01$). Some geographical effects were identified: inhabitants of Northern provinces bordering China increase their poultry consumption more during holidays, while living in the southern Mekong River Delta or in Ho Chi Minh City has the opposite effect ($p<0.01$).

Count of HPAI H5N1 outbreaks prior and after the Lunar New Year: The proportion of outbreaks notified in the 35 days prior to the Lunar New Year days is not high (10.5%). However, the 35 day periods following each Lunar New Year comprise 59.6%, 39.2% and 50.3% of outbreaks notified in the Southern, Central and Northern climatic zones, respectively (Fig. 4).

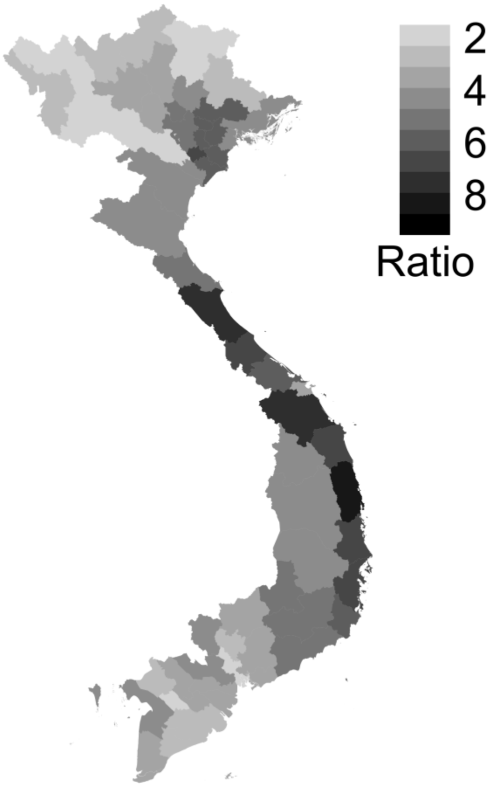


Fig. 3 Distribution of ratios of quantity of poultry consumed in holidays and the remaining parts of the year for each province of Vietnam (considering the number of celebration days equal to 22)

DISCUSSION

In this study, wavelet coherence analysis indicates that there is no consistent relationship between climatic variables and HPAI H5N1 outbreaks in the domestic poultry population across the whole country of Vietnam. Results differed between the 3 climatic zones, with absence of significant coherence in the North and phase differences between wavelet transforms varying between the two other climatic zones. The coherence with absolute humidity was particularly weak, considering that this variable has been mentioned as a critical factor for effective inter-individual transmission of influenza viruses (Shaman & Kohn, 2009). Because of synergistic effects, climatic factors might affect the spread of viruses in different ways in each of the climatic zones (Tamerius et al., 2013). Another interpretation is that other factors play a more crucial role in the dynamics of HPAI virus H5N1 in the domestic poultry population.

The Lunar New Year festival is characterised by an increased poultry consumption lasting 6 to 16 days. On such occasions, Vietnamese people prefer to consume native or cross-bred chickens rather than industrial breeds. Chickens of native breeds are mostly produced under backyard conditions (Hong Hanh et al., 2007), which explains the higher increase in home produced poultry consumption during holiday periods compared to purchased poultry consumption. It might also explain why rural households, who are more likely to raise poultry at home, have a particularly high increase in their poultry consumption during the holidays. Nevertheless, the quantity of purchased poultry meat also increases during the Lunar New Year

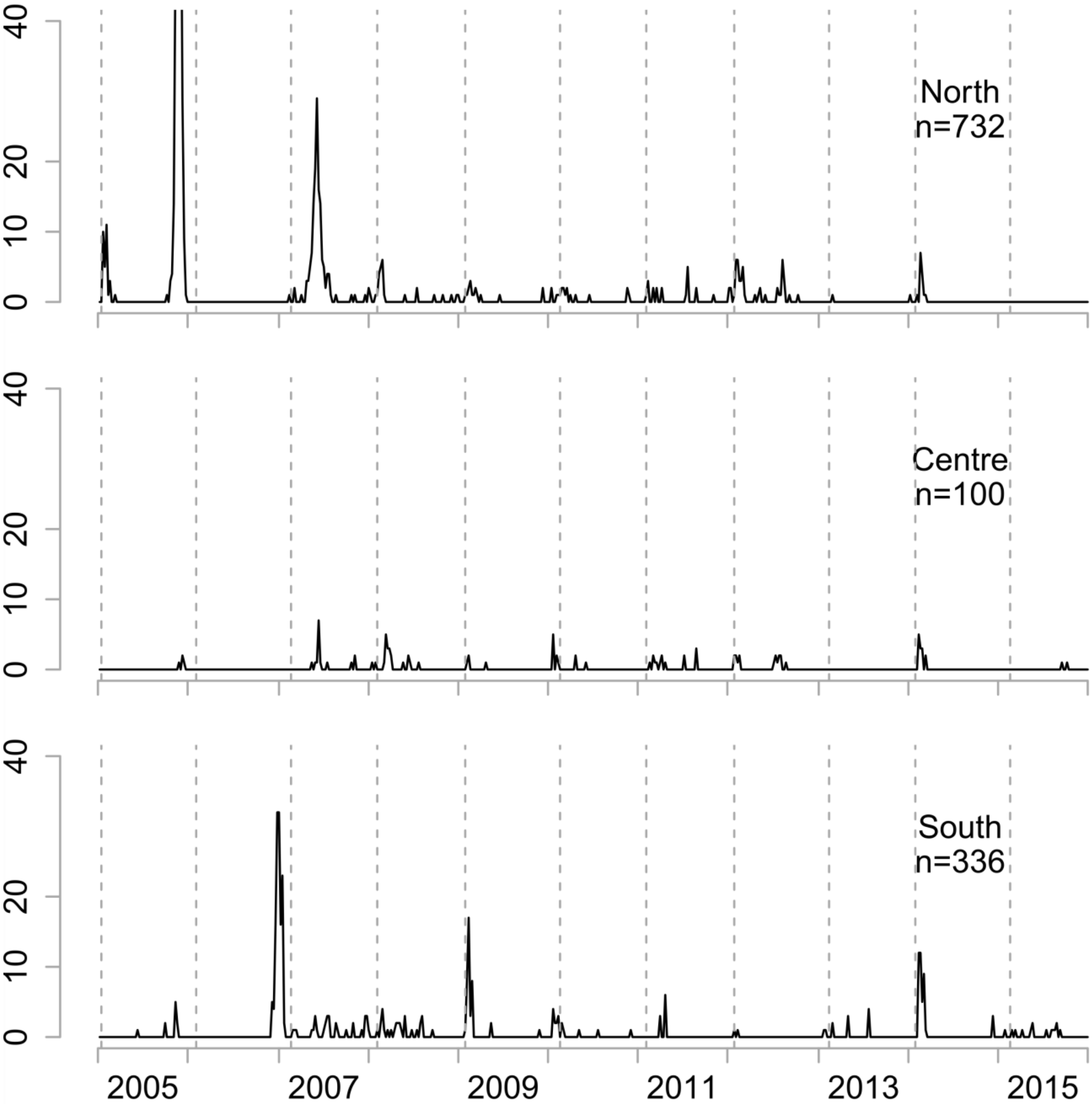


Fig.4 Weekly count of declared H5N1 outbreaks in the domestic poultry population of the three climatic regions of Vietnam. Dashed lines indicate Lunar New Years (n = total number of outbreaks)

period, meaning that the number of poultry produced for commercial purposes also increases before and during this period. Previous studies have demonstrated the contribution of commercial poultry movements to influenza virus spread (Fournie et al., 2013).

Half of the HPAI H5N1 outbreaks were notified during the five-week period following Lunar New Year in all 3 climatic zones. Outbreaks might actually occur long before and either remain undetected, or their notification is delayed (Walker et al., 2010, Minh et al., 2011). The fact that most outbreaks are recorded after, and not before, the Lunar New Year tends to suggest HPAI virus H5N1 spread is facilitated by poultry movements for sale during the festivities rather than the stocking of high numbers of poultry in farms during the months preceding the event. Another interpretation is a higher reluctance to report outbreaks during the days before Tet, as it would affect the commercial benefits made by farmers and other actors of poultry production at this time of the year. A previous sociological study showed that the fear of affecting the market price was one important reason why farmers as well as local government staff may not report avian influenza suspicions (Delabouglise et al., 2015).

The Lunar New Year festival appears to be a major contributor to the seasonality of HPAI H5N1 outbreaks in Vietnam and should be accounted for in the design of disease control strategies and their evaluation. The influence of culturally important holiday and festival periods on animal disease outbreaks is likely to be relevant also in other countries, such as China, and for other pathogens and host populations.

ACKNOWLEDGEMENTS

Alexis Delabouglise received funding from the European Union Seventh Framework Program (FP7 2007-2013) under grant agreement n°310806. Both Dirk Pfeiffer and Guillaume Fournié currently receive funding from the research programme ‘BALZAC, Behavioural adaptations in live poultry trading and farming systems and zonnoses control in Bangladesh’. This is one of 11 programmes funded under ZELS, a joint research initiative between Biotechnology and Biological Sciences Research Council (BBSRC), Defence Science and Technology Laboratory (DSTL), Department for International Development (DFID), Economic and Social Research Council (ESRC), Medical Research Council (MRC) and Natural Environment Research Council (NERC). The authors thank Dr Aaron Morris, from the Royal Veterinary College, London, UK, for his advice in the interpretation of the results of the wavelet coherence analyses.

REFERENCES

Bates, D., Maechler, M., Bolker, B.M. and Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7 [Online]. Available: http://CRAN.R-project.org/package=lme4 [Accessed August 10 2015]

Cannell, J.J., Vieth, R., Umhau, J.C., Holick, M.F., Grant, W.B., Madronich, S., Garland, C.F. and Giovannucci, E. (2006). Epidemic influenza and vitamin D. Epidemiol. Infect. 134**,** 1129-1140

Cauchemez, S., Valleron, A.J., Boelle, P.Y., Flahault, A. and Ferguson, N.M. (2008). Estimating the impact of school closure on influenza transmission from Sentinel data. Nature. 452**,** 750-754

Cazelles, B., Chavez, M., Berteaux, D., Ménard, F., Vik, J.O., Jenouvrier, S. and Stenseth, N.C. (2008). Wavelet analysis of ecological time series. Oecologia. 156**,** 287-304

Delabouglise, A., Antoine-Moussiaux, N., Phan, T.D., Dao, D.C., Nguyen, T.T., Truong, B.D., Nguyen, X.N.T., Vu, T.D., Nguyen, K.V., Le, H.T., Salem, G. and Peyre, M. (2015). The Perceived Value of Passive Animal Health Surveillance: The Case of Highly Pathogenic Avian Influenza in Vietnam. Zoonoses Public Health [Online]. Available: http://onlinelibrary.wiley.com/doi/10.1111/zph.12212/abstract [Accessed July 10 2015]

Eurostat. (2014). Meat production statistics [Online]. Available: http://ec.europa.eu/eurostat/statistics-explained/index.php/Meat_production_statistics [Accessed October 10 2015]

Fournie, G., Guitian, J., Desvaux, S., Cuong, V.C., Dung Do, H., Pfeiffer, D.U., Mangtani, P. and Ghani, A.C. (2013). Interventions for avian influenza A (H5N1) risk management in live bird market networks. Proc. Natl. Acad. Sci. USA. 110**,** 9177-9182

General Statistics Office of Vietnam (2004). Results of the Vietnam Living Standards Survey 2004. Statistical Publishing House, Hanoi, Vietnam, 712p

Gilbert, M. and Pfeiffer, D.U. (2012). Risk factor modelling of the spatio-temporal patterns of highly pathogenic avian influenza (HPAIV) H5N1: a review. Spat. Spatiotemporal. Epidemiol. 3**,** 173-183

Gouhier, T. (2014). biwavelet: conduct univariate and bivariate wavelet analyses (version 0.17.4) [Online]. Available: http://github.com/tgouhier/biwavelet [Accessed July 10 2015]

Hong Hanh, P.T., Burgos, S. and Roland-Holst, D. (2007). The Poultry Sector in Viet Nam: Prospects for Smallholder Producers in the Aftermath of the HPAI Crisis. Pro-Poor Livestock Policy Initiative Research Report. Food and Agriculture Organisation of the United Nations, Hanoi, Vietnam, 14p

Lipsitch, M. and Viboud, C. (2009). Influenza seasonality: Lifting the fog. Proc. Natl. Acad. Sci. USA. 106**,** 3645-3646

Lowen, A.C., Mubareka, S., Steel, J. and Palese, P. (2007). Influenza virus transmission is dependent on relative humidity and temperature. PloS Pathog. 3**,** 1470-1476

Minh, P.Q., Morris, R.S., Schauer, B., Stevenson, M., Benschop, J., Nam, H.V. and Jackson, R. (2009). Spatio-temporal epidemiology of highly pathogenic avian influenza outbreaks in the two deltas of Vietnam during 2003-2007. Prev. Vet. Med. 89**,** 16-24

Minh, P.Q., Stevenson, M.A., Jewell, C., French, N. and Schauer, B. (2011). Spatio–temporal analyses of highly pathogenic avian influenza H5N1 outbreaks in the Mekong River Delta, Vietnam, 2009. Spat. Spatiotemporal. Epidemiol. 2**,** 49-57

Montero, P. and Vilar, J.A. (2014). TSclust: An R Package for Time Series Clustering. J. Stat. Softw. 62**,** 1-43

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

R Core Team. (2014). R: a language and environment for statistical computing [Online]. Vienna, Austria. Available: http://www.R-project.org/ [Accessed October 8 2012]

Rosen, S., Murphy, K.M. and Sheinkma, J.A. (1993). Cattle Cycles. Cambridge, M.A., 24p

Shaman, J. and Kohn, M. (2009). Absolute humidity modulates influenza survival, transmission, and seasonality. Proc. Natl. Acad. Sci. USA. 106**,** 3243-3248

Steinfeld, H., Mooney, H.A., Schneider, F. and Neville, L.E. (2010). Livestock in a Changing Landscape: Drivers, Consequences and Responses. Island Press, Washington, D.C. 450p

Tamerius, J., Nelson, M.I., Zhou, S.Z., Viboud, C., Miller, M.A. and Alonso, W.J. (2010). Global Influenza Seasonality: Reconciling Patterns across Temperate and Tropical Regions. Environ. Health Perspect. 119**,** 439-445

Tamerius, J.D., Shaman, J., Alonso, W.J., Bloom-Feshbach, K., Uejio, C.K., Comrie, A. and Viboud, C. (2013). Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. PloS Pathog. 9**,** e1003194

Thai, P.Q., Choisy, M., Duong, T.N., Thiem, V.D., Yen, N.T., Hien, N.T., Weiss, D.J., Boni, M.F. and Horby, P. (2015). Seasonality of absolute humidity explains seasonality of influenza-like illness in Vietnam. Epidemics. 13**,** 65-73

Walker, P.G., Cauchemez, S., Metras, R., Dung Do, H., Pfeiffer, D. and Ghani, A.C. (2010). A Bayesian approach to quantifying the effects of mass poultry vaccination upon the spatial and temporal dynamics of H5N1 in Northern Vietnam. PLoS Comput. Biol. 6**,** e1000683

Wears, L.R. (2002). Advanced Statistics:Statistical Methods for Analyzing Cluster and Cluster-randomized Data. Acad. Emerg. Med. 9**,** 330-341

WHO. (2014). Avian Influenza [Online]. Geneva: World Health Organization. Available: http://www.who.int/mediacentre/factsheets/avian_influenza/en/ [Accessed October 10 2015]

Zhang, H., Ho, T.B., Zhang, Y. and Lin, M. (2006). Unsupervised Feature Extraction for Time Series Clustering Using Orthogonal Wavelet Transform. Informatica. 30**,** 305-319

RISK ASSESSMENT OF SEVEN EMERGING VECTOR-BORNE ANIMAL DISEASES FOR THE NETHERLANDS: A STRUCTURED APPROACH

C.J. DE VOS*, H.J.W. VAN ROERMUND, A.A. DE KOEIJER, E.A.J. FISCHER

SUMMARY

A structured risk assessment of seven vector-borne animal diseases (VBADs) indicated that the risk of all evaluated VBADs but Crimean-Congo haemorrhagic fever (CCHF) is moderate to high for the Netherlands. However, clear differences were observed when comparing the risk profiles of the VBADs based on probability of introduction and impact. Tularaemia and bovine babesiosis have a high probability of being introduced, but impact is low, whereas the probability of introduction of Rift Valley fever (RVF), epizootic haemorrhagic disease (EHD) and African horse sickness (AHS) is low, but impact is high due to trade restrictions (AHS and EHD) or zoonotic potential (RVF). West Nile has the highest overall risk with a high probability of introduction and moderate impact. The risk of CCHF is negligible because no competent vector is present in the Netherlands. It was concluded that the risk profiles provided useful information for risk management to prioritise diseases for preparedness.

INTRODUCTION

The Netherlands experienced two major epidemics of vector-borne animal diseases (VBADs) in the last decade resulting in severe economic losses for the Dutch livestock industry, viz. bluetongue in 2006-2007 and Schmallenberg in 2011-2012 (Velthuis et al., 2010; Koenraadt et al., 2014). This, combined with the increased incidence of vector-borne diseases such as West Nile and bluetongue in other European countries (Sambri et al., 2013; Zientara & Sánchez-Vizcaíno, 2013), has led to growing concern about the threat of VBADs for the Dutch livestock industry. Therefore, the risk of seven VBADs was assessed with the aim to prioritise diseases for preparedness and to identify common parameters among diseases that contribute most to the risk. A structured risk assessment approach was used, allowing for comparison and prioritisation of VBADs. The outcome of this risk assessment can be used to direct risk management to mitigate the risk of VBADs.

The selection of VBADs to include in the risk assessment was done in close cooperation with the Dutch Ministry of Economic Affairs, which is responsible for the prevention and control of exotic animal diseases in the Netherlands. To be included in the risk assessment, diseases had to be OIE listed (OIE, 2015) and had to be a perceived threat, either because of high morbidity and mortality in animals, expected economic impact, or geographic proximity to the Netherlands. The diseases selected were African horse sickness (AHS), bovine

---

* Clazien J. de Vos, Central Veterinary Institute, part of Wageningen UR, PO Box 65, 8200 AB Lelystad, The Netherlands. Email: clazien.devos@wur.nl

babesiosis (BB), Crimean-Congo haemorrhagic fever (CCHF), epizootic haemorrhagic disease (EHD), Rift Valley fever (RVF), tularaemia (TUL), and West Nile (WN). These diseases represent different types of pathogens (viruses, bacteria, protozoa) that are transmitted by a variety of arthropod vectors (ticks, mosquitoes, midges) and affect several vertebrate hosts (bovines, sheep, equines, wildlife). Five of the selected diseases are zoonotic.

MATERIALS AND METHODS

Structured approach

The risk assessment was performed using the FEVER framework (De Vos et al., 2011) and the MINTRISK calculation method (De Koeijer et al., 2014). FEVER is the acronym of a Framework to assess Emerging VEctor-borne disease Risks for livestock and offers a structured approach for qualitative risk assessment. MINTRISK is the acronym of a Method for INTegrated RISK assessment of vector-borne diseases and provides a calculation tool in Excel and Visual Basic for semi-quantitative risk assessment.

FEVER evaluates the probability of introduction of a disease pathogen into a predefined area at risk (here: the Netherlands), its subsequent spread and the consequences thereof. An outline of FEVER is given in Fig. 1, with the probabilities contributing to the risk at the left and the magnitudes of the consequences at the right. FEVER distinguishes six steps that contribute to the final risk estimate: (1) probability of entry, i.e., the probability that the pathogen causing the disease enters the area at risk, (2) probability of transmission, i.e., the probability that the pathogen is able to spread to susceptible hosts in the area at risk via a competent vector, (3) probability of establishment, i.e., the probability that the pathogen can spread from vector to host and vice versa given the conditions of entry into the area at risk (pathway, time and location), (4) extent of spread, i.e., the extent to which the pathogen is able to spread in the area at risk in time and space, (5) the likelihood of persistence, i.e., the likelihood that the pathogen will maintain itself in the area at risk for a prolonged period resulting in endemicity, and (6) impact of the disease on the livestock sector and – if zoonotic – on human health in the area at risk, including economic, socio-ethical, and environmental consequences.

MINTRISK is a semi-quantitative calculation method based on FEVER and uses the same steps to evaluate the introduction risk of VBADs. Input into the calculation tool is entered by scoring a set of questions for each step, choosing from five qualitative answer categories (very low, low, moderate, high, very high) with accompanying quantitative explanation. The latter ensures consistent answering of the questions, also when different risk assessors contribute to the risk assessment. For each question, the risk assessor can indicate his/her uncertainty in answering the question, choosing from three categories (low, moderate, high). Monte Carlo simulation is used to take this uncertainty into account when calculating the output for each step and the overall risk estimate. The output of MINTRISK is provided as semi-quantitative risk scores on a scale of 0 to 1 with associated uncertainty intervals, which are subsequently translated into qualitative risk terms.

Risk assessment

The risk of AHS, CCHF, and RVF for the Netherlands had recently been evaluated (Backer & Nodelijk, 2011; Hoek et al., 2011; Hoek et al., 2012; De Vos et al., 2012) and results of these studies were used to answer the questions in MINTRISK. For BB, EHD, TUL, and WN, a literature study was performed to answer the questions, the results of which were bilaterally discussed with disease experts to reduce uncertainty. Then, risk scores were calculated in MINTRISK using 1,000 iterations. The results of this semi-quantitative risk assessment were subsequently presented and discussed in two expert workshops after which the risk assessment was finalised.

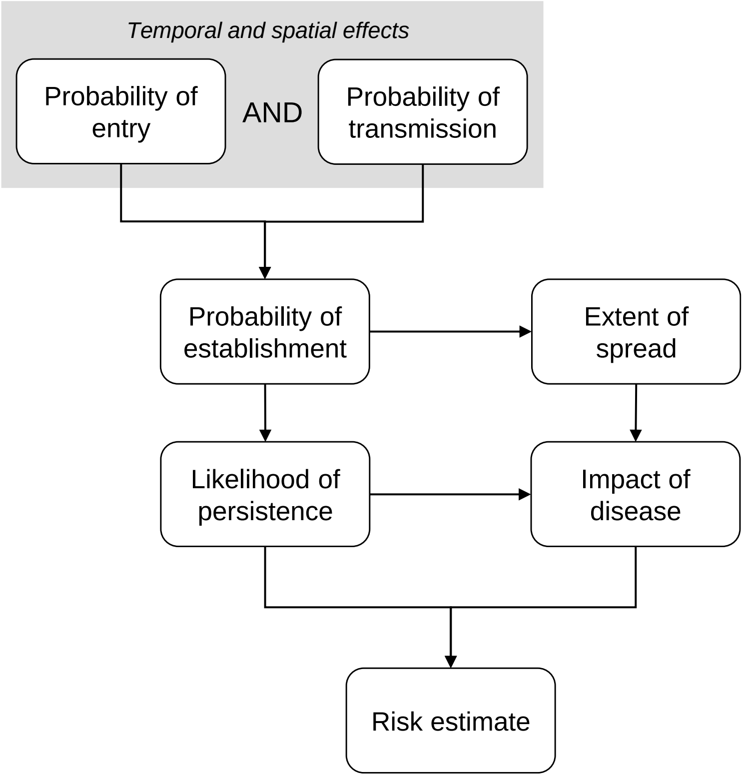


Fig. 1 Outline of FEVER: A Framework to assess Emerging VEctor-borne disease Risks for livestock

RESULTS

Hazard identification

The risk assessment evaluates the annual introduction risk of the selected VBADs for the Netherlands. An overview of the diseases, their pathogens, arthropod vectors, susceptible vertebrate hosts, and current geographical distribution is given in Table 1. The risk assessment of EHD was limited to EHD virus serotype 6 because of its recent occurrence in North-Africa and Turkey, where clinical disease in cattle has been reported (Savini et al., 2011). The risk assessment of TUL was limited to Francisella tularensis subs. holarctica because of its widespread occurrence in Europe (Gyuranecz, 2012; Hestvik et al., 2014).

Probability of introduction

The probability of introduction summarises the probability of entry (step 1) and the probability of establishment (step 3), the latter taking into account the probability of transmission (step 2). The probability of introduction thus indicates the probability of a successful entry, i.e. entry of the pathogen resulting in establishment in the area at risk and

Table 1. Overview of causing pathogens, vertebrate hosts, arthropod vectors, and current geographical distribution of seven VBADs[a]

Disease	Pathogen[b]	Vertebrate host	Vector	Zoonosis	Geographical distribution
African horse sickness	AHS virus (*Orbivirus*, *Reoviridae*)	Equines	*Culicoides* spp.	No	Sub Saharan Africa
Bovine babesiosis	*Babesia divergens* (*Babesia*, *Babesiidae*)	Bovines	*Ixodes ricinus*	Yes[c]	Europe, Tunisia, Turkey
Crimean-Congo haemorrhagic fever	CCHF virus (*Nairovirus*, *Bunyaviridae*)	Mammals	*Hyalomma* spp.	Yes	South-eastern Europe, Middle East, Central Asia, Arabian Peninsula, Sub-Saharan Africa
Epizootic haemorrhagic disease	EHD virus serotype 6 (*Orbivirus*, *Reoviridae*)	Deer, bovines, sheep	*Culicoides* spp.	No	Morocco, Algeria, Tunisia, Turkey, Reunion Island, Guadeloupe, Australia, USA
Rift Valley fever	RVF virus (*Phlebovirus*, *Bunyaviridae*)	Bovines, sheep, goats, wild ungulates, rodents	Mosquitoes of genera *Aedes*, *Culex*, *Anopheles*, *Ochlerotatus*	Yes	Sub Saharan Africa, Egypt, Arabian Peninsula
Tularaemia	*Francisella tularensis* subs. *holarctica* (*Francisella*, *Francisellaceae*)	Lagomorphs, rodents	Hard ticks (*Ixodidae*), mosquitoes, tabanids	Yes	USA, Scandinavia, Russia, Central and Southern Europe
West Nile	West Nile virus (*Flavivirus*, *Flaviviridae*)	Birds, equines	*Culex* spp.	Yes	South, Central and Eastern Europe, Middle East, Asia, Australia, North, Central and South America, Africa

[a] References

African horse sickness: Mellor & Hamblin, 2004; Coetzer & Guthrie, 2004; Boinas et al., 2009; MacLachlan & Guthrie, 2010
Bovine babesiosis: L’ Hostis & Seegers, 2002; Zintl et al., 2003; EFSA, 2010
Crimean-Congo haemorrhagic fever: Hoogstraal, 1979; Ergonul & Whitehouse, 2007; Bente et al., 2013
Epizootic haemorrhagic disease: MacLachlan & Osburn, 2004; EFSA, 2009; Savini et al., 2011; Sailleau et al., 2012
Rift Valley fever: Swanepoel & Coetzer, 2004; EFSA, 2005; Pepin et al., 2010; EFSA, 2013
Tularaemia: Petersen et al., 2009; Foley & Nieto, 2010; Gyuranecz, 2012; Carvalho et al., 2014; Hestvik et al., 2014
West Nile: Hayes et al., 2005; Reiter, 2010; Pradier et al., 2012; Roberts & Crabb, 2012; Sambri et al., 2013

[b] Genus and family of pathogen given between brackets

[c] Very low incidence, only in splenectomised people (Zintl et al., 2003)

subsequent spread. Establishment is most likely if entry of the pathogen occurs in the vector season and in regions where both vectors and hosts are present in sufficient numbers. An overview of the introduction probability of all seven VBADs for the Netherlands is given in Fig. 2. The pathways that contribute most to the probability of introduction of each VBAD are indicated in Table 2.

The probability of introduction of BB, TUL, and WN was estimated to be high to very high. This is in line with field observations for BB and TUL. Infections with Babesia divergens are diagnosed several times each year in the Netherlands (pers. comm. M. Holzhauer, GD Animal Health), some of which might be due to new introductions of the pathogen, whereas other infections result from existing foci of transmission. Trade in cattle contributes most to the probability of BB introduction. F. tularensis is present in countries neighbouring the Netherlands and infections have been observed in both hares and humans in the Netherlands in recent years (Rijks et al., 2013; Koene et al., 2015). Infected tick larvae and nymphs that enter the Netherlands via migrating wildlife or traded animals contribute most to the probability of TUL introduction, but other routes cannot be excluded. The probability of establishment of TUL in the Netherlands is high, since in most cases infected ticks or infected animals will be introduced in an area where both vectors and hosts are present in sufficient numbers. This is in contrast with BB, which has a low probability of establishment, because most cattle in the Netherlands are not grazing in areas where the tick vector of BB, Ixodes ricinus, is present.

The relatively high risk score for the probability of introduction of WN was unexpected. Infected mosquitoes that enter the Netherlands via containers contribute most to this probability of introduction, although the pathway of infected migratory birds was also evaluated to have a moderate to high probability of introducing WN. Establishment of WN that enters the Netherlands via this pathway is, however, less likely because most migratory birds originating from WN-infected regions arrive in early spring, i.e., before the start of the vector season.

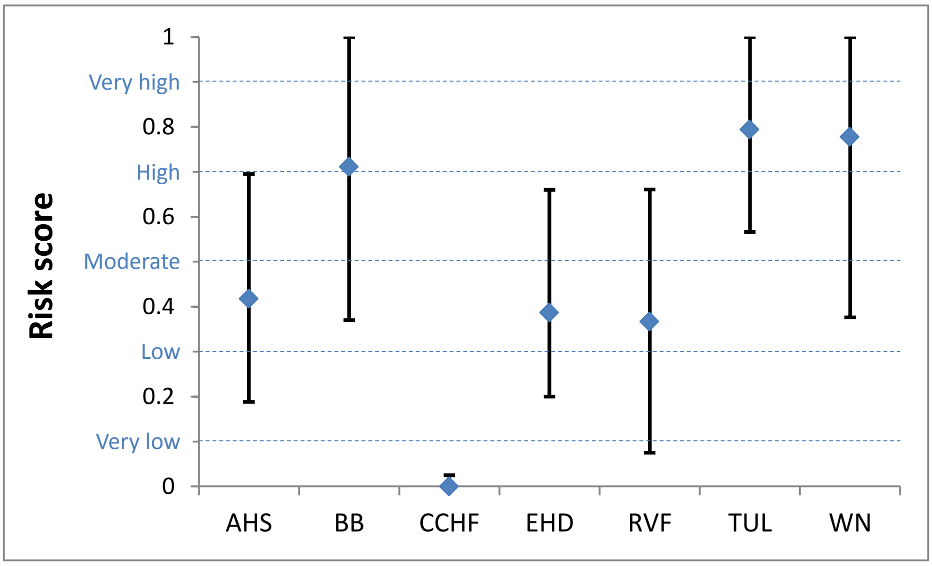


Fig. 2 Risk scores and their uncertainty for the probability of introduction of seven VBADs for the Netherlands

Table 2. Pathways contributing to the probability of introduction of each disease with the top ranking pathway or pathways indicated in bold

Group	Pathway	AHS	BB	CCHF	EHD	RVF	TUL	WN
Infected host animals								
	Legal trade in livestock		**X**	**X**		X		
	Illegal trade in livestock	X	**X**	**X**	**X**	X		X
	Trade in exotic animals							
	Trade in pet animals							
	Migration of wildlife						X	
	Migratory birds							X
Infected vectors (eggs, larvae, nymphs, adults)								
	Active flight							
	Passive flight (wind, air currents)							
	Transport vehicles (aircraft, ship, road transport)	X			X	X		X
	Containers on aircraft or ship					**X**		**X**
	Imported products (plant material, tires)					X		
	Traded animals (livestock, pets)	**X**	X	X	X		X	
	Migration of wildlife		X				X	
	Migratory birds		X	X			**X**	
Contaminated products								
	Products for consumption (meat, milk, eggs)				X			
	Genetic material (semen, ova, embryos)							
	Biological material (serum, plasma)				X			
	Vaccines	X	X					

[a] Infected humans were not evaluated as pathways for introduction because humans are considered dead-end hosts for all zoonotic infections evaluated in this risk assessment

The probability of introduction of AHS, EHD, and RVF was estimated to be low to moderate. For both AHS and EHD this estimate is based on the assumption that at least one of the Palearctic Culicoides species in the Netherlands is a competent vector for these viruses. Illegal trade in livestock from Mediterranean countries contributes most to the probability of EHD introduction, whereas infected adult vectors (Culicoides spp.) that enter the Netherlands via traded livestock contribute most to the probability of AHS introduction.

In assessing the probability of RVF introduction for the Netherlands, a mismatch was observed between the most likely pathways for entry of RVF and the most likely conditions in which establishment is feasible. Entry of RVF via infected mosquito vectors has a high probability, but this results in entry at places with a relatively low probability of establishment (e.g. airports and harbours). Entry of RVF via legal import of animals from RVF-infected countries is not very likely, but if this would happen, the probability of establishment is very high in the vector season. Entry of RVF via illegal import of animals has a higher probability of occurrence, but is less likely to result in establishment because the animals are more likely to be slaughtered or to end up in urban areas.

The probability of CCHF introduction was estimated to be negligible, i.e. entry resulting in establishment is expected not to occur. The main reason for this conclusion is the absence of a competent tick vector (Hyalomma spp.) in the Netherlands which inhibits transmission and establishment of CCHF. CCHF was therefore not addressed in the next steps of the risk assessment.

Impact of disease

The impact of disease depends on the extent of spread (step 4), i.e. the number of infected animals/herds and the geographical area affected, and the likelihood of persistence resulting in endemicity (step 5). Furthermore, parameters describing the severity of clinical disease in animals and – if zoonotic – in humans, the effects of control measures, and economic implications such as trade bans need to be considered. Impact of disease (step 6) was estimated taking into account economic losses, socio-ethical impact and environmental impact. The overall impact of AHS, EHD, and RVF was estimated to be high to very high, whereas the overall impact of BB, TUL, and WN was estimated to be low to moderate (Fig. 3).

Extent of spread: The estimated basic reproduction number R0 is higher for infections in which ticks contribute to transmission (BB and TUL) than for insect-borne infections (AHS, EHD, RVF, and WN). Despite these higher R0 values, spread of BB and TUL is expected to be slow because of the low number of infection generations per vector season. Hence, no large outbreaks are to be expected in the year of introduction for any of the evaluated VBADs, either because of a low R0 value or because of a long generation time. The extent of spread was estimated to be the highest for AHS and TUL, although the uncertainty of the assessment indicates that larger outbreaks are possible for all diseases but BB. Spread of BB is inhibited by the limited spatial overlap between its vector (I. ricinus in forest habitat) and its host (cattle on pastures). The probability of detection in the first vector season is higher for EHD and AHS than for BB, RVF, TUL, and WN and might limit the extent of spread of these Culicoides-borne infections if effective control measures are available.

Likelihood of persistence: The likelihood of persistence of a disease depends on the probability that natural fade-out of the infection does not occur in the first vector season, the probability that the infection is not detected (and controlled) in the first vector season, and the

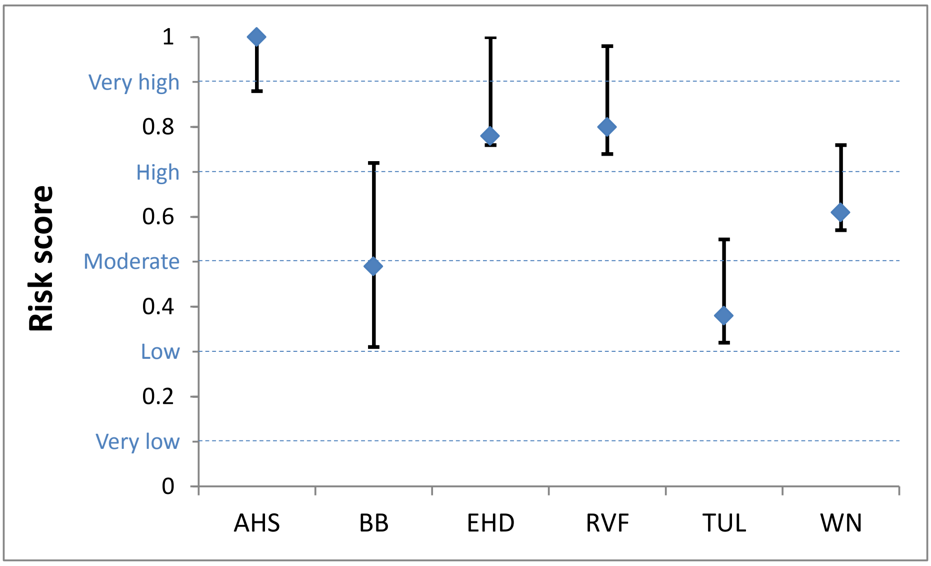


Fig. 3 Risk scores and their uncertainty for the impact of six VBADs for the Netherlands

probability that the infection is able to survive into the next vector season, i.e. that the infection overwinters in the Netherlands.

Fade out of the evaluated VBADs is not expected to occur in the first vector season, with the exception of EHD. Build-up of herd immunity is expected to be moderate for EHD, which, in combination with early detection, might result in fade out. Although detection of AHS is also likely to occur in the first vector season, the development of herd immunity will be slow due to the high case fatality rate in horses (> 70%) (Coetzer & Guthrie, 2004).

The probability of overwintering is highest for BB and TUL. Both pathogens have the opportunity to survive in their tick vector population. Furthermore, BB can also overwinter due to chronic infections in cattle (Zintl et al., 2003), whereas TUL has the opportunity to survive in the environment (water, carcasses, soil, dust, etc.) (Carvalho et al., 2014). For all insect-borne VBADs, overwintering is a critical issue for persistence and fraught with uncertainty. Overwintering of AHS, EHD, and WN is deemed most likely via a low-level continuous transmission cycle between vector and host (Mellor, 1993; Pradier et al., 2012), whereas vertical transmission in the vector is considered the most likely overwintering mechanism for RVF (Linthicum et al., 1985; Pepin et al., 2010).

Economic, socio-ethical and environmental impact: Economic losses contributed most to the overall impact. Indirect agricultural losses due to trade restrictions are to be expected in case of outbreaks of AHS, EHD, and RVF and are a major component of the economic losses incurred by these VBADs. Loss of animals due to infection or control measures adds to the economic losses of VBADs and are especially high for AHS due to the possible loss of expensive horses. Morbidity and mortality in horses also adds to the economic losses of WN, although to a lesser extent. Furthermore, direct and indirect costs resulting from human infections will contribute to the economic losses of WN and – to a lesser extent – of RVF and TUL. For RVF and TUL, less human cases are expected due to the limited contribution of vectors in transmitting these infections to humans. Human infections of RVF and WN might result in high socio-ethical impact, because of societal anxiety due to potentially fatal infections in humans. High morbidity and mortality in horses due to AHS infections is also expected to result in high socio-ethical impact, because of the close relationship of humans and horses in the Netherlands. Environmental impact is expected to be very low to negligible for all evaluated

VBADs, because neither the pathogens themselves nor vector control measures are thought to have a substantial impact on biodiversity and nature values.

Risk estimate

The overall risk estimate calculated by MINTRISK takes into account the results of all six steps of the FEVER framework. The resulting risk scores can be used to rank VBADs for their risk. The overall risk estimate was moderate to high for all evaluated VBADs but CCHF (Fig. 4). Establishment of CCHF is unlikely given the absence of a competent vector in the Netherlands. Although WN had the highest risk score, no ranking could be given because of overlapping uncertainty intervals. Hence, the overall risk estimate was not informative for prioritisation of the evaluated VBADs in this study.

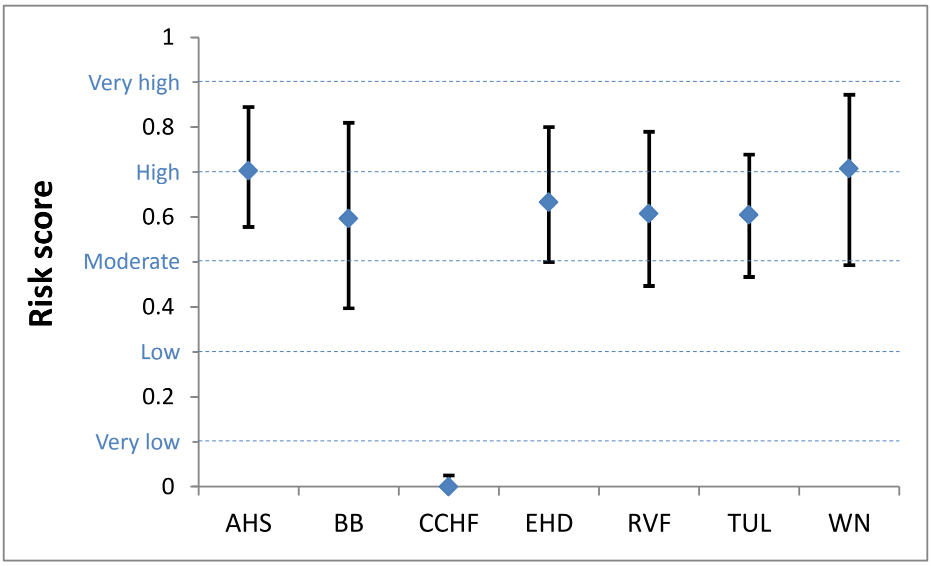


Fig. 4 Risk scores and their uncertainty for the overall risk estimate of seven VBADs for the Netherlands

The results of the risk assessment were therefore presented using a risk profile diagram (Fig. 5). In this diagram, the overall risk is presented by separating the probability of introduction and the impact of disease. Now, three groups of VBADs can be distinguished. The first group consists of AHS, EHD, and RVF that all have a low probability of introduction, but high to very high impact. The second group consists of BB, TUL, and WN with a high probability of introduction, but only low to moderate impact. The third group is CCHF that does not present a risk to the Netherlands in the absence of a competent vector. Based on this diagram, WN ranks top again being the only disease in the upper right quadrant, i.e., both the probability of introduction and impact exceed moderate.

DISCUSSION

This is the first study in which FEVER and MINTRISK were applied. The structured approach of these methods provided good guidance in consistently assessing the risk of all seven VBADs allowing for comparison of results. Feeding the MINTRISK calculation tool is

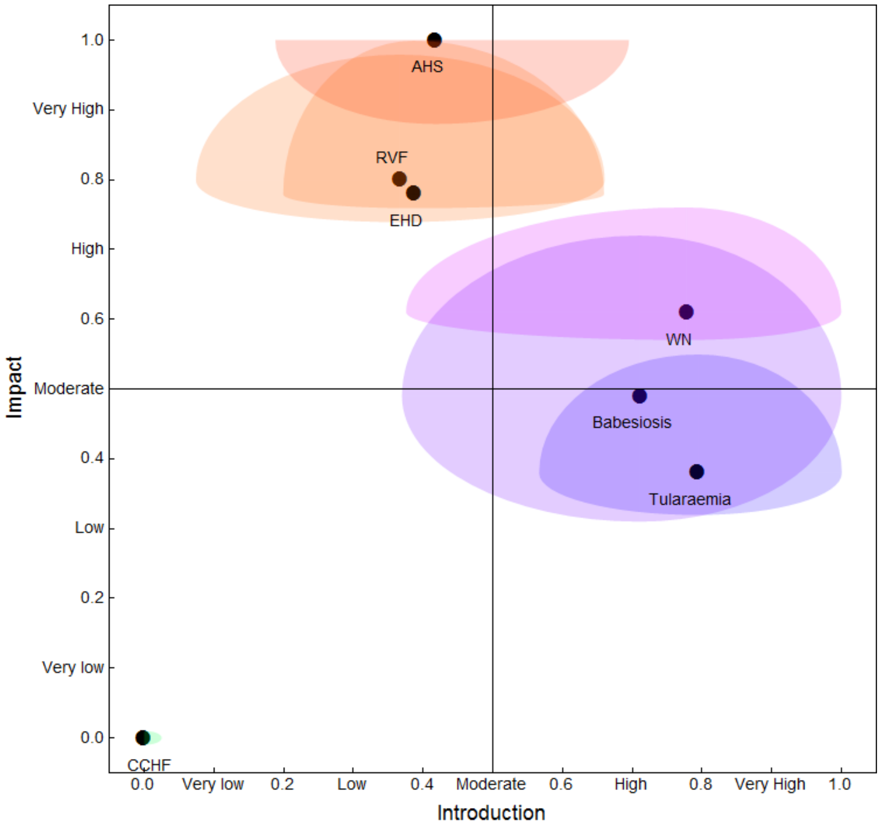


Fig. 5 Risk profiles of seven VBADs for the Netherlands indicating the risk scores for the probability of introduction and impact of disease. The dots in the figure indicate the point estimates of the risk scores and the surrounding surfaces indicate the two-dimensional uncertainty intervals

rather data-intensive, despite the fact that it is a semi-quantitative risk assessment method. Risk scores for the overall risk estimate were similar for six out of the seven evaluated VBADs. However, disentangling probabilities and consequences in a risk profile diagram (Fig. 5) provided useful information for risk management purposes.

The risk assessment indicated three clusters of VBADs (Fig. 5). AHS, EHD, and RVF all have a low probability of introduction but their impact is expected to be high to very high if introduced. BB, TUL, and WN, on the other hand, all have a high probability of introduction but with low to moderate impact only. The probability of introduction of CCHF was deemed negligible because no competent vector is present in the Netherlands and its impact was therefore not assessed. The risk of CCHF needs to be re-evaluated if Hyalomma ticks would establish in the Netherlands or if other tick species would be indicated as competent vectors for CCHF.

Probabilities of introduction were higher for those infections that are geographically most close to the Netherlands. AHS, EHD, and RVF are currently not present in Europe in contrast to BB, TUL, and WN. BB and TUL have even been observed in the Netherlands in recent years and are thus no longer exotic to the Netherlands. However, new introductions of those pathogens from abroad might result in larger outbreaks, especially if being introduced in naive herds (BB) or regions not affected yet (TUL). In this study, only the risk of such new introductions was assessed. Legally or illegally traded animals as well as migratory wildlife and birds are important entry pathways for all evaluated VBADs, either because the animals themselves might be infected or because they might carry infected vectors. The numbers of infected animals, vectors or commodities moved along the entry pathways determined to a large extent the estimated probabilities of entry and could not be estimated with certainty.

Establishment of VBADs is most likely if entry of the pathogens occurs in the vector season and in regions where both vectors and hosts are present in sufficient numbers. The establishment of tick-borne infections is less dependent on the arrival time of infection, because the infection can persist for a long period (years) in the vector population (Zintl et al., 2003). Hence, for BB, CCHF, and TUL, the location of introduction is most important for successful establishment, whereas for AHS, EHD, RVF, and WN timing of the introduction is most important. Mitigation of the risk should thus focus on the temporal component for insect-borne infections and on the spatial overlap of vector and host population for infections in which ticks contribute to transmission.

In the year of introduction, no large outbreaks are expected for any of the evaluated VBADs. This is due to the low number of infection generations per vector season, especially if transmission is tick-borne. Detection in the first vector season is only expected for AHS and EHD. That means that transmission of the other evaluated VBADs will go unnoticed during the first vector season and that no control measures will be implied. These infections hence might persist in the Netherlands if overwintering is possible.

The evaluated impact was especially high if presence of the infection would result in trade restrictions with associated large economic losses. This is expected for AHS, EHD, and RVF. Socio-ethical impact was highest for those infections resulting in horse morbidity and mortality (AHS and WN) or severe human illness (RVF and WN). Environmental impact was expected to be negligible and did not really contribute to the risk score for impact.

Most of the resulting risk scores had a wide uncertainty interval, especially those for the steps extent of spread and likelihood of persistence. Major uncertainties in estimating the risk of these seven VBADs included (illegal) trade volumes, vectorial capacity, and overwintering opportunities. Improved knowledge on these parameters will narrow the uncertainty intervals of the estimated risk.

ACKNOWLEDGEMENTS

This project was commissioned and financed by the Dutch Ministry of Economic Affairs (BO-20-009-026). We would like to thank Dr Rijks (UU), Dr Holzhauer (GD), Dr Van Maanen (GD), Mr Stroo (NVWA), Dr Van Rijn (CVI), Mrs Koene (CVI), Prof Jongejan (UU), Dr Van der Giessen (RIVM), and Dr Nodelijk (CVI) for their input into the risk assessment.

REFERENCES

Backer, J.A. and Nodelijk, G. (2011). Transmission and control of African horse sickness in the Netherlands: A model analysis. PLoS ONE 6, e23066

Bente, D.A., Forrester, N.L., Watts, D.M., McAuley, A.J., Whitehouse, C.A. and Bray, M., (2013). Crimean-Congo hemorrhagic fever: History, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antivir. Res. 100, 159-189

Boinas, F., Calistria, P., Domingo, M., Martínez-Avilés, M., Martínez-López, B., Rodríguez-Sánchez, B. and Sánchez-Vizcaíno, J.M. (2009). Scientific report on African horse sickness submitted to EFSA. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4e.pdf. Accessed November 26, 2015

Carvalho, C.L., Lopes de Carvalho, I., Zé-Zé, L., Núncio, M.S. and Duarte, E.L. (2014). Tularaemia: A challening zoonosis. Comp. Immunol. Microb. 37, 85-96

Coetzer, J.A.W. and Guthrie, A.J. (2004). African horse sickness. In: Coetzer, J.A.W. and Tustin, R.C. (Eds.) Infectious Diseases of Livestock, 2nd Edition. Oxford University Press Southern Africa, Cape Town, South Africa, pp. 1231-1246

De Koeijer, A., Hennen, W., Dhollander, S. and De Vos, C. (2014). Method for integral risk assessment of vector-borne livestock infections (MINTRISK). 8th Annual EPIZONE Meeting “Primed for tomorrow”, 23-25 September 2014, Copenhagen, Denmark

De Vos, C., Hoek, M., Fischer, E., De Koeijer, A. and Bremmer, J. (2011). Risk assessment framework for emerging vector-borne livestock diseases. Report 11-CVI0168. Central Veterinary Institute, part of Wageningen UR, Lelystad, The Netherlands. Available at: http://edepot.wur.nl/198115. Accessed November 26, 2015

De Vos, C.J., Hoek, C.A. and Nodelijk, H.A. (2012). Risk of introducing African horse sickness virus into the Netherlands by international equine movements. Prev. Vet. Med. 106, 108-122

EFSA (2005). Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to “The Risk of a Rift Valley Fever Incursion and its Persistence within the Community”. EFSA-Q-2004-050. The EFSA Journal 238, 1-128

EFSA (2009). Scientific opinion on epizootic hemorrhagic disease. EFSA Panel on Animal Health and Welfare (AHAW). EFSA Journal 7, 1418

EFSA (2010). Scientific opinion on geographic distribution of tick-borne infections and their vectors in Europe and the other regions of the Mediterranean Basin. EFSA Panel on Animal Health and Welfare (AHAW). EFSA Journal 8, 1723

EFSA (2013). Scientific opinion on Rift Valley fever. EFSA Panel on Animal Health and Welfare (AHAW). EFSA Journal 11, 3180

Ergonul, O. and Whitehouse, C.A. (2007). Crimean-Congo hemorrhagic fever. A global perspective. Springer, Dordrecht, The Netherlands. 328p

Foley, J.E. and Nieto, C.N. (2010). Tularaemia. Vet. Microbiol. 140, 332-338

Gyuranecz, M. (2012). Tularaemia. In: Gavier-Widen, D., Duff, J.P. and Meredith, A. (Eds.) Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Blackwell Publishing Ltd, Chichester, United Kingdom, pp. 303-309

Hayes, E.B., Komar, N., Nasci, R.S., Montgomery, S.P., O’Leary, D.R. and Campbell, G.L. (2005). Epidemiology and transmission dynamics of West Nile virus disease. Emerg. Infect. Dis. 11, 1167-1173

Hestvik, G., Warns-Petit, E., Smith, L.A., Fox, N.J., Uhlhorn, H., Artois, M., Hannant, D., Hutchings, M.R., Mattsson, R., Yon, L. and Gavier-Widen, D. (2014). The status of tularemia in Europe in a one-health context: A review. Epidemiol. Infect. 143, 2137-2160

Hoek, M.R., Fischer, E.A.J., De Koeijer, A.A., Bremmer, J. and De Vos, C.J. (2011). Risk assessment framework for exotic vector-borne livestock diseases: A Rift Valley fever case study. Report 11-CVI0412. Central Veterinary Institute, part of Wageningen UR, Lelystad, The Netherlands. 15p

Hoek, M., Fischer, E., Van den Hengel, R., Backer, J. and De Koeijer, A. (2012). Workshop on a risk assessment of Crimean Congo haemorrhagic fever in Western Europe, Lelystad, The Netherlands, 23-24 May 2012. Report 12-CVI0361. Central Veterinary Institute, part of Wageningen UR, Lelystad, The Netherlands. 39p

Hoogstraal, H. (1979). The epidemiology of tick-borne Crimean-Congo haemorrhagic fever in Asia, Europe and Africa. J. Med. Entomol. 15, 307-417

Koene, M., Rijks, J., Maas, M., De Rosa, M., Broens, E., Vellema, P., Engelsma, M., Pijnacker, R., Fanoy, E., Notermans, D., Van der Tas, P., Van der Giessen, J., Gröne, A. and Roest, H.J. (2015). Tularemie in Nederland, terug van weggeweest? Tijdschr. Diergeneesk. 140, 23-27 (in Dutch)

Koenraadt, C.J.M., Balenghien, T., Carpenter, S., Ducheyne, E., Elbers, A.R.W., Fife, M., Garros, C., Ibáñez-Justicia A., Kampen, H., Kormelink, R.J.M., Losson, B., Van der Poel, W.H.M., De Regge, N., Van Rijn, P.A., Sanders, C., Schaffner, F., Sloet van Oldruitenborgh-Oosterbaan, M., Takken, W., Werner, D. and Seelig, F. (2014). Bluetongue, Schmallenberg – what is next? Culicoides-borne viral diseases in the 21st Century. BMC Vet. Res. 10, 77

L'Hostis, M.L. and Seegers, H. (2002). Tick-borne parasitic diseases in cattle: Current knowledge and prospective risk analysis related to the ongoing evolution in French cattle farming systems. Vet. Res. 33, 599-611

Linthicum, K.J., Davies, F.G. and Kairo, A. (1985). Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. J. Hyg. Camb. 95, 197-209

Maclachlan, N.J. and Osburn, B.I. (2004). Epizootic haemorrhagic disease of deer. In: Coetzer, J.A.W. and Tustin, R.C. (Eds.) Infectious Diseases of Livestock, 2nd Edition. Oxford University Press Southern Africa, Cape Town, South Africa, pp. 1227-1230

MacLachlan, N.J. and Guthrie, A.J. (2010). Re-emergence of Bluetongue, African horse sickness, and other Orbivirus diseases. Vet. Res. 41, 35

Mellor, P.S. (1993). African horse sickness: Transmission and epidemiology. Vet. Res. 24, 199-212

Mellor, P.S. and Hamblin, C. (2004). African horse sickness. Vet. Res. 35, 445-466

OIE, 2015. OIE-Listed diseases, infections and infestations in force in 2015. Available at: http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2015/. Accessed June 17, 2015

Pepin M., Bouloy M., Bird B.H., Kemp A. and Paweska J. (2010). Rift Valley fever virus (Bunyaviridae: Phlebovirus): An update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet. Res. 41, 61

Petersen, J.M., Mead, P.S. and Schriefer, M.E. (2009). Francisella tularensis: An arthropod-borne pathogen. Vet. Res. 40, 07

Pradier, S., Lecollinet, S. and Leblond, A. (2012). West Nile virus epidemiology and factors triggering change in its distribution in Europe. Rev. Sci. Tech. OIE 31, 829-844

Reiter, P. (2010). West Nile virus in Europe: Understanding the present to gauge the future. Euro Surveill. 15, 4-10

Rijks, J.M., Kik, M., Koene, M.G., Engelsma, M.Y., Van Tulden, P., Montizaan, M.G., Oomen, T., Spierenburg, M.A., IJzer, J., Van der Giessen, J.W., Gröne, A. and Roest, H.J. (2013). Tularaemia in a brown hare (Lepus europaeus) in 2013: First case in the Netherlands in 60 years. Euro Surveill. 18, 20655

Roberts, H. and Crabb, J. (2012). West Nile virus: Potential risk factors and the likelihood for introduction into the United Kingdom. Qualitative Risk Assessment. Version No: 3.0. Date: 1 May 2012. Defra, UK. Available at: http://webarchive.nationalarchives.gov.uk/20140507133914/http://www.defra.gov.uk/animal-diseases/files/qra-wnv-120501.pdf. Accessed December 9, 2015

Sambri, V., Capobianchi, M., Charrel, R., Fyodorova, M., Gaibani, P., Gould, E., Niedrig, M., Papa, A., Pierro, A., Rossini, G., Varani, S., Vocale, C. and Landini, M.P. (2013). West Nile virus in Europe: Emergence, epidemiology, diagnosis, treatment, and prevention. Clin. Microbiol. Infec. 19, 699-704

Sailleau, C., Zanella, G., Breard, E., Viarouge, C., Desprat, A., Vitour, D., Adam, M., Lasne, L., Martrenchar, A., Bakkali-Kassimi, L., Costes, L. and Zientara, S. (2012). Co-circulation of bluetongue and epizootic haemorrhagic disease viruses in cattle in Reunion Island. Vet. Microbiol. 155, 191-197

Savini, G., Afonso, A., Mellor, P., Aradaib, I., Yadin, H., Sanaa, M., Wilson, W., Monaco, F. and Domingo, M. (2011). Epizootic haemorrhagic disease. Res. Vet. Sci. 91, 1-17

Swanepoel, R. and Coetzer, J.A.W. (2004). Rift Valley Fever. In: Coetzer, J.A.W. and Tustin, R.C. (Eds.) Infectious Diseases of Livestock, 2nd Edition. Oxford University Press Southern Africa, Cape Town, South Africa, pp. 1037-1070

Velthuis, A.G.J., Saatkamp, H.W., Mourits, M.C.M., De Koeijer, A.A. and Elbers, A.R.W. (2010). Financial consequences of the Dutch bluetongue serotype 8 epidemics of 2006 and 2007. Prev. Vet. Med. 93, 294-304

Zientara, S. and Sánchez-Vizcaíno, J.M. (2013). Control of bluetongue in Europe. Vet. Microbiol. 165, 33-37

Zintl, A., Mulcahy, G., Skerrett, H.E., Taylor, S.M. and Gray. J.S. (2003). Babesia divergens, a bovine blood parasite of veterinary and zoonotic importance. Clin. Microbiol. Rev. 16, 622-636

ESTIMATION OF H1N1 SWINE INFLUENZA A VIRUS TRANSMISSION PARAMETERS IN PIGS WITH DIFFERENT INITIAL IMMUNE STATUSES

C. CADOR*, S. HERVE, M. ANDRAUD, S. GORIN, F. PABOEUF, N. BARBIER, S. QUEGUINER, C. DEBLANC, G. SIMON AND N. ROSE

SUMMARY

Recurrent influenza infections occur mainly after weaning when piglets still have maternally derived antibodies (MDAs). A transmission experiment involving 72 specific pathogen-free piglets with or without MDAs was carried out to evaluate the impact of MDAs on H1N1 virus transmission. In each group (MDA+/MDA-), 2 seeder pigs were placed with 4 direct- and 5 indirect-contact piglets (3 replicates per group). Serological kinetics (ELISA test) and individual virus shedding (RT-PCR) were monitored. Duration of MDAs persistence was estimated by a non-linear mixed-effect model and survival analysis. Transmission parameters were estimated by maximum likelihood method. The time to MDA waning was 71.3 [52.8 – 92.1] days on average. The airborne transmission rate was 0.69 [0.33 - 1.18] days-1. The reproduction number estimated in MDA+ piglets (5.7 [1.2 - 12.8]), three times lower than in MDA- piglets (17.1 [8.9 - 29.4]), was significantly higher than 1, probably contributing to persistence of swine flu on farms.

INTRODUCTION

Swine influenza A viruses (swIAVs) are responsible for one of the most widespread respiratory diseases in swine worldwide. SwIAVs are polymorphic enveloped single-stranded RNA viruses from the *Orthomyxoviridae* family (Van Reeth et al., 2012). The main swIAVs affecting swine production worldwide are H1N1, H1N2 and H3N2 subtypes, which contain genetic components derived from both avian and human species leading to different lineages according to their geographical location (Kyriakis et al., 2013).

Clinical signs due to influenza infections are close to human flu, including hyperthermia, anorexia, apathy, coughing and nasal discharge. Growth retardation can also be observed. In its classical form, swine flu is known to be responsible for sporadic infections in swine herds, temporarily affecting a huge part of the pig population within an infected herd (Van Reeth et al., 2012). However, a potential persistence in-between epidemic phases has been more and more documented in European herds (Simon-Grife et al., 2012; Rose et al., 2013). In the recent years, the French National Surveillance Network for swIAVs (Résavip) reported almost 40% of swine flu cases corresponding to this endemic persistence (Hervé et al., 2014), also called recurrent flu. It occurs mainly after weaning, approximately at the same age and in successive

---

* Charlie Cador, Anses, Ploufragan-Plouzané laboratory, Swine Epidemiology and Welfare unit, Po Box 53, 22440 Ploufragan, France. Email: charlie.cador@anses.fr

batches, durably affecting the herds and favouring constant health disorders. Because of complications due to bacterial respiratory infections (Deblanc et al., 2012; Fablet et al., 2012), swIAV has been reported as a major contributing factor in the porcine respiratory disease complex (PRDC). Such complicated pulmonary disorders are difficult to manage and often motivate the use of antibiotic treatments. Such approaches can be extremely costly, and are also of concern for public health. Longitudinal studies have also highlighted the possibility for a piglet to experience consecutive or simultaneous infections by different subtypes of swIAVs (Simon-Grife et al., 2012; Rose et al., 2013). These concomitant infections can favour gene exchanges between viruses, leading to the emergence of novel reassortant viruses, potentially more pathogenic for pigs. The public health concerns should also be carefully considered, since swIAVs have zoonotic potential as illustrated by previous pandemic infections.

Based on epidemiological studies, the serological status of piglets at birth is presumed to be of primary importance for further spread of influenza among growing pigs. Vaccination of breeding sows is frequently implemented in swine farms to prevent from adverse consequences of swIAV infections on reproductive performance. Regular vaccination of breeding sows aims at transferring maternally derived antibodies (MDAs) to piglets, in order to prevent swIAV infection in early life. However, the role of passive immunity in piglets is controversial. Although MDAs provide temporary partial protection, interference between MDAs and post-infectious humoral response has been documented as early as 1975 (Renshaw, 1975). In addition, a follow-up study carried out in permanently infected herds highlighted that MDA-positive piglets born to routinely vaccinated dams experimenting an early infection showed an impaired post-infectious humoral response (Rose et al., 2013). This phenomenon could probably increase the likelihood of a second infection by a swIAV (Loeffen et al., 2003). Allerson et al. (2013) estimated a 15-fold reduction of the transmission rate in piglets having homologous MDAs. However, studies have also shown that piglets with heterologous MDAs do not show any significant protection against infection, and that the presence of MDAs can be associated with adverse effects when vaccinating young animals (Vincent et al., 2012). Other experimental studies have confirmed that MDA-positive piglets are not fully protected, restricting the benefit of MDAs to the reduction of clinical signs only (Loeffen et al., 2003; Kitikoon et al., 2006).

Considering the specific features of recurrent swine influenza virus infections (i.e. occurring in young piglets, at the same age, in successive batches), it is important to have quantitative data on epidemiological parameters associated with swIAV infection in presence of passive immunity. Those data will allow identifying the determinants of swine flu persistence within farrow-to-finish pig herds. The aim of this study was therefore to clarify the impact of maternal antibodies on the dynamics of infection in young piglets, both in terms of transmission and duration, by quantifying and comparing swIAV spread in piglets in presence and absence of MDAs under experimental conditions.

MATERIALS AND METHODS

A transmission experiment was conducted in the Anses level-3 biosecurity facilities using Specific Pathogen Free (SPF) piglets produced in our air-filtered biosecurity level-3 piggery to evaluate the protective impact of MDAs on swIAV transmission. The SPF sow herd is swIAV free. The experimental protocol was approved by the Anses/ENVA/UPEC Ethical Committee on animal experiments (agreement #16 with the National committee for Ethics in animal experimentation, legal notice 11/03/14-17).

Experimental design

Thirty-six piglets with swIAV-specific MDAs (MDA+ group), born to SPF sows vaccinated with a commercial inactivated trivalent vaccine (H1N1, H1N2, H3N2, Respiporc Flu® 3) during gestation, and 36 piglets without MDAs (MDA- group), born to unvaccinated SPF sows, were used. To produce MDA-positive piglets, SPF dams were primo-vaccinated 6 and 3 weeks before insemination by a 2 ml intramuscular injection, followed by three boosters 6, 3 and 2 weeks before farrowing in order to induce high levels of antibodies in the colostrum.

The contact structure involved two seeder pigs housed with four piglets in the same pen, and five piglets in the second pen, 30 cm apart (Fig. 1), to assess transmission through direct and indirect contact (no pig-to-pig contact allowed), respectively. This experimental design was replicated in three separate rooms for each experimental group (MDA+ and MDA-). Pen compositions were randomly balanced according to weight, sex and dam, as well as the MDA titre at 5 weeks of age for MDA-positive piglets. In each room, the two seeder pigs were intratracheally inoculated with 106 EID50 (Embryonic 50% Infectious Dose) of A/Sw/Cotes d'Armor/0388/09 strain, a European avian-like swine H1N1 (H1avN1) virus from the same lineage as the vaccine H1N1 antigen (in a total volume of 5ml) at 35 days of age (Day (D) 0). Piglets to inoculate were gathered in a separate room for the inoculation and put in contact with the other animals 24 h after inoculation. Three MDA-positive and three MDA-negative piglets were placed in two different pens in a seventh room, and were mock-inoculated with 5 ml of minimum essential medium. These pigs constituted the negative control groups. The experiment was ended at D28, corresponding to 63 days of age.

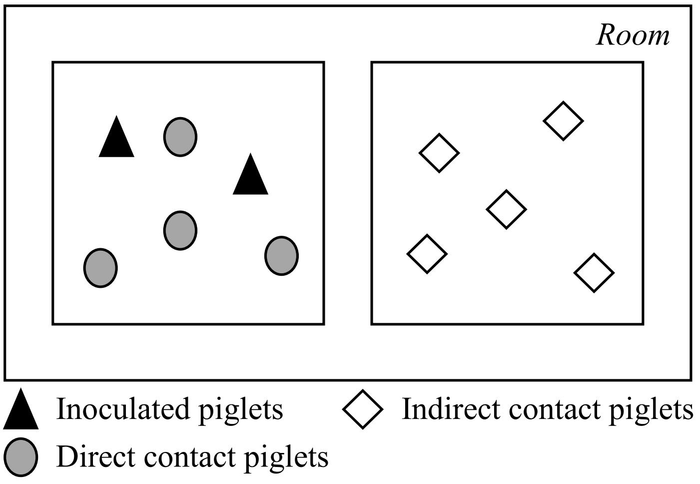


Fig. 1 Representation of the contact structure involved in the experimental design

Sampling collection and laboratory analysis

Strict measures were taken to prevent virus transmission through human and fomite contacts. Samples were first taken in the indirect contact pen, and secondly in the pen housing direct contact and inoculated piglets. Clothing and footwear were cleaned after each pen visit and changed between each room. A shower was taken before and after each sample collection. Individual-specific sampling materials were used for each pig.

As swIAVs are mainly shed in respiratory secretions, nasal swabs (MW951 sent, Virocult®, Corsham, England) were taken from all piglets on a daily basis during the two first weeks after inoculation and every 2 days thereafter to assess the frequency of H1avN1 shedding piglets over time. Nasal swab supernatants were subjected to a qualitative M gene real-time RT-PCR in duplex with β-actine gene amplification (Pol et al., 2011). Blood samples were collected by jugular vein puncture on D-3, 0, 4, 7, 11, 14, 18, 21 and 25 using evacuated tubes without additive (Vacuette, Dutscher SAS, Brumath, France). Antibodies against Influenza A virus were quantified in sera using LSIVet™ Porcine influenza - Serum ELISA Kit (VETSIV/I, Life Technologies, Courtaboeuf, France). Antibody titres were expressed as % IRPC (Relative Index Percent).

Statistical analysis and models

Duration of MDA persistence: A nonlinear mixed-effect model was used to estimate individual parameters governing antibody kinetics. Individual antibody kinetics were based on the ELISA results of the MDA-positive piglets. The decay of antibody titres was assumed to follow an exponential decrease ($dA/dt = -rA$). The model describing serological titre of individual i at observation time tij (with a constant residual error model) is given by Eq. (1) with $A_0^{(i)}$ the initial level of MDAs, ri the MDA decay rate and εij a vector of standardised random variables.

$$A_{ij} = A_0^{(i)}\, exp\big(-r_i t_{ij}\big) + a\varepsilon_{ij} \tag{1}$$

Individual parameters were assumed to be log-normally distributed. Population parameters were estimated using MLE by the SAEM algorithm for the hierarchical nonlinear mixed-effects model analysis using Monolix software (http://www.monolix.org). Using the parameter estimates, projections of each individual profile were made. Piglets were considered to have lost their maternal antibodies when they passed below the threshold of 10% IRPC. A parametric survival analysis was finally carried out to estimate the duration of MDA persistence, assuming a gamma distribution of survival times.

Estimation of transmission parameters: The virus transmission process was represented by a SEIR model (Susceptible – Exposed – Infectious – Removed), incorporating different transmission processes. The corresponding different transmission rates accounted for (i) the serological status of the piglets at the beginning of the experiment (MDA-positive or MDA-negative piglets) and (ii) the contact structure of the piglet with their roommates (direct contacts in the same pen, indirect contacts by airborne route otherwise). A piglet was considered infectious from the first until the last positive RT-PCR obtained from consecutive nasal swabs. In case a negative RT-PCR result was found in-between two positive results, the result was assumed to be a false negative and the piglet was assumed to be infectious at that time. The duration of the shedding period was estimated by parametric survival analysis. As the exact time when contact piglets got infectious was unknown, the distribution between the last negative and the first positive RT-PCR result was chosen to be uniform (same principle for recovery). Combining the results of previous experiments (Deblanc et al., 2012) and field observations (Rose et al., 2013), a duration of the latency period between 0.5 and 1.5 days was assumed. Thus, the interval in which pigs became infected was determined by subtracting 1.5 and 0.5 days from the last negative (i1) and the first positive RT-PCR (i2) respectively, giving an infection interval [e1, e2] for each contact-infected piglet calculated as [i1 – 1.5, i2 – 0.5] (Weesendorp et al., 2014).

The general routes of swIAV transmission include direct contact with infectious pigs, exposure to aerosols or contaminated fomites (Van Reeth et al., 2012). Two transmission routes have therefore been considered in this experiment, parameterised by the corresponding transmission rate parameter β: (i) virus transmission by direct contact within each pen, specific to the initial serological status of the piglet (MDA+ or MDA-), and (ii) virus transmission by airborne route within the entire room, including pen mates (βair). Those transmission rates represent the number of newly infected piglets per infectious piglet and per day. The global force of infection λk combines the two virus transmission routes. Hence, a piglet k can become infected by direct contact with an infectious pen mate with MDAs (transmission rate parameter βMDA+), or without (parameter βMDA-), or by contact with contaminated aerosol (parameter βair). The global force of infection λk for a piglet k is then calculated as follows (Eq. (2)), with Ip(ti) and Ir(ti) being the (time-dependent) number of infectious piglets in pen p or room r at time t and Np and Nr the total number of piglets in pen p or room r.

$$\lambda_k(t_i) = \beta_{MDA+} \frac{I_p(t_i)}{N_p - 1} + \beta_{MDA-} \frac{I_p(t_i)}{N_p - 1} + \beta_{air} \frac{I_r(t_i)}{N_r} \tag{2}$$

The probability for each piglet k to escape from infection when submitted to the global force of infection λk up to time e1 is $exp(- \int_0^{e_1,k} \lambda_k(t_i)\ dt)$. The probability to get infected during the time-interval [e1, e2] is therefore $1 - exp(- \int_{e_1,k}^{e_2,k} \lambda_k(t_i)\ dt)$. The product over all contact-infected piglets gives the full likelihood function L (Eq. (3)).

$$L = \prod_{k \in contact-infected} exp(- \int_0^{e_1,k} \lambda_k(t_i)\ dt) \left(1 - exp(- \int_{e_1,k}^{e_2,k} \lambda_k(t_i)\ dt)\right) \tag{3}$$

The different transmission rate parameters were then estimated by likelihood maximisation (Klinkenberg et al., 2002) and confidence intervals have been derived from the likelihood profile.

The impact of the contact structure and the initial serological status on (i) the onset of shedding and (ii) the duration of shedding period has been assessed using a semi-parametric Cox model. The reproduction numbers R0 specific to each group (MDA+ and MDA-) were finally estimated by multiplying the corresponding estimations of the transmission rate parameters and the duration of shedding period.

RESULTS

Serological results and persistence of MDAs

As the serological profiles of the MDA-positive piglets were heterogeneous at D-3, the MDA+ group was divided into two subgroups: piglets with a high initial antibody level (n=25) and piglets with a low initial antibody level (n=8). Piglets with a high initial antibody level showed a continuous antibody decay, without serological response after infection (Fig. 2). Conversely, piglets with a low initial antibody level were distinguished by having a weak serological response after infection, very close to MDA-negative piglet profiles (n=33). Owing to the limited number of piglets with a low initial antibody level (n=8) and the similarity to the post-infection serological profile of MDA-negative piglets, the same virus transmission characteristics and the same susceptibility to infection were considered for those two groups, respecting the spatial distribution of the initial experimental design, i.e. the localisation of the piglets with low initial antibody levels among piglets with high initial antibody levels. Moreover, the piglets with low initial antibody levels were not considered in the estimation of the duration of MDA persistence.

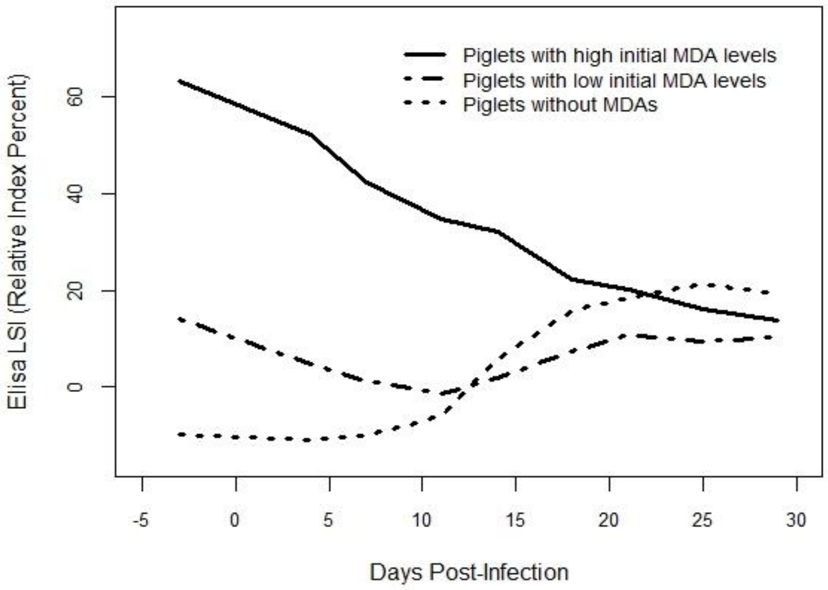


Fig. 2 Mean serological results of the piglets according to their group: piglets with high (solid line) or low initial MDA levels (dashed line) and piglets without MDAs (dotted line)

The individual duration of MDA persistence, based on the individual parameter estimates, was determined by projection of the antibody kinetics, leading to the estimation of 71.3 days (95% confidence interval: 52.8 – 92.1) of MDA persistence, on average, by survival analysis (Fig. 3).

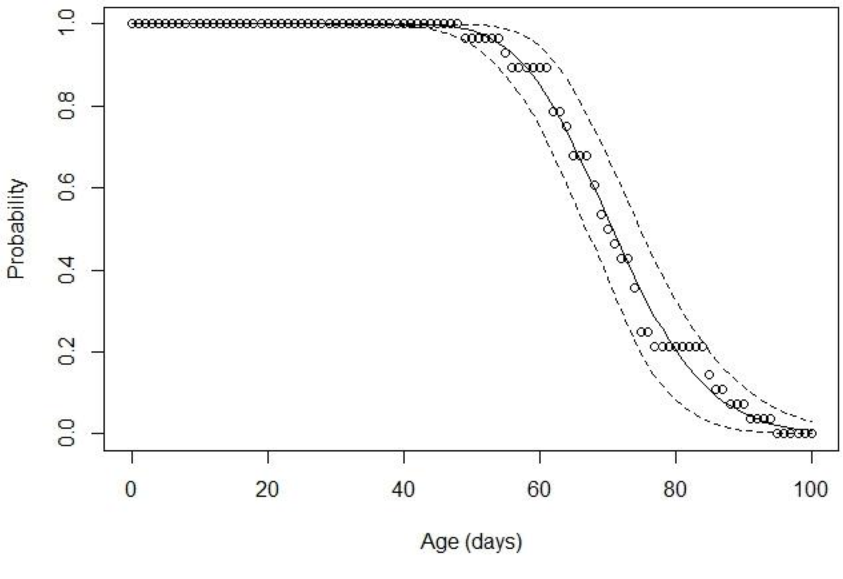


Fig. 3 Distribution of the duration of MDA persistence (days). Gamma distributions were assumed and maximum likelihood methods were used to estimate the shape and scale parameters (solid black line). Dashed lines represent 95% confidence intervals

Virus shedding and estimation of transmission parameters

Virus shedding: Virus shedding and seroconversion were not detected in the control piglets at any sampling time of the experiment (data not shown). All inoculated piglets began to shed virus between D2 and D4. All contact piglets were infected during the experiment with a variable time-delay according to (i) the contact structure and (ii) the immune status (Fig. 4). The spread was more extended in MDA-positive piglets than in MDA-negative piglets at the population scale. MDA-negative contact piglets began to shed virus more rapidly compared to MDA-positive piglets (Hazard Ratio = 3.36; p-value < 0.05). However, the individual duration of the shedding period was not influenced by the presence of MDAs in young piglets (HR = 1.39; p-value = 0.2), leading to a duration of shedding period of 6.1 days [5.9 – 6.4] for both groups (MDA+ and MDA-).

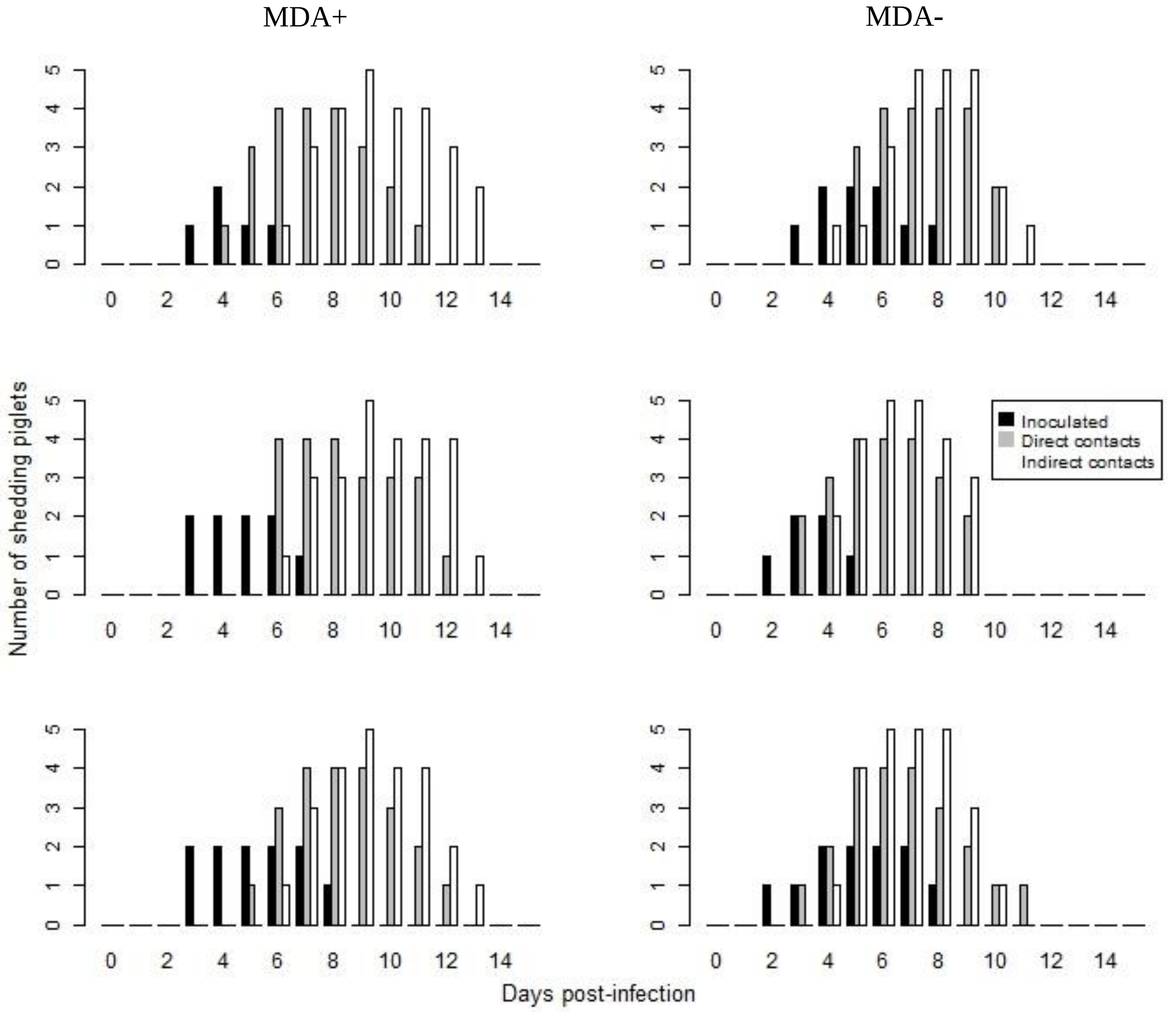


Fig. 4 Number of infectious piglets per room over time based on individual detection of the influenza A virus genome (M gene RT-PCR) according to the initial serological status (MDA+ on the left side, MDA- on the right side) and the contact structure (inoculated piglets in black, direct contacts in grey and indirect contacts in white)

Transmission parameters: Under experimental conditions, an MDA-negative piglet was able to infect 2.80 piglets per day by direct contact (βMDA- = 2.80 (1.5 – 4.6)), which was three times higher than for an MDA-positive piglet (βMDA+ = 0.93 (0.2 – 2.0)). Within a room, an infected piglet, with or without MDAs, was able to infect 0.69 piglets (0.3 – 1.2) per day via the airborne route. Transmission rates estimated in both groups (MDA+ and MDA-) were combined with a duration of the shedding period of 6.1 days (5.9 – 6.4), leading to estimated reproduction numbers of 5.7 (1.2 – 12.8) and 17.1 (8.9 – 29.4), respectively (Tab. 1). Although MDA-positive piglets were infected in presence of MDAs, they could transmit the virus to, on average, 5.7 piglets during their shedding period.

Table 1. Estimation of the transmission parameters according to the immune status of the piglets (MDA+ and MDA-)

	Duration of shedding period	Transmission rate β	Reproduction number R_0
MDA+	6.1 (5.9-6.4)	0.93 (0.2-2.0)	5.7 (1.2-12.8)
MDA-		2.80 (1.5-4.6)	17.1 (8.9-29.4)
Air		0.69 (0.3-1.2)	

DISCUSSION

Understanding the epidemiological processes responsible for swIAV endemic persistence within pig herds requires a quantitative approach to estimate viral transmission parameters within a pig herd population. Available data obtained from field studies suggested an interaction between the immune status of the animals at infection and the virus spread between batches (Rose et al., 2013). The present transmission experiment was designed to estimate transmission parameters under controlled conditions and compare those estimates between two populations differing only by their immune status. Such quantitative estimates of transmission parameters are essential to assess the consequences of swIAV infections in young animals still having MDAs, and to evaluate assumptions related to factors increasing the likelihood of those viruses to persist at the population level.

In the present study, two different virus transmission routes were considered in the same room: (i) a direct transmission, which can only occur between pen mates through pig-to-pig contacts and (ii) an indirect transmission via airborne route, which can occur between all the pigs in the room, including pen mates. The direct transmission parameters estimated in the present study for SPF piglets with or without passive immunity (0.93 (0.2 – 2.0) and 2.80 (1.5 – 4.6), respectively) are consistent with previous analysis of field data (Rose et al., 2013). They are also in line with data reported by Allerson et al. (2013), although the estimates in the present study were slightly lower in MDA-positive animals. Indeed, Allerson et al. (2013) estimated a virus transmission rate of 1.74 (1.18 – 2.46) in conventional piglets born to vaccinated sows and infected with a H1N1 strain of an American lineage. The difference between MDA-positive animals in the two studies could be due to differences in the vaccination protocols; in the Allerson experiment sows received a single vaccination, versus three boosters in this study. Consequently, the maternal immunity delivered to the piglets might have been weaker in the aforementioned case. In addition, it cannot be excluded that there could be differences related to the virus strain or the status of the animals (conventional piglets in Allerson’s study versus SPF pigs in the current experiment).

Inoculated piglets began to shed virus between D2 and D4. Previous data from published experimental studies corroborate this result (Romagosa et al., 2011; Deblanc et al., 2012). The intratracheal inoculation potentially differs from natural infection process (by aerosols), which might be faster. Under field conditions, duration of latency periods from 0.5 to 1.5 days have been estimated (Rose et al., 2013). Besides, the delay between the first virus shedding in inoculated- and contact piglets was sometimes less than a day in our experiment. Accounting for all of those components, a duration of latency period varying from 0.5 to 1.5 days was used.

The duration of the shedding period estimated in our study was longer than previously published experimental data (Allerson et al., 2013) but shorter than field-based estimations (Rose et al., 2013). The differences could be linked to the status of the piglets (SPF piglets in the present case) as well as the assessment and the characteristics of influenza outbreaks described under field conditions. In the latter case, duration of shedding was also estimated on the basis of M gene RT-PCR results, but could have been artificially increased due to the co-circulation of two virus subtypes, slightly delayed in time.

A short infectious period was observed in MDA-negative contact piglets at the population scale, owing to the high transmission rate and corresponding reproduction number. Although MDA-positive piglets displayed a maternal immunity against the H1avN1 subtype, they showed a high virus spread potential with a reproduction number significantly higher than 1, albeit smaller than for MDA-negative piglets. This reduced reproduction number led to an extended total duration of the infectious process at the scale of a maternally immune pig population. Transferred to field conditions, this extended infectious period could favour the within-herd persistence of the virus through permanent exposure of incoming susceptible piglets to shedding animals from other batches (Simon-Grife et al., 2012; Rose et al., 2013). Likewise, a longer period with shedding piglets at the herd level increases the probability of a concomitant multiple-strain circulation and thus generation of reassortant viruses, potentially more pathogenic for the pigs or possibly crossing species barriers.

Based on the individual profiles of maternal antibody decay in piglets born to vaccinated sows, a 71.3-day-duration of MDA persistence, on average, was estimated. A transition to a “more-susceptible-to-infection” status around 10 weeks seems realistic for SPF piglets born to multi-vaccinated sows. Indeed, under field conditions, MDAs were shown to persist for 11 weeks on average (Rose et al., 2013). Under experimental conditions, piglets born to conventional vaccinated sows showed a persistence of MDAs from 8 to 13 weeks-of age (Markowska-Daniel et al., 2011; Vincent et al., 2012). Nevertheless, but similar to those previous studies, heterogeneity between individual profiles has been observed in the present experiment. It could be linked to dam antibody titres as well as a different colostrum intake between piglets.

To our knowledge, this is the first quantification of swIAV transmission via the airborne route. Although several investigations have been carried out on this topic, either on the relationship between the number of infected pigs and the presence of the virus in the air, or the quantification of the virus in the air (Corzo et al., 2013), no transmission parameters have been estimated to date. The virus transmission rate for the airborne route estimated in the current study revealed that 0.69 pigs per day were newly infected via the air. This airborne transmission route must be added to the transmission route by direct contact, highlighting the importance to consider infectious aerosols in the swine flu within-herd spread management.

Influenza virus spread is modified by the piglets’ immune status and the contact structure between piglets. Although the presence of MDAs in weaned piglets significantly reduces swIAV transmission, the reproduction number is significantly higher than 1, highlighting the limited protection conferred by MDAs towards influenza virus spread. Negative effects are also observed: the spread process is slower compared to piglets without passive immunity, favouring the presence of shedding animals during a longer period of time at the population scale, which could enhance swine flu within-herd persistence. Thus, accounting for the immune status of the animals and the contact structure between weaned piglets (direct or by airborne route), appears pivotal to identify control measures aiming at preventing from recurrent swIAV infections in pig herds. At the farm level, practical management measures for swine flu within-herd control could be considered such as reducing breeding practices enhancing virus transmission by direct contacts (cross-fostering and mingling at weaning) and increasing segregation and internal biosecurity between batches.

ACKNOWLEDGEMENTS

The authors would like to thank A. Keranflec'h, J-M. Guionnet, N. Morin, Y. Bailly and G. Le Diguerher for their excellent technical work in animal facilities. Funding was provided by the Regional Pig Committee (CRP) of Bretagne, Pays de la Loire and Normandie, the National Pig Interprofession (INAPORC) and the Côtes d'Armor County Council.

REFERENCES

Allerson, M., Deen, J., Detmer, S.E., Gramer, M.R., Joo, H.S., Romagosa, A. and Torremorell, M. (2013). The impact of maternally derived immunity on influenza A virus transmission in neonatal pig populations. Vaccine 31, 500-505

Corzo, C.A., Culhane, M., Dee, S., Morrison, R.B. and Torremorell, M. (2013). Airborne detection and quantification of swine influenza a virus in air samples collected inside, outside and downwind from swine barns. PLoS One 8, e71444

Deblanc, C., Gorin, S., Quéguiner, S., Gautier-Bouchardon, A.V., Ferré, S., Amenna, N., Cariolet, R. and Simon, G. (2012). Pre-infection of pigs with Mycoplasma hyopneumoniae modifies outcomes of infection with European swine influenza virus of H1N1, but not H1N2, subtype. Vet. Microbiol. 157, 96-105

Fablet, C., Marois-Créhan, C., Simon, G., Grasland, B., Jestin, A., Kobisch, M., Madec, F. and Rose, N. (2012). Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: A cross-sectional study. Vet. Microbiol. 157, 152-163

Hervé, S., Garin, E., Rose, N., Marcé, C. and Simon, G. (2014). French network for the surveillance of influenza viruses in pigs (Résavip) - Results of the first three years of operation. B. Epidémiol. Sante Anim. Aliment. Anses-DGAl 63, 10-14

Kitikoon, P., Nilubol, D., Erickson, B.J., Janke, B.H., Hoover, T.C., Sornsen, S.A. and Thacker, E.L. (2006). The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination. Vet. Immunol. and Immunop. 112, 117-128

Klinkenberg, D., de Bree, J., Laevens, H. and de Jong, M.C. (2002). Within- and between-pen transmission of Classical Swine Fever Virus: a new method to estimate the basic reproduction ratio from transmission experiments. Epidemiol. Infect. 128, 293-299

Kyriakis, C.S., Rose, N., Foni, E., Maldonado, J., Loeffen, W.L.A., Madec, F., Simon, G. and Van Reeth, K. (2013). Influenza A virus infection dynamics in swine farms in Belgium, France, Italy and Spain, 2006-2008. Vet. Microbiol. 162, 543-550

Loeffen, W.L.A., Heinen, P.P., Bianchi, A.T.J., Hunneman, W.A. and Verheijden, J.H.M. (2003). Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus. Vet. Immunol. and Immunop. 92, 23-35

Markowska-Daniel, I., Pomorska-Mól, M. and Pejsak, Z. (2011). The influence of age and maternal antibodies on the postvaccinal response against swine influenza viruses in pigs. Vet. Immunol. and Immunop. 142, 81-86

Pol, F., Quéguiner, S., Gorin, S., Deblanc, C. and Simon, G. (2011). Validation of commercial real-time RT-PCR kits for detection of influenza A viruses in porcine samples and differentiation of pandemic (H1N1) 2009 virus in pigs. J. Virol. Methods 171, 241-247

Renshaw, H.W. (1975). Influence of antibody mediated immune suppression on clinical, viral, and immune responses to swine influenza infection. Am. J. Vet. Res. 36, 5-13

Romagosa, A., Allerson, M., Gramer, M., Joo, H., Deen, J., Detmer, S. and Torremorell, M. (2011). Vaccination of influenza a virus decreases transmission rates in pigs. Vet. Res. 42, 120-134

Rose, N., Hervé, S., Eveno, E., Barbier, N., Eono, F., Dorenlor, V., Andraud, M., Camsusou, C., Madec, F. and Simon, G. (2013). Dynamics of influenza a virus infections in permanently infected pig farms: Evidence of recurrent infections, circulation of several swine influenza viruses and reassortment events. Vet. Res. 44, 72-85

Simon-Grife, M., Martin-Valls, G.E., Vilar, M.J., Busquets, N., Mora-Salvatierra, M., Bestebroer, T.M., Fouchier, R.A., Martin, M., Mateu, E. and Casal, J. (2012). Swine influenza virus infection dynamics in two pig farms; results of a longitudinal assessment. Vet. Res. 43, 24-34

Van Reeth, K., Brown, I.H. and Olsen, C.W. (2012). Influenza Virus. In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.), Diseases of Swine 10th. 557-571

Vincent, A.L., Ma, W., Lager, K.M., Richt, J.A., Janke, B.H., Sandbulte, M.R., Gauger, P.C., Loving, C.L., Webby, R.J. and García-Sastre, A. (2012). Live attenuated influenza vaccine provides superior protection from heterologous infection in pigs with maternal antibodies without inducing vaccine-associated enhanced respiratory disease. J. Virol. 86, 10597-10605

Weesendorp, E., Backer, J. and Loeffen, W. (2014). Quantification of different classical swine fever virus transmission routes within a single compartment. Vet. Microbiol. 174, 353-361

SURVEILLANCE FRAMEWORKS

RISKSUR TOOLS: TAKING ANIMAL HEALTH SURVEILLANCE INTO THE FUTURE THROUGH INTERDISCIPLINARY INTEGRATION OF SCIENTIFIC EVIDENCE

A. COMIN*, B. HAESLER**, L. HOINVILLE, M. PEYRE, F. DÓREA, B. SCHAUER,
L. SNOW, K.D.C. STÄRK, A. LINDBERG, A. BROUWER, G. VAN SCHAIK,
C. STAUBACH, K. SCHULZ, B. BISDORFF, F. GOUTARD, J. PINTO FERREIRA,
F. CONRATHS, A. CAMERON, M. MARTINEZ-AVILES, J. SANCHEZ-VIZCAINO,
V.VARAN, D. TRAON, J. PINTO, J. RUSHTON, J. RIPPERGER AND D.U. PFEIFFER

SUMMARY

To enable wide-spread acceptance and adoption of risk-based surveillance approaches by stakeholders it is essential to provide those designing such systems with science-based frameworks guiding them through the systematic process of design and evaluation. The RISKSUR project has addressed this particular need through the development of integrated surveillance system design and evaluation frameworks and associated decision support tools (RISKSUR tools). This paper provides an overview of the RISKSUR tools and presents their application using several disease case studies relevant to EU member states. The RISKSUR tools provide user-friendly access to comprehensive, flexible and state-of-the-art integrated frameworks for animal health surveillance design and evaluation, thereby providing effective guidance during the complex decision making process. The tools will continue to be refined in response to user feedback and new methodological developments. Their availability in the public domain will facilitate access by users and allows widespread integration into training materials.

INTRODUCTION

The global demand for sufficient, safe and nutritious food continues to increase, requiring further intensification of livestock production, while at the same time recognising the need to protect our environment. The associated eco-social changes will increase the risk of emergence and spread of new and known infectious diseases affecting animals and humans. These developments lead to the need for conducting more effective disease surveillance, while they may reduce the availability of financial resources. Utilising knowledge about variation in risk of infection in exposed populations provides an opportunity for the animal health surveillance effort to be structured such that timely and maximum sensitivity of detection can be achieved while still being cost-effective. The development of such risk-based surveillance systems is complex, and requires an approach that is based on the most up-to-date knowledge effectively

---

* Arianna Comin, Department of Disease Control and Epidemiology, National Veterinary Institute, 75189 Uppsala, Sweden. Email: arianna.comin@sva.se
** Barbara Haesler, Leverhulme Centre for Integrative Research on Agriculture and Health, Royal Veterinary College, Hatfield AL9 7TA, UK. Email: bhaesler@rvc.ac.uk
Both authors contributed equally to this work

integrated between different scientific disciplines and transparent to stakeholders. Hence, for risk-based systems in particular, but also for non-risk based systems, surveillance design usually involves a process of comparing several options, which ideally should include a formal evaluation. These technical challenges and evaluation requirements mean that the process of surveillance design is best carried out by interdisciplinary teams. To enable wide-spread acceptance and adoption of surveillance approaches by stakeholders it is essential to provide those designing such systems with science-based frameworks guiding them through the systematic process of design and evaluation.

The RISKSUR project has addressed this particular need through the development of integrated surveillance system design and evaluation frameworks and associated decision support tools (RISKSUR tools) addressing four objectives: (i) early detection of exotic, new and re-emerging diseases, (ii) disease freedom documentation (iii) frequency estimation of endemic diseases, and (iv) detection of cases of endemic diseases. The project was conducted in 2012–2015 funded by the Seventh Framework Programme (FP7) of the European Union (EU). The Consortium involved 11 European partners and FAO, bringing together scientific expertise in veterinary medicine, veterinary epidemiology, statistical analysis, surveillance, risk assessment and animal health economics.

The objective of this paper is to provide an overview of the RISKSUR tools, and present their application using several disease case studies relevant to EU member states.

THE SURVEILLANCE DESIGN AND EVALUATION TOOLS

Preliminary activities

Initial activities informing the core of the surveillance design and evaluation tools included data collection to produce a high level overview of the animal populations, trade flows and animal health infrastructure, interviews with decision-makers, systematic literature reviews of surveillance systems and epidemiological methods targeting the different surveillance objectives, and information on ongoing surveillance systems in the EU.

Mapping and interviews: Secondary data on surveillance systems were collected in thirteen European countries (Belgium, Bulgaria, Czech Republic, Denmark, France, Germany, Great Britain, Ireland, Italy, the Netherlands, Spain, Sweden, and Switzerland) using data from public resources such as Eurostat, grey literature and websites of animal health services. Moreover, secondary data on critical infrastructure and primary data on existing decision-making processes for resource allocation to surveillance were gathered in France, Germany, Great Britain, the Netherlands, Spain, Sweden, and Switzerland. In these countries, 34 decision-makers were interviewed. Additionally, data on livestock and bee holdings in Europe, human and animal populations, gross domestic product, and farm values were collated from Eurostat. Data on trade were obtained from the EU's Trade Control and Expert System (TRACES), which records movements of live animals and livestock products in the EU. All data were entered into a database, cleaned and analysed descriptively (RISKSUR consortium, 2014).

Systematic reviews: The initial search forpublications was done on 'CabAbstract' and 'Scopus' databases using an algorithm defined by the RISKSUR consortium to ensure a homogeneous search across the surveillance objectives covered by the project. Screening of retrieved articles based on specific exclusion criteria resulted in 128, 132 and 69 articles used for the review of surveillance systems and methods aimed at early detection, disease freedom and endemic diseases, respectively (Comin et al., 2013; Schauer et al., 2013; Rodríguez-Prieto et al., 2014). Moreover, a systematic review was conducted using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to identify and compare the advantages and limitations of existing evaluation guidelines for surveillance (Calba et al., 2015).

Review of existing surveillance systems in the EU: Information on ongoing surveillance systems in nine European countries (Denmark, France, Germany, Great Britain, Italy, The Netherlands, Spain, Sweden and Switzerland) was collected from both public available documentation and through private contacts with the aim to describe basic epidemiological characteristics of current surveillance systems, detect variation in legislation and highlight similarities and differences (Comin et al., 2014).

Development of the tools

Development of the design tool: The first step was constructing the design framework to identify all elements that compose a surveillance system, i.e. all the small details a surveillance designer must think of and decide on when designing a surveillance system or it components. The surveillance steps were defined using the background work previously described as starting point. This was followed by several rounds of brainstorming among the group developing this task, other experts within the RISKSUR consortium, and also experienced surveillance designers within the partner institutions. Surveillance steps were then organized into a proper flow and grouped into broader surveillance design sections. A general framework was created first, with the intention of outlining the steps of surveillance that are common to all surveillance objectives. This framework was then refined to address the specific surveillance goals of early detection, disease freedom documentation, case detection and prevalence estimation.

The framework was implemented as a questionnaire in a Microsoft Excel® spreadsheet using real data of a case study for each of the above-mentioned surveillance goals. At various stages of development, the framework was tested and revised with the help of surveillance stakeholders from the various partner institutions who agreed to participate in exercises of surveillance design using the framework as a tool. Finally, the framework was refined with the help of surveillance stakeholders from outside the RISKSUR consortium. A workshop on surveillance design was carried out in conjunction with the annual meeting of the European Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) in 2015. Interested participants were encouraged to test the framework, and send feedback which was then used to refine the tool.

The design framework provides guidance for surveillance design via the wiki page at http://surveillance-design-framework.wikispaces.com/. The wiki content is based on the expertise of the RISKSUR consortium members developing the framework, and also incorporated feedback from user group workshops (provided via a standardised questionnaire) as well as comments from the wiki members. It also includes a glossary and links to statistical tools (e.g. sample size calculators) that can be used during the design process. The structure of the wiki is based on the Excel® tool, making it easy to retrieve the advice needed at each design step.

Development of the EVA tool: The aim of the tool is to produce protocols for an integrated epidemiological and economic evaluation. Based on the information collected through the preliminary activities, an initial conceptual framework and accompanying protocols were developed. This included online and face-to-face consultations with specialists within the consortium to capture guidance on the description of the evaluation context, the selection of appropriate evaluation questions, attributes, criteria and methods as well as communication of results. Eight case studies were used to support this process by providing input on terminology, suitable evaluation attributes and methods. The framework was then presented to, and approved by, the whole consortium and members of the advisory board. In a next step, the framework and protocols were applied to ten case studies so that users (consortium researchers) could test the logic of the tool and provide general feedback for refinement of the approach. Finally, an integrated epidemiological and economic evaluation of four case studies was conducted to validate the evaluation framework and tool.

Elicitation of expert opinion was used to identify the most relevant economic evaluation questions and evaluation attributes to be assessed according to each specific context. Detailed information on all the concepts and methods introduced in the EVA tool have been described in the dedicated wiki (http://surveillance-evaluation.wikispaces.com/). References to the wiki are available throughout the EVA tool to provide more in depth information and understanding on the process undertaken.

Creation of a web-based interface: Once the conceptual models of the two tools were available, a user-friendly web interface was developed, integrating essential information on the design process, general evaluation concepts, the development of the evaluation plan and the full evaluation process. The web-based interface allows the user to outline the structure of an animal health surveillance system, and to produce a protocol for full epidemiologic and economic evaluation of such a system (Varan et al., 2015). The tool is complemented by two Wikispace Classroom applications (http://www.wikispaces.com/content/classroom/about) which provide information for the design and evaluation processes, but also serve as educational platforms, as well as platforms for continuous updating of experiences, examples, methods and best practices.

Principles and structure of the tools

The surveillance design framework: The surveillance design framework aims at structuring the process of designing, documenting and re-designing animal health surveillance. The target users are expected to be “competent and technical level users who design, implement or evaluate surveillance strategies for infectious livestock diseases within the European Union”. Likely it will not be an individual, but rather a team, gathering knowledge in epidemiology and surveillance. The team is also expected to be supported by diagnostic experts and ideally an economic advisor.

The surveillance design framework has been structured in 14 main sections, as summarized in Fig. 1, each one comprising a separate worksheet in the Excel® questionnaire.

The first step in designing or documenting any surveillance activity is describing the surveillance system, which is defined as a collection of various surveillance components which all aim to “describe health-hazard occurrence and contribute to the planning, implementation, and evaluation of risk-mitigation actions”, for one health-hazard in particular, and in a defined region (Hoinville et al., 2013). Secondly, the surveillance designer is encouraged to think about all surveillance components that are/will be part of the surveillance system, and list them to have an overview. A surveillance component has been defined as a surveillance activity against the identified hazard, in a particular target population and geographical area, using a given data collection strategy (i.e. means of data acquisition, sample type, point of sample collection).

Finally, each identified component is fully characterized through the definition of target population, disease suspicion, surveillance enhancements, testing protocol, study design, sampling strategy, data generation (sample collection), data/sample transfer, data translation (sample analyses), epidemiological analyses, dissemination of results and surveillance review. Steps one and two represent the general characterization of a surveillance system and have been incorporated in the web-based interface as well, as they serve as starting point to further evaluate surveillance.

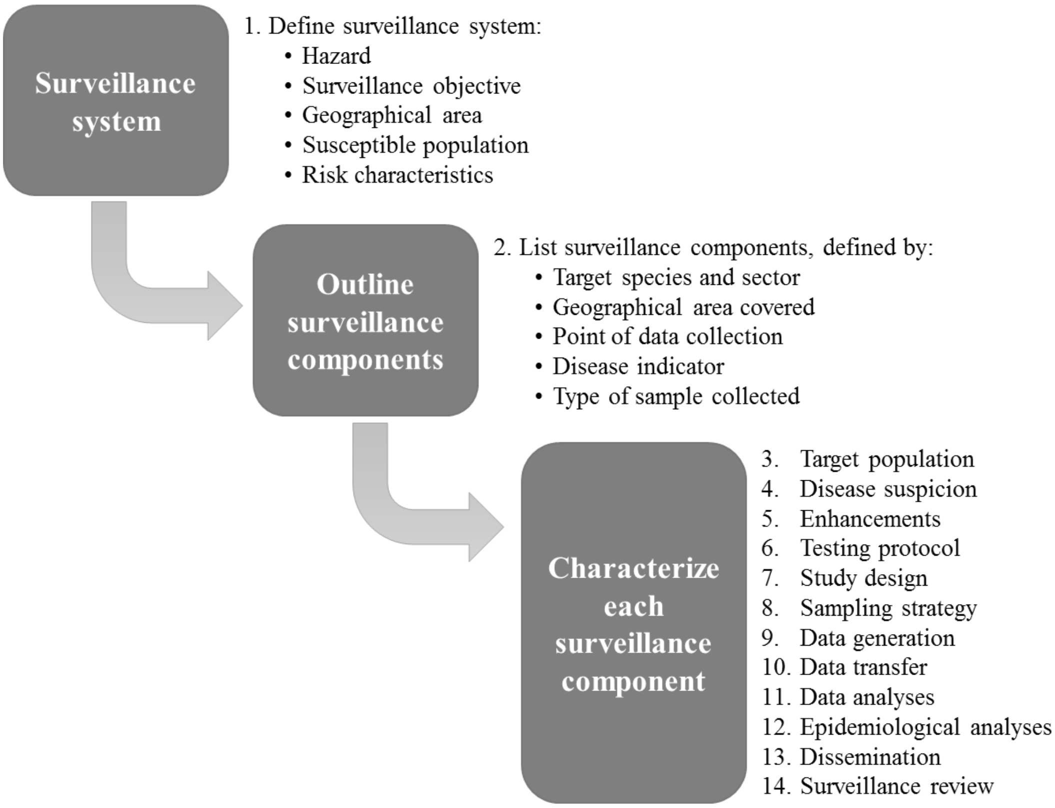


Fig. 1 Overall structure of the surveillance design framework

Although the focus of the framework is not on collecting information but on helping the users to define the necessary surveillance details, the Excel® spreadsheet provides the possibility to document the information entered, so that users can have a consistent documentation of the surveillance system design. This documentation can be used to assure transparency before international bodies or trading partners, or for later reference within the institution, representing the first step towards a harmonized and transparent reporting and documentation of design and achievements of animal health surveillance, which is currently missing in EU (Comin et al., 2014).

Many of the target users of the design framework will have surveillance activities already in place that they want to re-design in order to increase performance. Once a surveillance designer identifies that a particular performance attribute (e.g. sensitivity, or timeliness) needs to be improved (e.g. by applying the EVA tool, described below), the surveillance design framework offers the possibility to focus the re-design on that attribute. The user is then presented with the list of all the surveillance steps and advice on which ones can potentially impact the selected performance attribute.

The EVA tool: The EVA tool is a decision support tool for economic and/or epidemiological evaluation of surveillance components. It builds on existing evaluation framework methods and tools and provides specific guidance to the user to facilitate the selection of an evaluation approach according to their specific context of evaluation (e.g. surveillance system objective; economic context; decision maker needs), resource availability and expertise. The tool is organized into three main sections: The first section provides a general introduction to the tool and essential information on evaluation concepts, including evaluation attributes and economic methods. The second section provides guidance for the user on how to develop an evaluation plan. This section is organized into four main steps, namely Step 1: defining the evaluation context; Step 2: selecting the evaluation question; Step 3: selecting evaluation attributes and methods for the technical and economic assessments; Step 4: reviewing the summary of the evaluation plan. The third section provides guidance on how to perform the evaluation and how to report the outputs of the evaluation to decision makers.

After documenting the surveillance system under consideration, the user is asked to describe all the contextual elements that are critical in the elaboration of the evaluation plan (e.g. decision maker needs, legal requirements). A decision pathway leads the user step-by-step through a set of questions to help select a suitable evaluation question. The tool provides the list of evaluation attributes (i.e., effectiveness, functional and economic criteria) ranked in relation to the specific context (i.e. surveillance objective, evaluation context and evaluation question selected). The user is then guided to select the attributes to include in the evaluation plan and the method preferred for their assessment. Information on the pros and cons of including one or multiple attributes, and of the choice of one method over the other, is provided. This information is critical to assess the quality and limitations of the evaluation performed and to provide meaningful recommendations to decision makers according to i) the context of evaluation and ii) the extent of the evaluation plan. Finally, the EVA tool allows export of all the information from the web tool interface into a pdf document to support the implementation of the evaluation.

APPLICATION OF THE RISKSUR TOOLS TO CASE STUDIES

The developed tools were tested using case studies, as summarised in Table 1. Here we provide a short overview of the case studies conducted in relation to the two tools and highlight commonalities and differences across the case studies implemented.

Application of the surveillance design framework

The surveillance design framework was applied to design, redesign and document existing surveillance systems, aiming at assessing whether the structure of the tool is applicable to surveillance with various objectives – case detection, prevalence estimation, demonstrating freedom, or early detection – as well as multi-hazard surveillance. Eight case studies were selected for the purpose, covering endemic, exotic/re-emerging and absent animal diseases in several EU countries, namely: Salmonella in cattle in Sweden; Porcine Respiratory and Reproductive Syndrome (PRRS), Aujeszky’s Disease (AD) and Classical Swine Fever (CSF) in Sweden; Avian Influenza (AI) in the UK; African Swine Fever (ASF) in Poland; Bovine Viral Diarrhoea (BVD) in the UK, CSF in wild boar in Germany, Bovine Herpes Virus 1 (BHV1) in Germany and The Netherlands; and Bluetongue (BT) in Germany (Tab. 1).

Table 1. Overview of the case studies applied to test the two tools

Case studies	1	2	3	4	5	6	7	8
Hazard under surveillance[†]	*Salmonella*	PRRS, AD, CSF	AI	ASF	BVD	CSF	BHV1	BT
Target species	cattle	pigs	laying hens	pigs	cattle	wild boar	dairy cattle	ruminants
Surveillance goal:								
·Case finding	✓	–	–	–	✓	–	–	–
·Demonstrate freedom	–	✓	–	–	–	✓	✓	✓
·Early detection	–	–	✓	✓	–	–	–	–
·Prevalence estimate	✓	–	–	–	✓	–	–	–
Level	country	country	country	country	country	region	herd & country	country
Surveillance structure:								
·multi-component	✓	✓	✓	✓	✓	–	✓	✓
·single component	–	✓	–	–	–	✓	–	–
Use of the case study:								
·Document existing surveillance	✓	✓	✓	✓	✓	✓	✓	✓
·Design new surveillance	✓	–	✓	✓	–	✓	–	–
·Redesign surveillance	✓	–	✓	✓	✓	✓	✓	–
·Assess epidemiological performance	✓	–	✓	✓	–	✓	✓	–
·Multi-objective surveillance	–	✓	–	–	–	–	–	–
·Cost or economic evaluation	✓	–	✓	–	✓	✓	–	–
Descriptive assessment	OASIS	–	SWOT	–	SWOT	OASIS	–	–
Functional attributes evaluated:								
·Acceptability	–	–	–	–	✓	✓	–	–
·Availability	–	–	–	–	✓	–	–	–
·Engagement	–	–	–	–	✓	–	–	–
·Simplicity	–	–	–	–	✓	–	–	–
·Sustainability	–	–	–	–	✓	–	–	–

[†] PRRS = Porcine Respiratory and Reproductive Syndrome, AD = Aujeszky's Disease, CSF = Classical Swine Fever, AI = Avian Influenza, ASF = African Swine Fever, BVD = Bovine Viral Diarrhoea, BHV1 = Bovine Herpes Virus 1, BT = Bluetongue

Table 1. Cont’d

Case studies	1	2	3	4	5	6	7	8
Performance attributes evaluated:								
·Coverage	–	–	–	–	✓	–	–	–
·Detection fraction	✓	–	–	–	–	–	–	–
·Precision	–	–	–	–	✓	–	–	–
·Sensitivity	–	–	✓	–	–	✓	–	–
·Timeliness	–	–	✓	–	–	✓	–	–
Economic attributes evaluated:								
·Cost	✓	–	–	–	✓	✓	–	–
·Economic efficiency	✓	–	✓	–	–	✓	–	–

Multi-hazard surveillance is defined here as the secondary use of data/samples collected as part of a surveillance activity designed for one specific hazard (which will be called mother component) to make inference on additional hazards (which will be called child components). The way the framework deals with multi-hazard surveillance is to first document the surveillance system containing the mother component and then activate the multi-hazard tab to design/document the child components. This creates as many duplicates of the mother component as the number of child components to design, making it easy to perform the adjustments needed.

When designing or documenting a surveillance system, the individual surveillance components are described side by side, which allows an easy comparison of them. The framework includes specific sections for passive surveillance (i.e. Section 4, on the definitions of disease suspicion) and for active surveillance (i.e. Sections 7 and 8, on detailed sampling strategy), therefore being flexible in capturing different needs. The last sections (i.e. 9 to 14) are very descriptive and ask for information that are possibly not essential for the documentation of an existing surveillance system. However, when designing a new surveillance system, they provide useful insights into all the aspects to be considered when building an efficient and sustainable system. In general, the process of designing/documenting an existing surveillance system has been proved to be time demanding by nature but straightforward and comprehensive. Once a system has been designed/documented, adjustments as part of re-design are easy to implement.

The assessment of the epidemiological performance of newly designed surveillance components provided an evaluation of the compliance of the surveillance design framework in designing and improving surveillance system/components aiming at case finding, early detection and demonstration of freedom from disease. Risk-based surveillance played a central role in redesigning surveillance to improve its effectiveness. Risk-based surveillance components was shown to result in a better sensitivity for demonstrating freedom from CSF in wild boar in Germany and higher timeliness for early detection of AI in the UK. On the other hand, for case detection of Salmonella in cattle in Sweden a conventional surveillance approach would allow the detection of more infected herds in the short run. However, a risk-based surveillance would achieve the same effectiveness with much less resources in a longer time span.

Application of the EVA tool

The EVA tool was applied to four case studies (early detection of AI in the UK, freedom from CSF in wild boar in Germany, case detection of Salmonella in cattle in Sweden, and documentation of disease BVD in the UK) to do a descriptive evaluation of the system, to assess the performance and associated functional attributes and to judge the economic value of the newly designed or re-designed components.

The majority of the evaluation questions focused on achieving a higher effectiveness at lower or equal cost or to identify the least-cost option among components of similar effectiveness. Only one case study selected to identify the component (out of two or more) that would generate the biggest net benefit. All evaluators deemed their evaluation questions feasible and proceeded to select attributes and associated methods relevant for the selected question. For each case study 4-6 performance attributes were identified by the EVA tool and the user as relevant for the evaluation, but only 1-2 were assessed in the case studies (Tab. 1), which was mainly due to resource constraints. Of the functional attributes, the only attribute selected by more than one case study was ‘acceptability’.

All case studies conducted an assessment of the costs in comparison to one or more effectiveness criteria in either least-cost or cost-effectiveness analyses. One case study translated the effectiveness measures into a monetary benefit for inclusion in a cost-benefit analysis. Because all case studies looked at new designs to either complement or replace old designs, the analyses were prospective / ex ante. Regarding implementation of the economic evaluation methods, difficulties encountered included the estimation of fixed and variable costs, non-monetary benefits, and co-benefits resulting from using synergies, and the selection of a meaningful effectiveness measure. None of the case studies reported to use discounting, which may indicate limited awareness of the importance of this economic principle. Two cost-effectiveness analyses conducted faced challenges associated with the interpretation of the effectiveness metrics and demonstrated the importance of reflecting on the wider aspects of the surveillance and intervention efforts and to find out what the ultimate aim of the programme is to make an informed judgement.

The CSF case study highlighted the importance of considering more than one evaluation attribute to provide meaningful results and to discriminate between the different surveillance designs under evaluation. Indeed, most of surveillance designs (including the current one) reached the target effectiveness value defined in terms of surveillance system sensitivity. However, the timeliness, simplicity and acceptability differed between the different designs under evaluation. The combined analysis of all these different attributes allowed identifying the most preferable design in terms of function, performance and economic attributes.

Generally, the users were able to navigate the EVA tool without major difficulties and were able to set up an evaluation plan making use of the guidance provided. Several users reported that the information provided on suitable attributes and the limitations or requirements of methods helped them to structure their evaluation.

DISCUSSION

The surveillance design framework provides support for surveillance professionals in setting up appropriate animal health surveillance systems. It provides a tool to document their decisions for communication and review, and helps with the re-design of surveillance components when improvement of performance is needed. It needs to be emphasized that the framework itself does not suggest any preferential choice nor assess epidemiological performance; the user still makes all the decisions and carries out the epidemiological analyses. However, the step-by-step process of the design framework aims at helping users navigate through the variety of tools and information available to make such decisions. The choice of providing advice and guidance through a wiki was motivated by the intention of keeping it constantly updated by the input from the surveillance community. Members of the wiki can edit advice, post questions and examples and engage in discussions: all fundamental activities to make the surveillance design framework a living tool. Since its first release in May 2015, the wiki already counts 62 members, 43 of which are outside of the RISKSUR consortium (to 15 December 2015).

Feedback from pilot testers highlighted room for improvement of the surveillance design tool. Some of the suggested improvements have already been scheduled (e.g. improvement of the section on sampling strategy, objective-specific advice for redesign), while some other must be accepted as limitations. In particular, the balance between complexity and simplicity is an important issue. The surveillance design framework has been chosen to be reasonably simple and generic, so that it can be used to design surveillance targeted at many possible hazards. This in turn penalizes more complex designs (e.g. a three-stage sampling design) or uncommon situations (e.g. when there are two equally important surveillance objectives). Furthermore, the framework has not been assessed for the design of particular surveillance systems such as those for aquatic organisms and bees. Nevertheless, the framework was successfully used for a wide range of case studies, representing current surveillance activities for diseases that are present, absent or exotic/re-emerging in important terrestrial species in EU member states.

Evaluation of surveillance activities is receiving increasing attention from both veterinary services as well as other providers such as private industry. Some countries started to integrate evaluation into the policy cycle of surveillance. While there is published guidance for conducting evaluation in general, there is a lack of information to specifically support the evaluation of animal health surveillance. Also, there are not yet many published reports of completed evaluation projects in the public domain. The tool and documents provided by RISKSUR provide an attempt to fill these gaps.

Similarly as in the design of surveillance activities, it is important to define the expectations and focus of evaluation projects clearly at the very beginning. This requires a specific evaluation question to be agreed upon and understood by all parties involved from the outset. The selection of evaluation attributes and criteria will be driven by this question, but it will also be strongly influenced by the resources that are available. Some quantitative attributes require extensive data collection and skills that may not be feasible or accessible. To assure data availability, it is recommended to consider evaluation at an early stage such that the required information can be collected prospectively, ideally during the surveillance design stage. Particularly, economic information is often found to be patchy and difficult to collect in retrospect.

Along with specific guidance on the choice of evaluation question and attributes, the EVA tool provides general information on evaluation concepts, evaluation attributes and economic evaluation methods along with practical example of surveillance system evaluation. This is necessary for promoting the understanding of the evaluation process and the documentation of the quality of the data generated by the evaluations in order to better inform the decision making process. The EVA tool was developed to integrate the different level of evaluation and degree of complexity and to guide the users in the development of their evaluation plan and framing the boundaries of their evaluation. The objective of the tool was to promote the use of comprehensive evaluation including economic evaluation by providing detailed information on the available methods and relevance according to a specific evaluation question and context.

Sometimes, the selection criteria for effectiveness and economic efficiency may not be the same and it is important to think thoroughly about the potential outcomes of the analysis and what information these outcomes will provide. This is complicated by the fact that it is rare to find users that possess both advanced technical and economic knowledge and skills. Assuming that the target user for the evaluation tool would be a person with strong technical surveillance expertise, but limited economic knowledge, it was decided to provide an introduction to critical concepts, suitable methods, data and time requirements for the economic evaluation of surveillance. This approach is expected to nurture the use of economics applied to surveillance, which is still in its infancy. In particular, the three-variable relationship between surveillance, intervention and loss avoidance (Howe, Häsler & Stärk, 2013); value of information (Stärk & Häsler, 2015), and non-monetary benefits are elaborated and linked to economic analysis methods commonly used in animal health (Rushton, Thornton & Otte, 1999). In the long term, increased awareness and understanding of the economic theory underpinning the economic evaluation of surveillance as well as an appreciation of challenges that can accrue from application of differing paradigms is expected to increase professional capacity and help to address the problem of resource allocation for surveillance to the benefit of all.

CONCLUSIONS

The RISKSUR tools provide user-friendly access to comprehensive, flexible and state-of-the-art integrated frameworks for animal health surveillance design and evaluation, thereby providing effective guidance during the complex decision making process. The use of the surveillance design framework should be immediately compatible with the needs of veterinary authorities, while the evaluation framework makes a new and essential dimension of the process accessible to decision makers. This inclusion of structured evaluation in both short- and long-term surveillance implementation cycles will enhance the validity of surveillance system outputs and stakeholder acceptance of the utility of animal health surveillance. With tools now being available, the aim will be to expose stakeholders from around Europe and beyond to the RISKSUR tools and the underlying concepts. During this phase, the tools will continue to be refined in response to user feedback and new methodological developments. Their availability in the public domain will facilitate access by users and allows widespread integration into training materials.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 310806.

REFERENCES

Calba, C., Goutard, F.L., Hoinville, L., Hendrikx, P., Lindberg, A., Saegerman, C., and Peyre, M. (2015). Surveillance systems evaluation: a systematic review of the existing approaches. BMC Public Health 15, 448. doi:10.1186/s12889-015-1791-5.

Comin, A., Dórea, F., Rosendal, T., Vergne, T., Frössling, J., Nöremark, M., Wahlström, H. and Lindberg, A. (2013). Systematic review of surveillance systems for endemic disease, including information on the extent of multi-objective surveillance activities. RISKSUR Deliverable 4.14, 1–52. Available at http://www.fp7-risksur.eu/sites/default/files/documents/Deliverables/RISKSUR%20%28310806%29_D4.14.pdf (accessed 15 December 2015).

Comin, A., Dórea, F., Martínez-Avilés, M., Rodríguez-Prieto and Schauer, B. (2014). Review of surveillance systems in place in the European Union. RISKSUR internal report.

Hoinville, L.J., Alban, L., Drewe, J.A., Gibbens, J.C., Gustafson, L., Häsler, B., Saegerman, C., Salman, M. and Stärk, K.D.C. (2013). Proposed terms and concepts for describing and evaluating animal-health surveillance systems. Prev. Vet. Med. 112, 1–12.

Howe, K. S., Häsler, B. and Stärk, K.D.C. (2013). Economic principles for resource allocation decisions at national level to mitigate the effects of disease in farm animal populations. Epidemiol. Infect. 141, 91–101.

RISKSUR consortium. (2014). Mapping of surveillance systems, animal populations, trade flows, critical infrastructure and decision making processes in several European countries. Research brief, No. 1.2. Available at http://www.fp7-risksur.eu/sites/default/files/documents/Deliverables/RISKSUR%20Research%20Brief%20N%C2%B01.2.pdf (accessed 15 December 2015).

Rodríguez-Prieto, V., Vicente-Rubiano, M., Sánchez-Matamoros, A., Rubio-Guerri, C., Melero, M., Martínez-López, B., Martínez-Avilés, M., Hoinville, L., Vergne, T., Comin, A., Schauer, B., Dórea, F., Pfeiffer, D.U. and Sánchez-Vizcaíno, J.M. (2014). Systematic review of surveillance systems and methods for early detection of exotic, new and re-emerging diseases in animal populations. Epidemiol. Infect. 143, 2018-2042.

Rushton, J., Thornton, P. K. and Otte, M.J. (1999). Methods of economic impact assessment. Revue Scientifique et Technique (International Office of Epizootics), 18, 315–42.

Schauer, B., Staubach, C., Vergne, T., Comin, A., Fröhlich, A., Höreth-Böntgen, D., Stärk, K.D.C., Cameron, A. and Conraths, F. (2013). Review of epidemiological methods used for surveillance systems certifying freedom from disease. RISKSUR Deliverable 3.10, 1–34. Available at http://www.fp7-risksur.eu/sites/default/files/documents/Deliverables/RISKSUR%20%28310806%29_D3.10_0.pdf (accessed 15 December 2015).

Stärk, K.D.C. and Häsler, B. (2015). The value of information: Current challenges in surveillance implementation. Prev. Vet. Med. 122, 229-234.

Varan, V., Peyre, M., Hoinville, L., Njoroge, J., Häsler, B., Schauer, B., Staubach, C., Brouwer, A., Comin, A., Sargent, E. and Dórea, F. (2015). Web-based decision support tools, RISKSUR Deliverable 6.25, 1–14. Available at http://www.fp7-risksur.eu/sites/default/files/documents/Deliverables/RISKSUR%20%28310806%29_D6.25.pdf (accessed 15 December 2015).

THE COMPARISON OF EXISTING AND ALTERNATIVE SURVEILLANCE STRATEGIES TO PROVE FREEDOM OF BHV1 IN DAIRY FARMS: A CASE STUDY WITHIN THE RISKSUR PROJECT

A. VELDHUIS*, I. SANTMAN-BERENDS, B. SCHAUER, F. WALDECK, J. MARS, C. STAUBACH AND G. VAN SCHAIK

SUMMARY

This study aimed at the application of the RISKSUR surveillance design framework to describe and redesign the surveillance program of bovine herpesvirus type 1 (BHV1) as laid down by EU-legislation. Scenario-tree analyses were carried out to determine surveillance system sensitivities (and components thereof) and the monthly herd-level confidence of freedom with two different surveillance designs. At a within-herd design prevalence of 10%, the conventional (EU) design led to a varying probability of freedom between 99.6-100% in an endemic situation, compared to a constant probability of freedom of >99.8% in the alternative design. In a disease-free situation, both designs performed equally well. The RISKSUR surveillance design framework provided easy-to-use guidance to describe and redesign the BHV1 surveillance program, potentially contributing to a standardisation of surveillance documentation. The assessment of various surveillance designs could be highly useful to support decision-making towards a more risk-based approach of animal health surveillance.

INTRODUCTION

This study was conducted within the context of the RISKSUR project, which was an international research project aiming at developing an integrated surveillance system design and evaluation framework. The project was conducted between 2012 and 2015 and funded by the Seventh Framework Programme of the European Union (http://www.fp7-risksur.eu). Within the RISKSUR project, a surveillance design framework (SDF) was developed to guide scientists and policymakers in the development of disease surveillance systems by structuring the process of designing, documenting and redesigning the system. We used bovine herpesvirus type 1 (BHV1) in dairy farms as a case study for the use of the SDF. BHV1 is a member of the alphaherpesvirinae and causes infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/ balanoposthitis (IPV/IPB). EU Member States have the possibility to obtain an official BHV1-free status as laid down in EU Directive 64/432/EC. This directive allows Member States with a BHV1-free status to impose restrictions on the importation of cattle from countries or regions that are not free from BHV1. The current study aimed at (i) the application of the SDF to describe and redesign the surveillance program of bovine herpesvirus type 1

---

* Anouk Veldhuis, GD Animal Health, PO Box 9, 7400 AA Deventer, Netherlands. Email: a.veldhuis@gdanimalhealth.com

(BHV1) in dairy farms as laid down in EU legislation, and (ii) to compare the epidemiological performance of the conventional and alternative design using scenario tree modelling in both an endemic and disease-free situation.

MATERIALS AND METHODS

Surveillance design

By using the SDF, the user is presented with the surveillance decisions that need to be made when designing a surveillance system, such as defining the target hazard and surveillance objective, target population, surveillance enhancements, testing protocol, study design, sampling strategy, sample collection, data/sample transfer, sample analyses, epidemiological analyses, dissemination of results and surveillance review. The framework is available in Excel and can be downloaded in the educational Wikispace created for the framework at: https://surveillance-design-framework.wikispaces.com. In the current study, the SDF was used to describe the BHV1 program as prescribed by EU Decision 2004/558/EC and 2007/584/EC.

Conventional design: The BHV1 surveillance regime as required according to EU legislation is described as follows:

- Obtaining the BHV1-free status (OBTconv): All cattle >9 months of age should be tested serological negative twice with a maximal interval of five to seven months.
- Monitoring the free status (MONconv): All cattle >24 months of age should have negative test results following serological investigation at intervals of not more than 12 months[1].

According to the EU legislation, certified holdings are not allowed to have animals in the herd that originate from non-free herds. Therefore, purchase testing is not part of the conventional surveillance design (i.e. it was assumed that animals from non-free herds will not be introduced into the free herd).

Alternative design: An alternative surveillance approach based on monthly bulk milk testing was designed. A risk-based component based on additional testing of cattle purchased from non-free herds was added. This design is based on the voluntary BHV1 control program for dairy herds that is currently operational in the Netherlands[2]. The alternative surveillance design consists of three components and can be described as follows:

- Obtaining the free status (OBTalt): All cattle older than 12 months should be subjected to a serological test (gE-ELISA) after which animals with positive test results should be removed from the holding. After removal of seropositive animals (if any), the free status of the herd is validated within 4-8 weeks by a bulk milk test.

---

[1] In addition, EU Decisions 2004/558/EC and 2007/584/EC describe alternative control regimes to attain and maintain a BHV1 free status, which were not considered in this study.

[2] In addition to the components included in the alternative design, the BHV1 control program in the Netherlands consists of a passive clinical surveillance component including testing of aborting cows.

- Monitoring of free status (MONalt): Seronegative bulk milk test (gE-ELISA) results need to be obtained from the holding, at least nine times per year[3], with intervals of at least one month. The gE-ELISA test in bulk milk is 98% sensitive given a prevalence of at least 10% in the group of lactating cows (Wellenberg et al., 1998). A positive test result should be followed by a new bulk milk test within seven days. The free status of the holding is maintained if the second test result is negative. If the second sample also tests positive then the free status of the holding is suspended.

- Purchase testing (PURalt): Cattle purchased from non-free herds[4] need to be tested for serology within eight weeks after being introduced in the herd. Cattle with positive test results must be removed from the holding. After removal of seropositive animals (if any), the free status of the herd is validated within 4-8 weeks by either a bulk milk test or a random sample of three blood samples (depending on the age of the purchased animal).

Scenario-tree model

A scenario tree analysis method was applied to the conventional and alternative surveillance designs to estimate (i) the sensitivity of the surveillance system's components to detect an infected herd and (ii) the monthly probability of disease freedom during one year monitoring of a herd's free status. In this study, sensitivity is defined as the probability of detecting an infected herd given that BHV1 is prevalent in the herd at the level of the design prevalence (PstarA). A stochastic scenario-tree model was developed for each surveillance component, as described by Martin et al. (2007a,b). Briefly, in these models, the probability that a single unit (eg. animal) will yield either a positive or a negative outcome when subjected to the testing protocol laid down in the component is calculated.

In each scenario tree, we used a within-herd PstarA of 10%, which has been used previously by EFSA (EFSA, 2006). Within the component MON_{alt}, PstarA was transformed to a group level probability of infection, as the testing protocol (bulk milk testing and its test sensitivity) applies to the pool of lactating cattle. To do so, we assumed that with an animal-level prevalence of 10%, the prevalence within the group of lactating cattle will be 10% as well (i.e. the disease is distributed homogenously throughout the herd), thus classifying the group of lactating cattle as infected.

The monthly probability of introduction of the virus into a herd (PIntro) was assumed to be influenced by purchase of cattle originating from non-free herds. In addition, in an endemic environment (country or region), BHV1 could be introduced in a herd via over-the-fence contacts, aerosols, neighborhood contacts, persons or material. In this study, we used different values of PIntro depending on the disease status at country level (free or endemic) and whether or not cattle from non-free herds were purchased by the farmer (in an endemic environment). In an endemic situation, a baseline PIntro was estimated to be 0.04% per month, based on a breakdown rate of 0.5% per year in certified herds in the Netherlands in 2014 (GD Animal Health, unpublished data) (Table 1). PIntro was increased by a hazard rate of 1.1 per purchase. In a disease-free situation, PIntro was assumed to be constant at 0.01% per month.

---

[3] Preferably 12 times a year. In 2014, 12 bulk milk samples per herd were tested in 81% of the certified dairy herds.

[4] Or cattle that have been in contact with cattle from non-free herds (on a fair of exhibition)

The model was developed using @RISK 5.7.1 (Palisade Corporation) in Excel (Microsoft) and outputs were based on 5,000 iterations, which appeared sufficient to obtain stable output values (mean and variance). Spread sheets were created in Microsoft Excel 2010 to represent each surveillance component (OBT_{conv}, MON_{conv}, OBT_{alt}, MON_{alt} and PUR_{alt},). The corresponding scenario trees are illustrated in Figures 1-4.

Table 1. Input parameters used in the scenario tree model to proof freedom of BHV1 in dairy herds in the Netherlands

Description of input parameter	Value	Source
Within-herd design prevalence (PstarA)	0.10	EFSA, 2006
Baseline monthly probability of introduction of BHV1 into a certified holding (PIntro)		Data from the BHV1 control program in the Netherlands (GD Animal Health) and expert opinion
in an endemic country	0.004	
in a disease-free country	0.001	
Proportion of risk factors and their relative risk (RR)		
Relative risk of seropositivity in animals ≥ 24 months of age vs. animals < 24 months of age	3	Van Wuyckhuise et al., 1993; Mars et al., 2001
Hazard rate per purchased animal for introduction of BHV1 into dairy farms	1.1	Van Schaik et al., 1999
Test sensitivities for serology and virus detection		
BHV1 gE-specific ELISA		Perrin et al., 1996; Van Oirschot et al, 1997; Wellenberg et al., 1998
on serum samples	0.87[b,c]	
on bulk milk samples	0.98[d]	
BHV1-specific real-time duplex PCR	0.99	Internal validation at GD Animal Health

[a] Specificity is assumed to be 100%

[b] Average of estimates from Perrin et al., (1996), Van Oirschot et al., (1997) and Wellenberg et al., (1998).

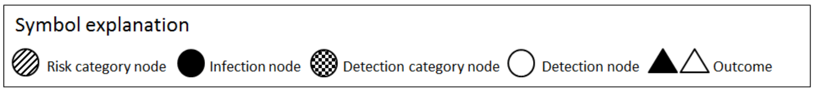
[c] When used on a sample of three animals, a herd sensitivity of 23,9% is reached given a design prevalence of 10% in an infinite population.

[d] When used on bulk milk samples, the gE-ELISA test is 98% sensitive to detect an infected herd provided that the animal level prevalence in the group of lactating cows is 10% or more (Wellenberg et al., 1998)

Component sensitivities: The probability that a single unit (animal) yields a positive outcome when subjected to the testing protocol laid down in the component, was calculated (component sensitivity at unit level, CSeU for component *i*). The $CSeU_i$ is calculated by summing up the limb probabilities for all limbs with positive outcomes in the scenario tree. $CSeU_i$ was transformed to an overall estimate of the component sensitivity (CSe_i), representing the probability of a positive outcome at herd level when all units (n) within component i are subjected to the testing protocol:

$$CSe_i = 1 - (1 - CSeU_i)^{n_i} \tag{1}$$

with i being components OBTconv, MONconv, OBTalt, MONalt or PURalt. Appropriate distributions were chosen for ni, based on movement and herd population data derived from the Dutch Identification and Registration database in 2014.



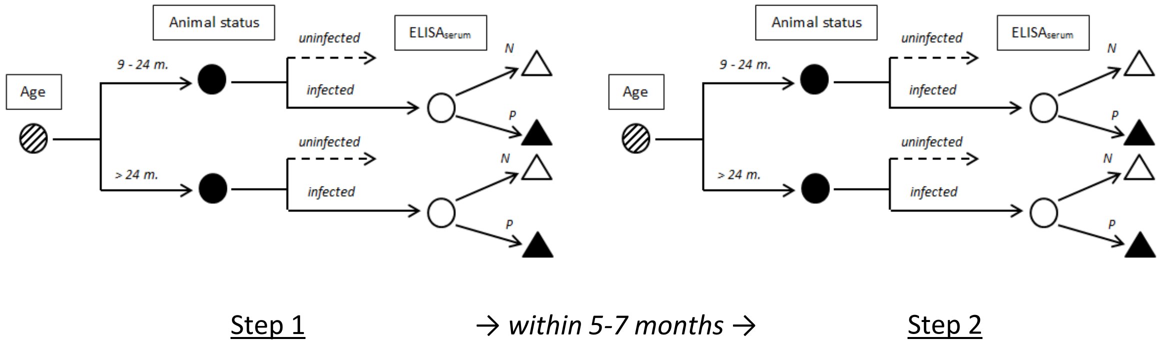


Fig. 1 Scenario tree illustrating the surveillance system component for obtaining the BHV1-free status in the conventional BHV1 surveillance design (OBT_{conv})

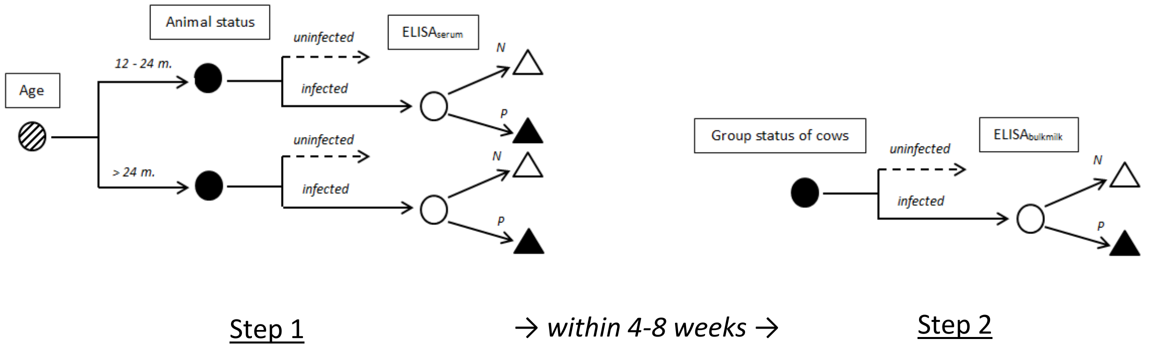
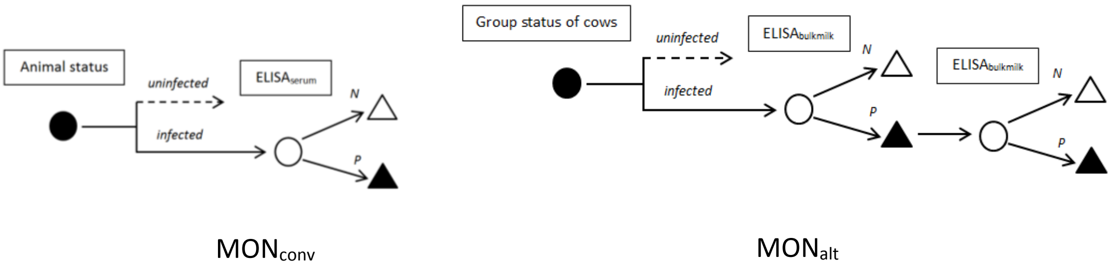


Fig. 2 Scenario tree illustrating the surveillance system component for obtaining the BHV1-free status in the alternative BHV1 surveillance design (OBT_{alt})



MON_{conv} MON_{alt}

Fig. 3 Scenario trees illustrating the surveillance system component for monitoring the BHV1-free status in the conventional (left) and alternative BHV1 surveillance design (right)

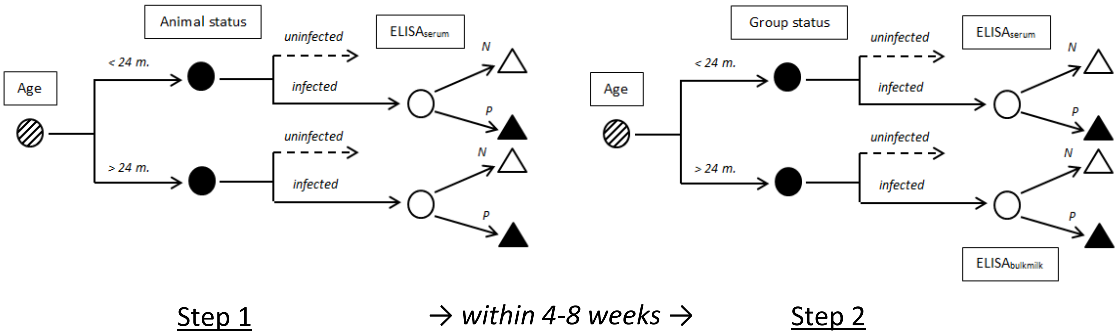


Fig. 4 Scenario tree illustrating the surveillance system component for testing of animals that are purchased from non-free farms in the alternative BHV1 surveillance design (PUR_{alt})

The sensitivity of the total surveillance program (SSe) is a combination of the CSe's of the different components in place. SSe is equal to OBTconv or OBTalt when a herd has entered the program to obtain a free status. During the phase in which the free status is monitored, the component MONconv or MONalt represents the SSe. If in addition purchase testing is carried out, the component PURalt is in place as well. If multiple surveillance components are in place, the sensitivity of the total surveillance program is calculated as:

$$SSe_t = 1 - (1 - CSe_1)(1 - CSe_2) \tag{2}$$

with CSe_1 and CSe_2 being the component sensitivities of the surveillance components in place in month *t*.

Probability of freedom: The probability of freedom from BHV1 per month t (PostPFreet) was calculated for the phase in which a herd's free status is monitored, with t=1 being the first month after the free status was obtained. PostPFreet was calculated based on the probability of infection at the beginning of the month (PriorPInft) and the sensitivity of the total surveillance system in that month. PostPFreet was estimated in time steps with a 1-month interval, by the following formula:

$$PostPFree_t = \frac{1 - PriorPInf_t}{1 - PriorPInf_t + (PriorPInf_t \text{ X } (1 - SSe_t))} \tag{3}$$

This formula corresponds to the standard calculation of a negative predictive value when assuming that the specificity of the surveillance system is equal to 1. $PriorPInf_t$, the prior probability that the herd is infected at the beginning of month *t*, is calculated as:

$$PriorPInf_t = PostPInf_{t-1} + PIntro - (PostPInf_{t-1} \text{ x } PIntro) \tag{4}$$

where $PostPInf_{t-1}$ is the estimated posterior probability of infection in the preceding month, adjusted for the introduction of BHV1 in between (PIntro) and is described by:

$$PostPInf = 1 - PostPFree \tag{5}$$

At t=1, the posterior probability of infection after obtaining the free status was used as PriorPInf.

<u>Scenarios</u>: The monthly probability of freedom from BHV1 was calculated for three different scenarios, depending on the probability of introduction of BHV1 in the herd:

A. BHV1-free herd without purchase of animals from non-free herds, assuming BHV1 is endemic at country level
B. BHV1-free herd without purchase of animals that originate from non-free herds[‡], in a situation in which the country is free from BHV1
C. BHV1-free herd that purchases animals that originate from non-free herds, assuming BHV1 is endemic at country level

<u>Sensitivity analyses</u>: To determine whether the model outcome was sensitive to the probability of introduction assumed in this study, the model was re-run with a probability of introduction that was double the default value (0.08% per month).

To assess the effect of (passive) clinical surveillance on the probability of freedom, a clinical surveillance component (CLIN) was added to both surveillance designs. Observation of typical BHV1-related clinical signs (high fever, drop in milk production, nasal discharge, etc.) in cattle must be notified to the competent authority as soon as possible, followed by immediate sampling (nasal swab) of the affected cattle and subsequent virus detection by PCR. The free status of the holding is terminated in case of at least one positive test result. Affected cattle with negative test results must be subjected to serology within 4-8 weeks after the suspicion to validate the holding's free status.

It was deemed unrealistic to assume that all cattle within a herd are continuously subject of clinical surveillance. Therefore it was decided to include lactating animals only. Additional input parameters were defined, describing the probability of clinical disease after BHV1 infection (assumed to vary between 0 and 0.4 with a most likely value of 0.1) and disease awareness of the farmer (assumed to vary between 0.2 and 0.9 with a most likely value of 0.75). The clinical surveillance component is illustrated in Figure 5.

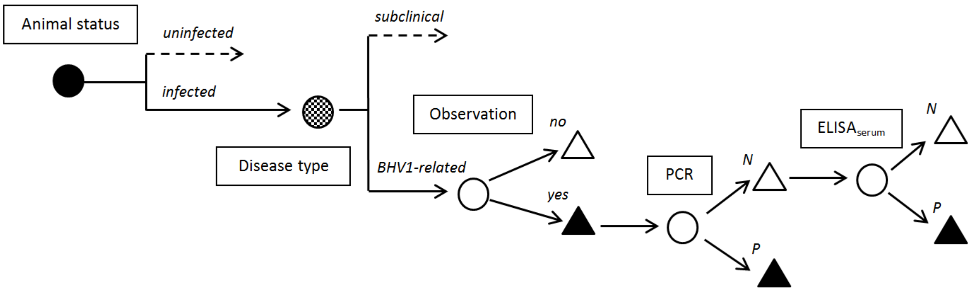


Fig. 5 Scenario tree illustrating the surveillance system component for clinical surveillance of BHV1 (CLIN)

RESULTS

Surveillance re-design feedback

The performance attributes of interest for redesign were sensitivity, timeliness and the negative predictive value of the system. Regarding these attributes, the SDF provided several

---

[‡] In the Netherlands, the free status of a BHV1-free dairy herd is temporarily suspended between the day of purchase and the test result. For this study it was assumed that this interval is minimal (i.e. several days).

pieces of advice regarding testing protocol and sampling strategy that were applicable to the conventional surveillance design, the alternative design, or both (Table 2).

Table 2. Advice of the surveillance design framework (SDF) which was applicable to mprove timeliness, sensitivity and the negative predictive value of conventional and alternative surveillance system designs to detect and proof freedom of BHV1. For each advice it was indicated whether the conventional and alternative design lacked (✗) or comprised (✓) the advice

Advice provided by SDF	Inclusion in design		Comment
	Conv.	*Alt.*	
Timeliness			
Increased sampling frequency will reduce the time to detection.	✗	✓	Yearly sampling (conventional) vs. ≥ 9 times per year (alternative)
Choosing a sampling point that allows frequent and easy access of the target population may improve timeliness.	✓	✓	Sampling at the herd in both designs, which allows frequent and easy access to the target population
Sensitivity			
Risk-based targeting can increase sensitivity	✗	✓	No risk-based sampling (conventional) vs. sampling of purchased cattle originating from non-free herds (alternative)
Negative predictive value (NPV)			
NPV can be increased by taking differences in the risk of introduction into account.	✗	✓	No difference in risk of introduction (conventional) vs. Increased risk of introduction through purchase is accounted for (alternative)
Pooling will increase the NPV when inference is made at animal level, but it will reduce it in case the inference is made at herd level.	✓	✗	Individual sampling of cows (conventional) vs. testing of a (pooled) bulk milk sample (alternative).

Surveillance sensitivities

Both designs reached 100% sensitivity with the intake procedure to obtain a free status (OBT) (Table 3). The sensitivity of the component to monitor a herd's free status (MON), i.e. to classify a herd as positive when infected at an within-herd design prevalence of 10%, was 99% for the conventional design and 96% for the alternative design. In the alternative design, the purchase testing component (PURalt) led to a component sensitivity of 55%. The total surveillance system sensitivity of a herd increased from 96% to 98% when additional testing of purchased animals was done besides monitoring the free status in the alternative design. When additional passive clinical surveillance was in place, both designs led to a total system sensitivity of 100% when monitoring the free status of a holding.

Table 3. Mean sensitivity values (with 5th percentile and 95th percentile values) of conventional and alternative surveillance system components to detect a BHV1 infection at herd level based on a within-herd design prevalence of 10% and total system sensitivity when monitoring the free status of a herd

	Conventional design	Alternative design
Component sensitivity		
OBT	1.00 (1.00-1.00)	1.00 (1.00-1.00)
MON	0.99 (0.93-1.00)	0.96[a]
PUR	n.a.	0.55 (0.23-0.93)
Total system sensitivity		
Monitoring free status	0.98 (0.91-1.00)	0.96[a]
Monitoring free status + purchase	n.a.	0.98 (0.97-1.00)
Monitoring free status + clinical surv.	1.00 (1.00-1.00)	1.00 (1.00-1.00)

[a] Single output value as all input parameters were fixed.

Probability of freedom

Conventional design: In an endemic situation (Scenario A), the conventional design led to a mean probability of freedom of 99.6 - 100%, depending on the time since the serological investigation of all lactating cattle (Fig. 6). When the probability of introduction (PIntro) was doubled to 0.08% in de endemic situation (not shown), the resulting mean probability of freedom varied from 99.8% in the first month after obtaining the free status to 99.2% in the month before serology on all lactating animals[§]. In a situation where the country is free from BHV1 (Scenario B), the conventional design led to a mean probability of freedom of 99.9% or more throughout the year (Fig. 6).

Alternative design: In an endemic situation (Scenario A), the alternative design led to a probability of disease freedom of >99.9%, even in the months without bulk milk testing (Fig. 7). The increased risk of introduction when purchasing animals from non-free herds (Scenario C) resulted in a slight reduction of the monthly probability of freedom but did not result in a mean monthly probability of freedom of <99.9%. In a situation where the country is free from BHV1 (Scenario B), the alternative design led to a constant 99.99% probability of freedom per month (Fig. 7). When the probability of introduction (PIntro) was doubled to 0.08% in the endemic situation, the resulting mean probability of freedom decreased to 99.8% in months without bulk milk testing, 99.9% in months with bulk milk testing and 99.9% in months with bulk milk testing and purchase (not shown).

DISCUSSION

During the last decade, scenario tree methods have been widely used to evaluate surveillance systems (Martin et al. (2007a,b); Frössling et al. (2009); Blickenstorfer et al. (2011); Calvo-Artavia et al. (2013)). The models we used in this study focussed on sensitivity of detection on herd level given a new introduction in a BHV1-free herd and proofing freedom from disease, and could easily be adapted to country level.

[§] Arbitrarily chosen to be carried out in month 10.

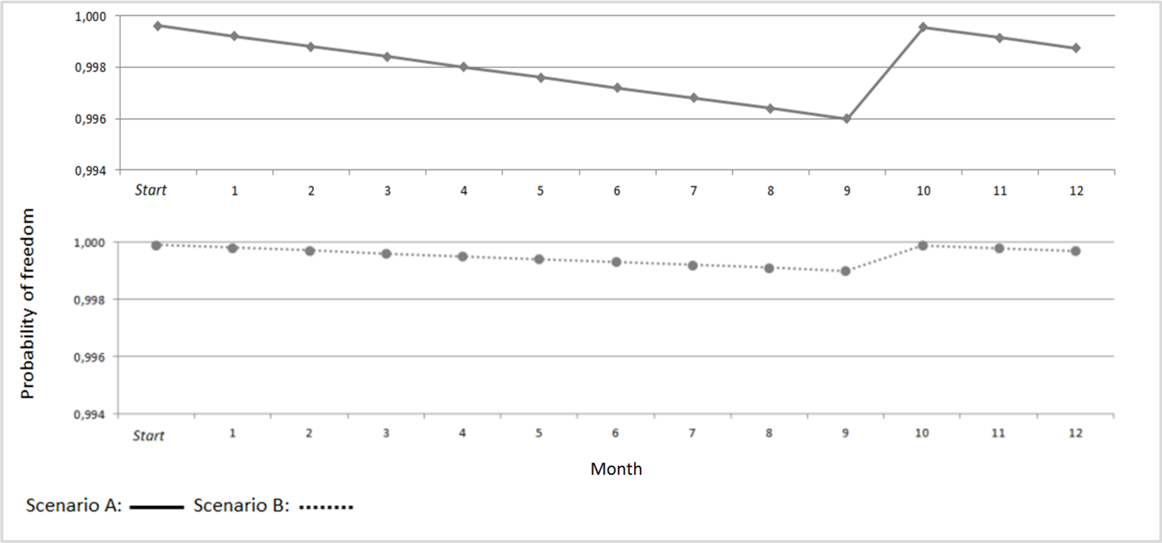


Fig. 6 Mean monthly probability of freedom of certified dairy herds as estimated based on a scenario-tree model of the conventional surveillance system for BHV1 infections, i.e. yearly serology on all lactating animals (in month 10 here). Estimations were made for the situation in which BHV1 is endemic at country level (Scenario A; top) and for the situation in which the country is disease-free (Scenario B; bottom)

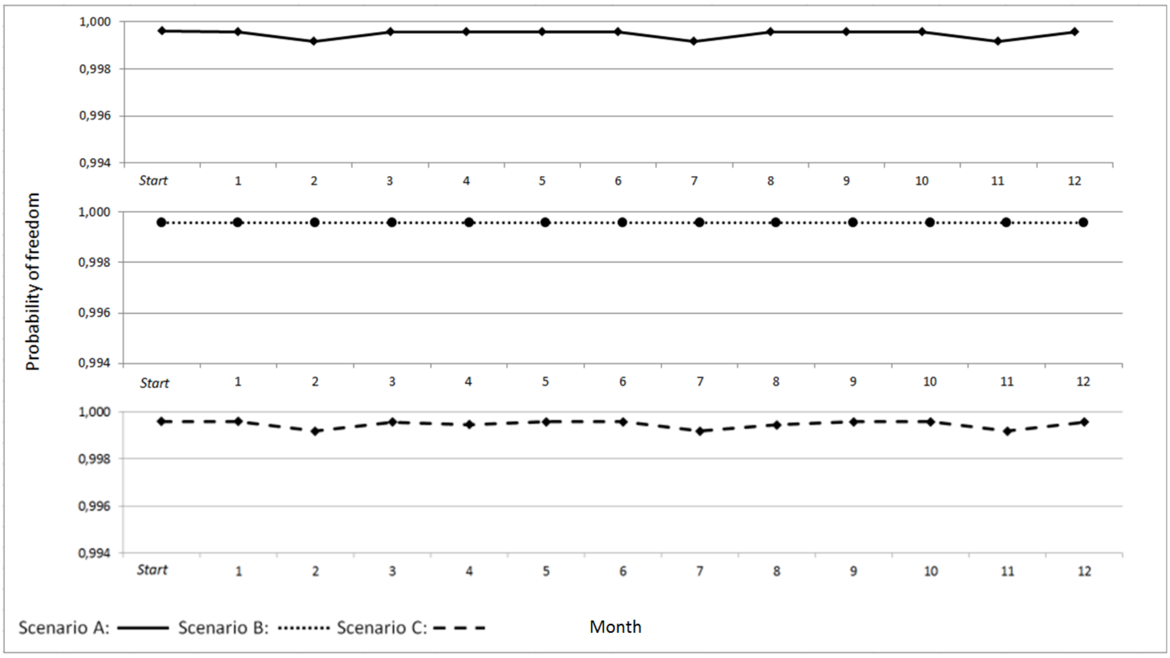


Fig. 7 Mean monthly probability of freedom of certified dairy herds as estimated based on a scenario-tree model of the alternative surveillance system for BHV1 infections, i.e. bulk milk testing is carried out 9 times a year (months 2, 7 and 11 are omitted). Estimations were made for the situation in which BHV1 is endemic at country level (Scenario A; top), for a disease free situation (Scenario B; middle) and for the situation in which BHV1 is endemic at country level + animals from non-free herds are purchased in months 4 and 8 (Scenario C; bottom)

The alternative surveillance design described in this study was based on the voluntary BHV1 control program in the Netherlands. In a disease free situation, the conventional and alternative designs performed equally well in terms of substantiating disease freedom at a within-herd design prevalence of 10%. It would therefore be interesting to assess differences in costs related to these two designs, which was no included in the current study. In an endemic situation, assuming a monthly probability of introduction of BHV1 of 0.04% at herd level, the alternative design led to a constant probability of freedom of minimum 99.8%. This is sufficient according to the recommendations of the Terrestrial Animal Health Code of the OIE and was superior to the conventional design as described by EU legislation. However, if the design prevalence were to be lower, eg. 5%, the performance of the alternative design might be inferior to the conventional design, as the bulk milk test is not sensitive to detect small outbreaks (Wellenberg et al., 1998). Minor outbreaks (without clinical signs) may therefore remain undetected until they reach a within-herd prevalence of 10% or more. Nevertheless, given BHV1's high reproduction ratio (Bosch, 1997), within-herd prevalences will rapidly reach levels of 10% or more. The alternative surveillance design based on monthly bulk milk testing has been reported before as being adequate to detect BHV1 infection timely, thus reducing spread of infection after introduction (Graat et al., 2001).

The efficacy of any animal health program relies on timely case detection. Timeliness of detection was not assessed in the current study but is related to the length of the period between sampling moments. The low frequency of testing in the BHV1 control regime as described by EU-legislation could be considered as a drawback of the system in terms of timeliness of detection. In addition, this study showed that in an endemic situation, the monthly confidence of freedom in the EU-design decreased to the minimum of 99.8% (as laid down by the Terrestrial Animal Health Code of the OIE) within 5 months after the serological investigation of all cattle >24 months of age. Nevertheless, once the serological investigation is carried out, it is likely that each infected individual will be detected by the testing protocol, given its high level of sensitivity.

Risk-based veterinary surveillance can be defined as a form of surveillance in which exposure and risk assessment methods have been applied together with traditional approaches to assure appropriate and cost-effective data collection (Stärk et al., 2006). In this study, the conventional and alternative surveillance systems to substantiate disease freedom in certified dairy herds were both targeted at adult (lactating) cattle, as seropositive animals are most often observed in this age cohort. In addition, we extended the alternative design with a testing scheme for cattle that are purchased from non-free herds, as they have an increased risk of infection. This risk-based surveillance component resulted in an increase in total surveillance sensitivity. However, due to an increase in probability of introduction, the probability of freedom decreased slightly in months in which animals were purchased.

The RISKSUR surveillance design framework provided easy-to-use guidance to describe and redesign BHV1 surveillance programs, potentially contributing to a standardisation of surveillance documentation. The results of this study provided a description of two surveillance designs that could be used to control BHV1 infection in dairy herds, including a comparison of the sensitivity and negative predictive value of these systems. Such an assessment of various surveillance designs could be highly useful to support decision-making towards a more risk-based approach of animal health surveillance.

ACKNOWLEDGEMENTS

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7 / 2007-2013) under grant agreement number 310806.

REFERENCES

Blickenstorfer, S., Schwermer, H., Reist, M., Doherr, M. G. and Hadorn, D.C., 2011. Using scenario tree modeling for targeted herd sampling to substantiate freedom from disease. BMC Veterinary Research, 7:49

Bosch, J.C., 1997. Bovine herpesvirus 1 marker vaccines: Tools for eradication? Ph.D thesis, University of Utrecht, Netherlands, 122 pp.

Calvo-Artavia, F. F., Alban, L and Nielsen, L.R., 2013. Evaluation of surveillance for documentation of freedom from bovine tuberculosis. Agriculture, 3, 310-326.

Frössling, J., Ågren, E.C.C., Eliasson-Selling, L., and Sternberg Lewerin, S., 2009. Probability of freedom from disease after the first detection and eradication of PRRS in Sweden: Scenario-tree modeling of the surveillance system. Prev. Vet. Med. 91, 137-145

Graat, E.A.M., de Jong, M.C.M., Frankena, K. and Franken, P., 2001. Modelling the effect of surveillance programmes on spread of bovine herpesvirus 1 between certified cattle herds. Vet. Microbiology, 79, 193-208

Mars, M.H., de Jong, M.C.M., Franken, P., and van Oirschot, J.T., 2001. Efficacy of a live glycoprotein E-negative bovine herpesvirus 1 vaccine in cattle in the field. Vaccine, 19, 1924-1930

Martin, P.A., Cameron, A.R. and Greiner, M., 2007a. Demonstrating freedom from disease using multiple complex data sources 1: A new methodology based on scenario trees. Prev. Vet. Med. 79, 71-97

Martin, P.A., Cameron, A.R. and Greiner, M., 2007a. Demonstrating freedom from disease using multiple complex data sources 2: Case study – Classical swine fever in Denmark. Prev. Vet. Med. 79, 98-115

Metz, J.A.J., 1978. The epidemic in a closed population with all susceptibles equally vulnerable; some results for large populations and small initial infections. Acta Biotheoretica, 27, 75-123

Van Oirschot, J.T., Kaashoek, M.J., Maris-Veldhuis, M.A., Weerdmeester, K. and Rijsewijk, F.A.M., 1997. An enzyme-linked immunosorbent assay to detect antibodies against glycoprotein gE of bovine herpesvirus 1 allows differentiation between infected and vaccinated cattle. Journal of Virology Methods, 67, 23-34

Perrin, B., Perrin, M., Moussa, A., and Coudert, M., 1996. Evaluation of a commercial gE blocking ELISA test for detection of antibodies to infectious bovine rhinotracheitis virus. Veterinary Record, 138, 520

Van Schaik, G., Schukken, Y.H., Nielen, M., Dijkhuizen, A.A. and Huirne, R.B.M., 1999. Application of survival analysis to identify management factors related to the rate of BHV1 seroconversions in Dutch dairy farms. Livestock Production Science, 60, 371-382

Stärk, K.D.C., Regula, G., Hernandez, J., Knopf, L., Fuchs, K., Morris, R.S. and Davies, P., 2006. Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. BMC Health Services Research, 6:20

Wellenberg, G.J., Verstraten, E.R.A.M., Mars, M.H. and Van Oirschot, J.T. (1998). ELISA detection of antibodies to glycoprotein E of bovine herpesvirus 1 in bulk milk samples. Vet. Rec. 142, 219-220

Wellenberg, G.J., Verstraten, E.R.A.M., Mars, M.H. and Van Oirschot, J.T. (1998). Detection of Bovine Herpesvirus 1 Glycoprotein E Antibodies in Individual Milk Samples by Enzyme-Linked Immunosorbent Assays. Journal of Clinical Microbiology, 36, 409-413

Van Wuyckhuise, L., Bosch, J., Franken, P., Hage, J.J.,Verhoeff, J. and Zimmer, G., 1993. The prevalence of Infectious Bovine Rhinotracheitis (IBR) in the Netherlands. In: Elbers, A.R.W. (ed), Proceedings 6th meeting of the Dutch Society for Veterinary Epidemiol. Economics. Boxtel, 7-16

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

PAST VENUES AND ORGANISERS OF ANNUAL MEETINGS

Year	**Venue**	**Organiser(s)**
1983	Southampton	Davies & Thrusfield
1984	Edinburgh	Thrusfield
1985	Reading	Thrusfield
1986	Edinburgh	Thrusfield
1987	Solihull	Thrusfield
1988	Edinburgh	Thrusfield
1989	Exeter	Howe
1990	Belfast	McIlroy
1991	London	Jones
1992	Edinburgh	Thrusfield
1993	Exeter	Howe
1994	Belfast	Menzies
1995	Reading	Paterson
1996	Glasgow	Reid
1997	Chester	Clarkson
1998	Ennis, Ireland	Collins
1999	Bristol	Green
2000	Edinburgh	Thrusfield & Mellor
2001	Noordwijkerhout, Netherlands	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny, Switzerland	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli, Finland	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes, France	Fourichon & Hoch
2011	Leipzig, Germany	Thulke & Lange
2012	Glasgow	Parkin & Others
2013	Madrid, Spain	Martínez-López & Vargas Alvarez
2014	Dublin, Ireland	More & Collins
2015	Ghent, Belgium	Mintiens & Dewulf

PAST PRESIDENTS

1982-’83	G. Davies
1983-’84	P.R. Ellis
1984-’85	G. Gettinby
1985-’86	R.W.J. Plenderleith
1986-’87	M.V. Thrusfield
1987-’88	K.S. Howe
1988-’89	A.M. Russell
1989-’90	S.G. McIlroy
1990-’91	J.E.T. Jones
1991-’92	J.M. Booth
1992-’93	E.A. Goodall
1993-’94	R.G. Eddy
1994-’95	J.T. Done
1995-’96	G.J. Gunn
1996-’97	M.S. Richards
1997-’98	J.D. Collins
1998-’99	F.D. Menzies
1999-’00	K.L. Morgan
2000-’01	S.W.J. Reid
2001-’02	A.D. Paterson
2002-’03	L.E. Green
2003-’04	J.L.N. Wood
2004-’05	E.G.M. van Klink
2005-’06	D.J. Mellor
2006-’07	E. J. Peeler
2007-’08	J. R Newton
2008-’09	L. Alban
2009-’10	D.U. Pfeiffer
2010-’11	L.A. Kelly
2011-’12	C. Fourichon
2012-’13	T.D.H. Parkin
2013-’14	K.L.P. Verheyen
2014-’15	K. Mintiens
2015-’16	H.H. Thulke

EXECUTIVE COMMITTEE 2015-2016

H-H. Thulke (President), K. Mintiens (Senior Vice-President), A. Lindberg (Junior Vice-President), R. Christley (Honorary Secretary), M. Brennan (Honorary Treasurer), D. Brodbelt, F. Calvo-Artavia, M. McIntyre, L. Rosenbaum Nielsen, B. van den Borne, T. Halasa (co-opted)

Honorary Auditors: Dominic Mellor & Fraser Menzies

LIFE MEMBERS

J.M. Booth, M.J. Clarkson, J.D. Collins (deceased), P. Cripps, G. Davies, J.T. Done (deceased), R.G. Eddy, P.R. Ellis, E.A. Goodall, G. Gettinby (deceased), K.S. Howe, M.E. Hugh-Jones, W. Martin, F. Menzies, A.M. Russell, M.V. Thrusfield, J. Wilesmith, K. Morgan

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2016	Bernhard Url The foundation of science-based risk assessment for decision support on food safety and animal health in EU	Mirjam Nielen Evidence-based veterinary medicine needs clinical epidemiology
2015	Piet Vanthemsche Preventive Veterinary Medicine as an essential part of sustainable animal production	Crawford Revie Hype and Hysteria: Should veterinary epidemiologists really care about Big Data?
2014	Ian Gardner, Bridging the gap in infectious disease epidemiology between aquatic and terrestrial food animals: challenges and future opportunities	Nils Toft Confessions of a wannabe Bayesian
2013	Andreas Hensel Dioxins, EHEC and strawberries: Risk assessment and risk communication in practice	José Manuel Sánchez-Vizcaíno The Spanish experience on the control and eradication of infectious diseases: from the old to the current system
2012	Stuart Reid Evidence-based prevention: well done or rare	Didier Boichard Genomic selection: an opportunity for improving health of farm animals
2011	Karin Schwabenbauer From science to policy - the case of classical swine fever (CSF) control	Dominic Mellor The trouble with epidemiology: the tyranny of numbers
2010	David Waltner-Toews Beyond one world, one health and ecohealth…what's out there?	James Wood From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard The interaction between veterinary science, legislation and management in animal disease control in the European Union	Katharina Stärk Food safety challenges in a global market – are we ready?
2008	Paul Fine Infectious disease eradication – meanings and implications	Kenton Morgan For the benefit of Mr Kite

2007	Yrjö Gröhn Food supply veterinary medicine: Modelling of production, health and food safety	Laura Green Improving Animal Health
2006	David Galligan From partial budgets to real options - concepts in animal health economics	Nigel French Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More Towards eradication of bovine tuberculosis in Ireland: A critical review of progress
2004	Ulrich Kihm BSE and the stable to table concept	Gary Smith Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox The current state of statistical science	Ynte Schukken Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby Informatics and epidemiology – the first 400 years	Bryan Grenfell Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston Science politics and animal health policy: epidemiology in action	Mart de Jong Design and analysis of transmission experiments
2000	Jim Scudamore Surveillance – past, present and future	Dirk Pfeiffer Spatial analysis – a new challenge for veterinary epidemiologists
1999	Aalt Dijkhuizen The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse Understanding the epidemiology of scrapie
1998	Wayne Martin Art, science and mathematics revisited: the role of epidemiology in promoting animal health	

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

APPLICATION FOR MEMBERSHIP

Name ....

Address ....

....

....

....

Telephone: ....

Fax: ....

E-mail: ....

Signed .... Date ....

Please enclose the membership fee (£40 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr Marnie Brennan
School of Veterinary Medicine and Science
Sutton Bonington Campus
College Road
Sutton Bonington
Leicestershire LE12 5RD
UK

TEL +44 (0) 115 951 6577
FAX +44 (0) 115 951 6415
Email: marnie.brennan@nottingham.ac.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. 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 Honorary Treasurer of a completed application form and subscription equivalent to the rate for two calendar years at first application or subsequent application following an elapsed subscription. Subsequent annual 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 five ordinary elected members. However, the Committee will have powers of co-option. Elected officers and ordinary members of the committee have normal voting rights at committee meetings but co-opted and ex-officio members (e.g. the proceedings editors) do not

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. The Treasurer 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 an elected Committee member.

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.

Laid down April, 1982
Revised March, 1985; April, 1988; November 1994, March 2014
Corrected January 1997; April 2002