

EVALUATION OF SCENARIOS FOR REDUCING HUMAN SALMONELLOSIS THROUGH HOUSEHOLD CONSUMPTION OF FRESH MINCED PORK MEAT

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1. INTRODUCTION

Despite its declining incidence, Salmonellosis is the second most frequently reported zoonotic disease in the European Union (EU) and its control and prevention is prioritized by the EU and its member states. For Belgium, reducing the *Salmonella* contamination in the pork production might be a good human Salmonellosis mitigation strategy. Indeed, following EU baseline surveys on the prevalence of *Salmonella* in slaughter pigs in 2006-2007, higher prevalences are observed for Belgium compared to the other EU member states and a similar result holds for the *Salmonella* contamination at post-processing. In the literature, several strategies aiming to reduce the *Salmonella* contamination in the pork production have been suggested, e.g. the use of acidified feed for slaughter pigs and improved slaughter hygiene. In order to evaluate the effectiveness of such potential mitigation strategies, policy makers are increasingly using scenario-analysis (or 'what-if'-analysis) as a tool to aid decision making. In the current study, a scenario analysis is carried out using the METZOON-model, being a modular 'farm-to-fork' risk model recently developed following the Codex Alimentarius Principles. The METZOON-model is introduced and described in detail in [1.].

2. MATERIALS AND METHODS

2.1. Selected Mitigation Strategies

Several strategies aiming to reduce the number of human salmonellosis cases due to home consumption of fresh minced pork meat are investigated. In total, an exhaustive list of 14 *Salmonella* mitigation strategies, that can be evaluation without substantially changing the METZOON-model, is considered. The strategies are implemented at different stages of the minced pork meat production and consumption. The description of the strategies are given in Table 1.

2.2. Scenario-analysis and experimental design

A scenario-analysis can be thought off as an scientific experiment, hence involving a careful consideration on experimental design. In the context of scenario-analysis, two types of designs are particularly applicable, i.e. the completely randomized (CR) design and the randomized complete block (RCB) design. In a CR design, the effect of a factor is evaluated by systematically changing that factor without controlling for potential nuisance factors. In a scenario-analysis, this comes down to repeatedly running independent iterations of the risk model while systematically changing the variable corresponding to the mitigation strategy of interest, typically over a finite set of values $P = \{p_1, p_2, \dots, p_n\}$. However, although its commonly done, this is not a powerful design if, given a specific value p_i , the results of the iterations are still highly variable. In this case, the RCB design could remedy. In such a design, the effect of the variable of interest is investigated while known nuisance

factors are controlled for. In a scenario-analysis, this translates to running an iteration of the model for each value p_i from $P = \{p_1, p_2, \dots, p_n\}$ in turn while fixing nuisance factors, like the input variables in a risk model. As such, one homogeneous 'block' of risk outcomes is generated. This process is then repeated several times, each time fixing the 'blocked' input parameters to other randomly selected values, yielding independent 'blocks' of dependent iterations.

2. 1. Evaluating Scenarios using Effect Size

Typically, the results of a scenario-analysis are analyzed using t-tests comparing the effectiveness of a particular mitigation strategy with the baseline. However, in simulation studies, the effect size can be chosen arbitrarily large, rendering even negligible differences significant. An other common measure to present the results of a scenario-analysis is the relative reduction, which is only based on differences in means, and as such, ignores the variability in the outcome variable. An alternative measure that does not suffer from above mentioned shortcomings, is the effect size expressed as a standardized difference in means or $ES = (\mu_{scenario} - \mu_{baseline}) / \sigma_{baseline}$, indicating whether the observed difference is large enough to be of substantial interest. Corresponding on the design used (CR or RCB), the corresponding confidence intervals are to be calculated differently. Details can be found in [2.]

Table 1. Description of the Salmonella mitigation strategies evaluated using the METZOON-model.

Stage	Nb.	Description scenario
Primary production	1.	Reducing the probability that pigs are seropositive at primary production with 10%, 25%, 50% and 75%.
	2.	Reducing the probability that pigs are internally infected at lairage with 10%, 25%, 50% and 75%.
Transport-lairage	3.	Reducing the probability that pigs are externally infected at lairage with 10%, 25%, 50% and 75%.
	4.	Reducing the probability that a carcass is contaminated after killing with 10%, 25%, 50% and 75%.
Slaughterhouse	5.	Reducing the probability that a carcass is contaminated after singeing with 10%, 25%, 50% and 75%.
	6.	Reducing the probability that a carcass is contaminated after polishing with 10%, 25%, 50% and 75%.
	7.	Reducing the probability that a carcass is contaminated after evisceration with 10%, 25%, 50% and 75%.
	8.	Reducing the probability that a carcass is contaminated after chilling with 10%, 25%, 50% and 75%.
Post-processing	9.	Reducing the number of <i>Salmonella</i> CFUs in a meat mix with 10%, 25%, 50% and 75%.
Distribution-storage	10.	Avoiding microbial growth due to temperature abuse during transport from retail to home.
	11.	Avoiding microbial growth due to temperature abuse during storage at home.
Preparation-consumption	12.	Reducing the probability of not hand washing during cooking with 10%, 25%, 50% and 75%.
	13.	Reducing the probability that the same cutting board is used after meat handling with 10%, 25%, 50% and 75%.
	14.	Reducing the probability of undercooking with 10%, 25%, 50% and 75%.

3. RESULTS

In the METZOON-model, uncertainties were modeled by using uncertainty distributions for the input parameters rather than ignoring them by arbitrarily restricting the input parameter space. However, this resulted in a huge variability between iterations of the model when adopting the CR-design and as such, no meaningful results could be obtained within reasonable computation time. Therefore, we opt to use the RCB design creating homogeneous 'blocks' by fixing all input parameters. The different scenarios given in Table 1 are evaluated using several outcome variables, calculated using $R = 1000$ iterations of the model, which is repeated $B = 100$ times in order to obtain a distribution for each of the outcome variables. The results of the 10 most effective scenarios are summarized in Table 2.

Table 2. Summary of the predicted number of annual cases for the 5 most effect scenarios: Average number of annual cases (95% confidence intervals) and effect size (95% confidence interval).

Nb.	Short description	Annual cases	Effect Size
6.	Decreasing contamination after polishing with 75%.	08861, [03071, 20509]	-1.395, [-1.716,-1.074]
7.	Decreasing contamination after evisceration with 75%.	05536, [00602, 12038]	-1.436, [-1.778,-1.094]
8.	Decreasing contamination after chilling with 50%.	10374, [03704, 23816]	-1.388, [-1.714,-1.061]
8.	Decreasing contamination after chilling with 75%.	05655, [01187, 13175]	-2.151, [-2.561,-1.740]
9.	Reducing <i>Salmonella</i> CFUs in meat mix with 75%	06768, [02620, 12335]	-1.727, [-2.100,-1.353]

4. DISCUSSION

The results of this study indicate that the most effective scenarios are the ones taken at the end of the slaughter line and during post-processing. Improving consumer awareness is found to be effective as well. The METZOON-model and the results obtained from the scenario-analysis may help policy makers formulate new regulations. Indeed, the output of the different scenarios can provide realistic microbiological targets (criteria) to be implemented. Due to the modular approach in the METZOON-model the criteria can be set, if opted by the decision makers, at each level of the pork production and consumption chain.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

- [1.] Bollaerts, K., Mesens, W., Delhalle, L., Aerts, M., Van der Stede, Y., Dewulf, J., Quoilin, S., Maes, D., Mintiens, K. and Grijspeerdt, K. (2009). Development of a Quantitative Microbial Risk Assessment for human salmonellosis through Household consumption of fresh minced pork meat in Belgium. *Risk Analysis*, 29, 821-840.
- [2.] Bollaerts, K., Mesens, W., Aerts, M., Dewulf, J., Maes, D., Grijspeerdt, K. and Van der Stede, Y. (2009). Evaluation of scenarios for reducing human salmonellosis through Household consumption of fresh minced pork meat. *Risk Analysis*, submitted.

EPIDEMIOLOGICAL ANALYSIS OF THE TRICHINELLA INFECTION SITUATION IN BELGIUM

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As part of a request from Belgium to be officially recognised by the European Commission as a region where the risk of *Trichinella* in domestic swine is negligible, the Scientific Committee is asked to perform an epidemiological study of the Belgian *Trichinella* situation and to propose a risk-based determination of the number of domestic swine (slaughter pigs raised under controlled housing conditions and pigs at risk; this latter category comprises outdoorreared pigs and breeding pigs) and indicator animals (foxes) to be tested annually in the case the recognition is attributed, in accordance with Regulation (EC) No 2075/2005.

Based on official data obtained with the digestion method, the real prevalence of *Trichinella* in domestic swine in Belgium is estimated at 0% (IC 95% : 0% - 0%, n = 136.311.723, exact binomial distribution) for the period from 1992 to 2008. This is less than one case per million pigs, which constitutes a negligible risk. The prevalence in horses is estimated at 0% (IC 95% : 0% - 0,0014%; n = 208.717) for the period from 1993 to 2008. The prevalence in wild boars is estimated at 0,0025% (IC 95% : 0,0003% - 0,0089% ; n = 81.042) for the period from 2001 to 2008. The prevalence in foxes is estimated at 0,2% (IC 95% : 0,0051% - 1,11% ; n=499) for the period from 2003 to 2009. In other domestic and/or wild animal species, the prevalence is zero. In man, the last case of trichinellosis caused by consumption of pork dated from 1893, and the last case caused by consumption of wild boar meat dated from 1978.

The sensitivity of the current surveillance system is higher than 99%, and the results of the ring tests do not lower this sensitivity under these 99%.

The Scientific Committee has quantitatively determined the risk level of *Trichinella* in domestic swine in Belgium, with two methods. The methodology described by Alban et al. (2008) has been used to compare the situation in Belgium and in Denmark, which obtained in 2007 the official recognition status as region with negligible risk of *Trichinella* based on this method. Based on this method, it was determined that the probability that the Belgian domestic swine population is free of *Trichinella*, based on the current surveillance program (testing all the pigs from all the categories), amounted to 98,91% (IC 95% : (98,69% – 99,1%). This can be considered as a negligible risk. Based on the risk-based surveillance program (testing only the swine population at risk, the probability amounted to 97,50% (97,13% - 97,82), what can also be considered as a negligible risk. However, the Scientific Committee makes comments on the methodology described by Alban et al. (2008) and proposes an alternative method based on scenario analyses. Based on this method, the probability that Belgium is currently free of *Trichinella* is 98,5%, which can also be considered as a negligible risk.

This indicates that an alleviated surveillance program aimed at the pig categories at risk can be proposed. The Scientific Committee recommends to continue to systematically test all domestic swine at risk (337.973 pigs, in accordance to estimations of 2008), all wild boars (cfr. wild fauna and cases in 2004 and 2007) and all horses (cfr. import risk), which is statutory laid down for the latter species. The scenarioanalysis method allows to evaluate the probability of detection of an eventual introduction of *Trichinella* in the population in function

of different testing options of the slaughter pigs raised under controlled housing conditions. These options inform the risk managers on the choices to be made for the monitoring of this risk.

Concerning the wild fauna, the Committee recommends to test annually 2.922 foxes, also rats captured during other monitoring programs, and approximately fifty samples from other wild carnivores. The Scientific Committee underlines also the importance of the strict respect of the biosecurity measures, notably concerning the feeding of pigs, and concerning the measures aimed at avoiding introduction of the parasite in pig farms from outside and from the wild fauna.

PREDICTING THE SPREAD OF BTV1 BY WIND FROM SOUTHERN FRANCE IN 2009

Els Ducheyne, Yves van der Stede, Estelle Meroc and Guy Hendrickx

Introduction

During the BTV8 epidemic in 2006 a wind model has been developed which showed a strong link between spatial spread patterns of the epidemic and the occurrence of potentially infective wind events originating from pixels with infected farms depicted as weekly cumulative wind density maps (Hendrickx *et al*, 2008). This model whilst being descriptive, correctly predicted the exponential spread of the epidemic in 2007. Based on extracted spatial spread parameters a third-generation predictive simulation modelling approach has now been developed (Ducheyne *et al*, submitted). This predictive model will now be applied to evaluate the risk of natural introduction of bluetongue in Belgium and the possible impact of vaccination on the total number of cases and the spatial extent of the epidemic.

Material and methods

A full model description is given in Ducheyne *et al* (submitted). The model is briefly described in the following paragraph. The number of cattle and ruminant farms per municipality was obtained from AFSSA. This was spatially joined to the spatial data layer of the French municipalities. The spatial denominator layer was overlaid with the CORINE land cover (JRC, 2000). The frequency of farms per land cover class as derived from the CORINE data set was determined and land cover classes without farms were eliminated. The farms were then randomly distributed within each municipality and within the valid land cover classes to obtain the individual estimated location of the farm. The cases per municipality were then randomly selected from the farms. When the impact of vaccination is included, farms were randomly selected according to the percentage of vaccination at department level. Three scenario's were included: (i) random select per department, (ii) select the farms that were infected then redistribute the vaccine over the other farms (minimum level) and (iii) assume that the farms that were infected are immune and thus do not require vaccination and redistribute the vaccines over the other farms within the department.

In order to identify different spatio-temporal clusters, a retrospective space-time permutation model at municipality level was used to analyse the data (Kulldorff *et al*, 2005). Incidence was derived from the epidemiological curve and fitted using a least square estimator to the Pearl-Verhulst growth function. The distinction between short (8km), medium (40km) and long distance (> 40 km) spread was estimated using the nearest infected farm procedure as described in Hendrickx *et al* (2008). The local infection probability is determined as the ratio of number of infected farms within the radius of the short distance spread over the total number of farms. Finally, the wind data, obtained for 2008 from the ECMWF, was used to derive the wind probability for each farm within the medium distance spread.

The model was initially seeded using the cases prior to July 28, 2008 (13 cases). The cases for week *t* were then selected using a Monte Carlo Markov Chain procedure, for the local (50%) and the medium distance (45%) spread separately.

Results

Modelling the 2008 epidemic

South-west France

Three distinct spatio-temporal clusters are identified within the dataset. The initial cluster ($p=0.001$) starting on July 17 and finishing on August 20, 2008 had a relative risk ratio of

5.02. The second cluster starts on August 14 and ends on September 3 had a relative RR of 1.88. The final cluster starts on September 11 and ends on December 3. While the epidemic curves in the first and second cluster follows a near-Gaussian distribution, the third cluster has a peak in the beginning of September.

When this clustering is taken into account, the model predicts an outbreak similar to the observed data (Fig 1). In the predicted output, there is a high density of cases in the initial zone of introduction, a second high-density area starting from South-central France along the Pyrenees and thirdly a cloud of cases northwards of the first two zones. There is a large gap of cases in the Landes. Also there is higher density of cases at the bottom of the Massif Central. From Fig. 1 also follows that when the surveillance zone would be delineated starting from the predicted cases, the spatial extent is 100 km more northwards than the actual observed zone.

Brittany and Normandy

In Brittany, three cases were observed during the autumn of 2008. Because they arose later in the year these presumably did not give rise to a recorded spread though non-reported other clinical cases have been observed (Lancelot, personal communication). To simulate spread should the initial case have occurred at the beginning of the 'bluetongue season' the model was ran starting in July. If the cases had started earlier, the entire peninsula of Bretagne would have been covered. The simulation was repeated in Normandy. In this case a seed was selected near the border with Brittany. The predicted spread is mainly along the coast, consistent with the wind patterns. It can also be observed that in this case the restriction zone would extend towards Belgium, thus suggesting that Belgian farms near the French border could have been at risk.

Modelling the 2009 epidemic using vaccination data

South-west France

Given the vaccination status in May 2009 and the current known locations of cases, the number of cases in 2009 will be maximum 2500. The spatial extent could increase further northwards over a distance of 300 km. No cases will be found in the Massif Central even though there is a high density of bovine and ovine in that area. The distance from the restriction zone to the Belgian border will be at least 300km.

Modelling cases from 2009

On September 2009, a new case was found in Alliers. Although this was a non-symptomatic case, we run the model given this seed and the vaccination coverage. The model predicts 220 cases. This may be due to the high amount of bovine vaccination coverage in the departments surrounding Alliers and the high amount of vaccination coverage within the department of Alliers. Given this there was no imminent threat to Belgium.

Discussion

This model is the first to predict the spread of bluetongue before the 'bluetongue season'. Other models such as the atmospheric dispersion models by Gloster *et al* (2006, 2007) are used to analyse in retrospect the possible introduction of the disease within a previously disease-free region. However, this is not used operationally for spread of disease modelling after the initial introduction.

The predicted outcome of the model with three clusters for 2008 correctly identified the entire area that was covered by the disease. In the counties of Haute-Pyrénées and Gers however, the model predicts a high density of cases, which were not observed. This may be explained by the fact that at the onset of the epidemic vaccination was conducted in

these areas (Hooyberghs, personal Gloster et al. Will bluetongue come on the wind to the United Kingdom in 2007?. The Veterinary Record (2007) vol. 160 pp. 422-426communication), thus explaining why there is a gap in the observed data.

In the prediction there is a higher density of cases around the Massif Central, whilst at the same time no cases are predicted within the Massif Central. This is linked to the impact of slope on the spread of the disease (Bishop *et al.*, 2004; Hendrickx *et al.*, 2008) The higher density of infected *Culicoides* in combination with the presence of hosts can lead to higher density of cases in those areas.

In the first simulation exercise in Brittany, we simulated a scenario where the disease started in July instead of in late autumn. It can be seen from the obtained output that under these conditions there is a uniform spread across the peninsula. It remains uncertain how long the infected cattle was ill before they were diagnosed (oral comm. Lancelot, 2009), thus potentially having caused other, as yet undiagnosed, infected cattle that may initiate a new outbreak in 2009.

In retrospect, it seems that the model is overestimating the actual number of cases in 2009. Up until now, no clinical cases are observed. This may be due to the vaccination effort. It seems that vaccination can prevent build up from a few cases but will not hamper once the disease is established. Earlier analysis of the effect of vaccination in the BTV8 epidemic (Ducheyne *et al.*, submitted) indicated that in order to reduce the geographical extent of bluetongue the level of vaccination should be at least 80%.

References

- Ducheyne, E., De Clercq, E. M., Goossens, E. and Hendrickx G., A stochastic predictive model to predict neighbourhood and wind spread of BTV8. Submitted to Plos One
- Hendrickx, G., Gilbert, M., Staubach, C., Elbers, A., Mintiens, K., Ducheyne, E., 2008, A wind density model to quantify the airborne spread of *Culicoides* species, *Prev. Vet. Med.*, 87, 162-18
- Gloster, J., Mellor, P.S., Burgin, L. Sanders, C., Carpenter, S., 2006, Will bluetongue come on the wind to the United Kingdom in 2007? *The Veterinary Record*, 160, 422-426
- Kulldorff, M., Heffernan, R., Hartman, J., Assunção, R., Mostashari, F., 2005, A space-time permutation scan statistic for disease outbreak detection. *PLoS Medicine*, 2(3): e59

SPATIAL RISK FACTOR ANALYSIS FOR BLUETONGUE IN NORTHERN EUROPE

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1. Introduction

Bluetongue serotype 8 (BTV-8) was introduced into North-West Europe in 2006 and spread to several European countries. The 2006 BTV-8 outbreak in Northern Europe affected cattle and sheep farms in large parts of Belgium, the Netherlands, Germany and Luxemburg. It is important to understand why farms in some areas are more affected as compared to other areas. It is clear that there is not a single risk factor that determines the risk for farms in a region to be infected with BTV-8, but that it is an interplay between many factors. This presentation focuses on a spatial and spatio-temporal multivariable risk factor analysis of the 2006 BTV-8 outbreak in Northern Europe.

2. Material

The risk factors that are considered in the analysis are climate, land use, farm-and animal-density, altitude, movement of animals (from area of first infection) and wind. Analysis is performed at the municipality level. In the geographical analysis we concentrate on the differences among areas, without accounting for the time trend.

In a geographical analysis, the risk for a farm to be infected with BTV-8 during the year 2006 can be estimated as the proportion of the number of infected farms with the total number of farms per municipality. In this analysis, both sheep and cattle farms are considered (together). Since however the total number of sheep farms in Germany is not available at the municipality level, but only at the district level, the total number of sheep farms at municipality level is approximated by a proportion of the total number of sheep farms in the district, accounting for the size of the municipality.

The information on the climate is given as daily mean temperature, daily altitude-adjusted temperature and daily precipitation at 198 weather stations in the study area. From these measurements, averages from May 1 to November 30 are calculated per weather station. Since the risk factor analysis will be performed at the municipality level, a prediction model is used to estimate the average temperature and precipitation per municipality. The centroids of the municipality are used as a representation of the municipality. A tensor product spline model is used as prediction model, based on all weather stations with no missing values in the period May 1 to November 30. Weather stations that do not have temperature or precipitation measurements during some days or weeks in the study area were not included in the analysis, since a simple average over the available measurements could yield biased estimates.

Environmental information per municipality is available as the proportion of forest, crop, pasture and urban areas per municipality. Since these variables are (almost) linearly related, using all these variables in a multivariable analysis will yield problems with multicollinearity. A univariate analysis suggests no effect of the proportion of crop areas on the risk for farms in a municipality to be infected with the BTV-virus. Therefore, the forest, crop and pasture variables are used in the multivariable risk factor analysis, with the proportion of crop as baseline category. Other environmental information per municipality is the altitude, farm-density and animal-density. However, all these variables are highly correlated with the land coverage variable, and thus, contain the same type of information on the

environmental differences among regions. Therefore, these variables are discarded from the multivariable risk factor analysis.

Both transport and wind are possible risk factors for the spread of BTV-8. The cumulative number of transported animals from the area of first infection in the period 2006, and the cumulative number of wind events from an infected farm are available, and are used in the multivariable analysis.

3. Methods

Some methods for the statistical analysis of counts of infectious diseases in small areas have been proposed in literature (Held et al. 2005, Knorr-Held and Richardson 2003, Mugglin et al. 2002, Paul et al. 2008). We model the number of infected farms Y_i in municipality i as a binomial probability with the number of farms n_i as the number of events

$$Y_i \sim \text{Binomial}(n_i, \pi_i),$$

and π_i the probability for a farm in municipality i to get infected in 2006. The probability to get infected is modeled as

$$\text{logit}(\pi_i) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \lambda \sum_{j \in \mathfrak{N}_i} y_j,$$

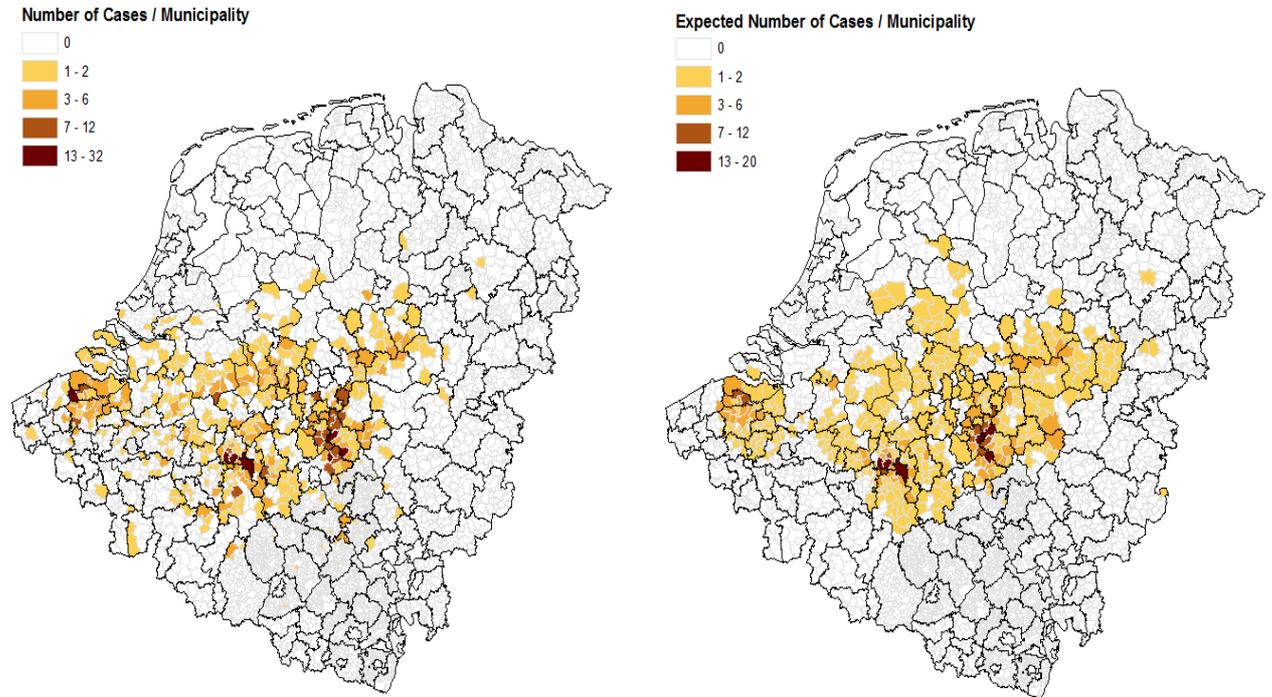
with \mathfrak{N}_i the set of neighboring municipalities of municipality i . A pair of municipalities is said to be neighbors if the distance between the centroids of the municipalities is smaller than 10 km. The disease probability is thus separated in two parts: a risk factor component $\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots$ containing all risk factors (and pairwise interactions) and an epidemic component $\lambda \sum_{j \in \mathfrak{N}_i} y_j$, a dependence on the number of outbreaks in neighboring municipalities, describing the local spread of the disease (within 10 km distance). Since more variability is expected as assumed under a binomial model, an overdispersion factor is accounted for into this model.

All the previously described risk factors are considered as main effects in the model, together with all possible pairwise interactions. Both backward and forward model selection methods were used to select the best model based on a Bayesian Information Criterion (BIC). The BIC penalizes the likelihood with the number of parameters, accounting for the number of observations in the data.

4. Conclusions and discussion

From the final model we can conclude that the land use in a municipality is a very important risk factor. It is the combination of different land types (especially forests together with pasture) which makes an area to be a high-risk area for infection. Also a large precipitation increases the risk of pasture areas. The local spread, reflected by the epidemic component, shows a significant increasing effect corresponding to an increase of cases in the neighborhood of the municipality. There is a significantly increasing effect of the risk with the number of wind events from infected areas, although areas with mainly forests are less sensitive to the spread due to wind events. The spread due to animal movements is also significant, with an odds ratio of 1.011 corresponding to 10 extra animals transported from the area of first infection. Also high temperature together with a high precipitation enforces the risk of infection. A risk map based on the results has been made, together with the

predicted number of events accounting for the number of farms in a municipality. The predicted number of events seems to match quite well the observed outbreak, meaning that the risk factor model describes most of the variability.



Vet-geoTools, a new spatial decision support system to manage disease outbreaks more rapidly and efficiently

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Introduction

Infectious and vector-borne disease outbreaks cause a heavy burden on an already stressed livestock sector. Therefore the rapid control of animal disease outbreaks is essential to limit the amount of direct and indirect economic losses caused by such diseases. In some cases it may also be essential to limit their potential impact on public health. During the last decade, the economic impact of both contagious diseases such as Classical Swine Fever, Foot-and-Mouth Disease and Avian Influenza (Dewulf *et al*, 2005), as well as (emerging) vector-borne diseases such as bluetongue had an enormous economic impact. The direct cost of even a small outbreak such as the 1997 CSF outbreak, which was limited to 8 infected farms and 64 contacts farms, was estimated at 11M€ (Mintiens *et al*, 2001). Bluetongue spread in three years over a total area 2.25 million km² within temperate Europe (Hendrickx, 2009). The spread of zoonoses such as the highly pathogenic H5N1 Avian Influenza and more recently Mexican Flu is furthermore increasing the fear of a pandemic with a major public health impact. Once a disease has been detected, a key factor to limit the disease impact will be how efficiently high quality data in general, and geo-referenced data in particular, are acquired, processed and analyzed as part of the decision making process. In this paper we describe a series of key functionalities of Vet-geoTools, a newly developed spatial decision support system (SDSS) that aims at managing infectious disease outbreaks more rapidly and more efficiently. Vet-geoTools is a server based software package which includes a fully operational GIS engine, an EU certified "from farm to fork" traceability database and a set of functionalities to assist with all spatial aspects of disease monitoring and control.

Adopted approach

Spatial Decision Support Systems

A Spatial Decision Support System (SDSS) is a system of hardware, software and procedures to facilitate the management, manipulation, analysis, modelling, representation and display of geo-referenced data to solve complex problems regarding planning and management of resources (NCGIA¹, 1990).

The power of a SDSS comes from the ability to relate information in a spatial context and to reach a conclusion about this relationship. Infectious diseases spreading from one farm to another, either through direct contact, wind, import via transport networks, or dissemination by arthropod vectors are prime candidates for such a spatial analysis approach.

Defining the functional requirements

A performing SDSS is dependent on high quality input data and the availability of tailor-made functionalities. Data quality largely depends on the livestock system and available resources. Western European high input animal husbandry systems usually feature a wealth of information contained in multiple databases. It is essential to identify these data sources and to link this information within a centralized database management system (DBMS). To

¹ National Center for Geographic Information and Analysis, US

identify additional data needs and functional requirements of Vet-geoTools a stepwise approach was followed. First, a series of available Belgian and European contingency plans and guidelines for the control of infectious diseases was consulted and all information related to spatial data needs and analysis requirements were listed. Then the acquired information was grouped by operational functionality and a series of use cases were designed. At various stages throughout this one year process, expert meetings were organized in Belgium with representatives of the Food Safety Agency (FAVV-AFSCA), the Veterinary and Agrochemical Research Centre (CODA-CERVA), Dierengezondheidszorg Vlaanderen (DGZ) and the Epidemiology group of the Veterinary Faculty at Ghent University (UGent).

Developing the system

As part of the preparatory phase described above a three month KMO *Innovatiestudie* was conducted with support from the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT). The aim of this preliminary study was to make key ICT choices related to the core architecture of Vet-geoTools. As outcome “Intertrace TM” from PAN-Livestock Services², Reading University, UK and “Orbit-GIS TM” from Eurotronics³, Lokeren, Belgium were selected as core DBMS and GIS engine respectively.

Finally Vet-geoTools was developed as part of a two year KMO *Innovatieproject* (IWT) in collaboration with Eurotronics. Based on the identified requirements a series of ten work packages were designed:

1. *Systems analysis* to further refine the systems analysis and set the final list of functionality priorities.
2. *InterTrace extension* to include the required spatial related tables to the Intertrace central database.
3. *OrbitGIS extension* to include raster tools and other additional functionalities to the central GIS engine.
4. *Zonation tool*: discussed in more detail below.
5. *Tracing tool*: discussed in more detail below.
6. *Logistics tool*: discussed in more detail below.
7. *Premises tool*: to digitize and include as part of the system maps of premises.
8. *Spatial epidemiology tool*: discussed in more detail below.
9. *Data tools en control panels* to establish the link between the system components in a user friendly way.
10. *Validation*: beta testing stage with representatives from the user community (FAVV, CODA UG).

Functionalities

The functionalities of Vet-geoTools include four key modules who are discussed here and which have a major impact on the improved spatial management of infectious disease outbreaks in high input animal husbandry systems: the zonation, tracing, logistics and spatial epidemiology module.

It is important to recall that systems such as Vet-geoTools are highly dependent on the quality of the input data. In case high quality data are not available, *e.g.* in low input extensive animal husbandry systems in developing countries, a spatial disease management system should focus first on acquiring denominator and other relevant spatial data sets in the most cost efficient way. Such a Vet-geoTools “extensive” package is currently being developed by Avia-GIS in South Africa as part of the EPISTIS research project by the Belgian Science

² <http://www.panlivestock.com/AboutITSystems/InterTrace.htm>

³ <http://www.eurotronics.com/?c=software/orbit.htm>

Policy Office (Belspo) and in six African countries starting in Uganda as part of the ICONZ RTD project funded by the European Commission (FP7).

Zonation module

The zonation module enables to generate complex sequential disease management risk zones (e.g. quarantine zone, protection zone, restriction zone...). These zones can automatically be adapted to landscape infrastructure such as roads, railways, rivers, forest edges, to ease field operations. Automated reports can be generated as required on either of those, e.g.: farm lists per zone with status, cost evaluation stamping out, crossroad lists for warning signs, etc.

Tracing module

The tracing module enables to store and analyse retro-actively farm forward tracing data related to direct and indirect disease contamination risk. The main objective here is to rapidly include information gathered by official vets regarding the spatial risk of spread of the epidemic. The various risk zones can be automatically adapted to this new information.

Logistics module

The logistic module enables to manage lowest risk routing of a variety of interveners between the different risk zones to mitigate as much as possible contamination risk. Practical examples include: weekly visit routes for official vets, planning delivery of animal feed, planning of milk collection and the planning of pick-up of carcasses. The tool also manages complex logistic questions such as: how to collect bulk milk in a given area with a known number of farms and a given volume of the milk tanks. Minimum risk routes can be calculated centrally and GPS compatible files (*.gpx) can be sent by e-mail to drivers.

Spatial epidemiology module

The spatial epidemiology module enables to analyse disease data and produce epidemiological reports according to a series of national and international formats. As a standard the weekly Bluetongue newsletter developed as part of the EFSA BTV epidemiology study (EFSA, 2007) was used. Depending upon requirements (e.g. OIE, ADNS, EFSA...) templates can be designed which enable automated reporting using the latest available epidemiological data.

The module also includes specific tools which enable to calculate required sample sizes, denominator kernel density and epidemiological curves, as well as to conduct spatio-temporal cluster analysis (SaTScan™). In peacetime the tool can be used to analyse epidemiological data of previous epidemics and simulate various control options.

Discussion

To our knowledge no other systems similar to Vet-geoTools are currently available. Whilst several of the proposed functionalities can be conducted by a skilled GIS operator using standard GIS software and scripts, the strength of the proposed system is to (i) integrate both data and functionalities in the same environment, and (ii) propose a complete series of tailored tools specifically adapted to the objective of more rapid and efficient disease outbreak management. The main added value is that during a crisis operators at various operational levels and from various disciplines have access to the same data in the same software environment and therefore can focus more rapidly on solving problems and proposing solutions.

At this stage Vet-geoTools was successfully beta tested in collaboration with the epidemiology groups of UG and CODA using a set of exercises developed with data from the CSF outbreak of 1997 in Belgium.

References

- Dewulf, J., Koenen, F., De Clercq, K., Van Den Berg, T., Ribbens, S., De Kruif, A (2005) Uitbraken en bestrijding van klassieke varkenspest, mond-en klauwzeer en hoog pathogene aviaire influenza in de Europese unie. *Vlaams Diergeneeskundig Tijdschrift* 74: (2) 103-116
- EFSA (2007) Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe: pp. 42, 9 annexes.
- Hendrickx, G. (2009) The spread of bluetongue in Europe. *Small Ruminant Research* (in press)
- Mellor, P.S. and Wittmann, E.J. (2002) Bluetongue virus in the Mediterranean basin, 1998 – 2001. *Veterinary Journal* 164: 20-37.
- Mintiens, K., Deluyker, H., Laevens, H. Koenen, F., Dewulf, J., De Kruif, A. (2001) Descriptive epidemiology of a Classical Swine Fever outbreak in the Limburg Province of Belgium in 1997. *Journal of Veterinary Medicine series B-Infectious Diseases and Veterinary Public Health* 48: (2) 143-149.
- Thiry, E., Saegerman, C., Guyot, H., Kirten, P., Losson, B., Rollin, F., Bodmer, M., Czaplicki, G., Toussaint, J.F., DE Clercq, K., Dochy, J.M., Dufey, J., Gilleman, J.L., Messeman, K. (2006) Bluetongue in Northern Europe. *Veterinary Record* 159: 327.

WELKE EPIDEMIOLOGISCHE TOOLS HEEFT HET BELEID NODIG?

Hooyberghs J, Houdart Ph.

Het Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV) is verantwoordelijk voor de organisatie en de uitvoering van de officiële controles in de ganse voedselketen, met inbegrip van de dierenziekten. Waar dierenziektebestrijding in het verleden vooral empirisch werd aangepakt, wordt nu zowel op Belgisch als op Europees niveau in toenemende mate beroep gedaan op epidemiologische hulpmiddelen. Ook het controleprogramma van het FAVV is gebaseerd op epidemiologische principes.

In het verleden werden vooral prevalentiestudies georganiseerd. Een voorbeeld hiervan waren de jaarlijkse prevalentiestudies voor de ziekte van Aujeszky. Met behulp van steekproefsgewijs serologisch onderzoek kan de vordering van een eradicatieprogramma op een betrouwbare wijze gevolgd worden.

Op basis van deze prevalentiestudies en gegevens beschikbaar in bestaande gegevensbanken of uit specifieke questionnaires worden risicofactorenanalyses uitgevoerd. Op die manier kunnen nieuwe interventiestrategieën uitgewerkt worden. De voorbije jaren werden op Europees niveau een reeks base line studies uitgevoerd voor Salmonella, Campylobacter en MRSA. Voor België werden deze surveys uitgevoerd door het FAVV. Naast een prevalentieschatting, nodig voor het vastleggen van Europese doelstellingen voor de bestrijding van Salmonella in de primaire sector, heeft EFSA ook risicofactorenanalyses uitgevoerd. Mede op basis hiervan werd bijvoorbeeld de vaccinatie van leghennen tegen Salmonella enteritidis verplicht in alle Europese lidstaten met een hoge prevalentie.

Voor bepaalde ziekten, zoals aviaire influenza, moet volgens internationale normen de afwezigheid aangetoond worden. Na het voltooiën van eradicatieprogramma's (varkenspest, Aujeszky, blauwtong, ...) moet eveneens de afwezigheid van de ziekte met voldoende betrouwbaarheid aangetoond worden. Hiervoor is een specifieke aanpak vereist.

De voorbije jaren werd Europa geconfronteerd met een aantal nieuwe ziekten (blauwtong, hoog pathogene aviaire influenza, ...). Om deze risico's beter te kunnen voorspellen en beheren zijn er aangepaste hulpmiddelen nodig, zowel om de risico's op introductie van nieuwe ziekten zo goed mogelijk te controleren als om bij introductie nieuwe ziekten zo snel mogelijk vast te stellen (early warning).

Om bestrijdings- en bewakingsprogramma's voor dierenziekten te evalueren en te optimaliseren kunnen risicomodellen uitgewerkt worden. Voor aviaire influenza, *Brucella abortus* en enzoötische runderleucose heeft dit al aanleiding gegeven tot min of meer drastische aanpassingen teneinde deze programma's efficiënter te maken. In de toekomst zullen alle belangrijke bestrijdings- en bewakingsprogramma's op een gelijkaardige manier geëvalueerd worden en zo nodig bijgestuurd worden.

De bestrijding en bewaking van dierenziekten en andere risico's in de voedselketen kunnen aanzienlijke financiële inspanningen vergen. Vooraleer programma's opgestart worden moeten dan ook de kosten en baten tegen elkaar afgewogen worden. Het draagvlak van programma's is mede afhankelijk van een goede kosten baten verhouding. Bij het uitwerken en eventueel bijsturen van programma's zijn ook hier betrouwbare gegevens noodzakelijk.

Bij risicomanagement is tijd een belangrijke factor. Vaak is onvoldoende tijd beschikbaar om een gevaar op al zijn aspecten te onderzoeken. Het is dan essentieel dat de beschikbare gegevens op korte tijd geanalyseerd worden, zodat snel een advies kan gegeven worden. Het FAVV doet hiervoor beroep op het Wetenschappelijk Comité en financiert het Centrum voor Coördinatie van de Diergeneeskundige Diagnostiek (CCDD) van het CODA waar meerdere epidemiologen studies voor het FAVV uitvoeren.

QUANTIFICATION OF BIOSECURITY STATUS IN BELGIAN PIG HERDS USING AN ONLINE SCORING SYSTEM

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INTRODUCTION

Biosecurity gains importance for the health management of pig farms. In order to quantify the biosecurity situation on pig farms, a scoring system was developed by the Veterinary Epidemiology Unit of the faculty of Veterinary Medicine, Ghent University and incorporated in a free online application (www.biocheck.ugent.be) (Ribbens et al., 2008).

MATERIALS AND METHODS

The scoring system takes both external (preventing pathogens from entering the herd) and internal biosecurity measures (reducing within herd spread of the infection) into account. Both parts are divided into 6 subcategories each consisting of 2 to 13 questions. The different subcategories in external biosecurity are: purchase of animals and sperm, removal of manure and dead animals, feed and water supply, personnel and visitors, vermin and bird control and environment & region. Internal biosecurity is divided in: disease management, suckling period, nursery unit, fattening unit, measures between compartments and working lines, material management and cleaning and disinfection.

Each question in a subcategory and each subcategory on its own receive a weight based on literature on pathogen transmission and general knowledge of infection risks (Ribbens et al., 2008). To calculate the total score for external or internal biosecurity, the scores for the subcategories are added up. A score between 0 and 100 is obtained for both external and internal biosecurity, with zero being the worst possible situation and 100 being the best possible situation. The mean of both scores gives the overall biosecurity score. The scoring system is adapted to be appropriate for every type of pig unit (fattening herd, breeding herd, mixed herd, etc). The questionnaire is initially developed in Dutch but will soon be translated into French.

RESULTS

From December 2008 until August 2009, 99 herds (i.e. 12 breeding herds, 5 fattening herds and 82 mixed herds) had voluntarily filled in the questionnaire. The distribution of these herds between the different provinces matched the distribution of pig herds in Belgium. On 27% of the farms, other animals were kept for professional use (of which 65% has cattle). The average score for external biosecurity was 65 (min 29; max 95). The score for internal biosecurity was lower in most farms with an average of 50 (min 18; max 89). The overall biosecurity score was on average 58 (min 28; max 84).

Some selected results relating to external biosecurity showed that 82% of the herds purchasing new breeding animals use quarantine facilities for an average period of 36 days and that 71% of these farms performed all-in all-out in the quarantine stable. Sperm is purchased on 90% of the mixed and breeding farms and 66% knows the health status of the

farm of origin. Farm-specific clothing and footwear is provided to visitors in 95%. Carcass removal can be done from the road on 77% of the farms, but only 53% of the farmers regularly perform cleaning and disinfection of the carcass storage. In 29%, the farmers never clean and disinfect hands after handling carcasses or wears gloves when handling carcasses. A sanitary transition zone isn't available or used in 23% although it's legally obliged and only 36% makes visitors wash and disinfect their hands. Although most farmers are very strict concerning hygienic measures taken before entering the stables for visitors, only in 50% of the herds, the farmer and/or personnel carry out these hygienic measures themselves before entering the stables. On 47% of the farms, cats and dogs are allowed in the stables. On 10% of the farms, the transporter of live animals has entrance to the stables and in 60% of these cases, the driver didn't wear farm-specific clothing. Loading of the animals is done directly from the stable or central corridor in 81%. Only half of the farmers examine the quality of the drinking water used for the pigs every year.

Concerning internal biosecurity, all-in all-out management is practiced in 85% of the herds in the nursery unit, 71% of the herds in the fattening unit. From all the herds, 88% mostly cleans and disinfects every stable after a production round, but only very few (5) verifies the efficiency of these measures. Only in 34% of the herds diseased animals are housed in separate hospital pens and 50% manipulates the diseased animals after the healthy ones. Suckling piglets are transferred between sows on 99% of the herds, of which 29% does this more than once and 44% keeps on performing this operation after 4 days post partum. In 73%, the farmer never changes clothing and 74% never washes hands between the different compartments. Only 58% of the farmers always works from the younger to the older pigs. In spite of the use of disposable needles, the needle is only changed after 101 animals on average. Although most farmers practice all-in all-out management, 24% mixes pigs of different ages in order to obtain pens with pigs of similar weight in the nursery and/or fattening unit. On 58% of the farms, a sanitary stand empty period is applied after each production round. Only 36% of the herds have a foot bath with disinfectant at the entry of the farm, although this is also legally obliged.

In general there is a positive correlation between the scores for external and internal biosecurity (figure 1). The correlation between the overall biosecurity and herd size is slightly positive in relation to the number of sows and slightly negative in relation to the number of fattening pigs.

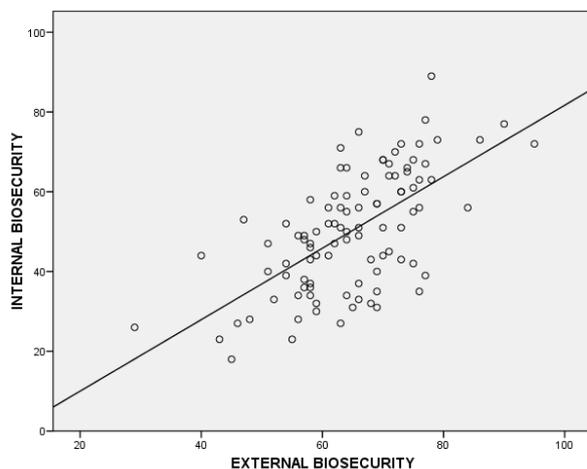


Figure 1. The correlation between the scores for external and internal biosecurity on Belgian pig farms.

DISCUSSION AND CONCLUSIONS

It needs to be emphasized that these 99 farms voluntarily filled in the questionnaire and scoring system and therefore it is to be expected that these herds are not a representative sample from the entire population but rather the herds with an interest for biosecurity. Therefore it is likely that the biosecurity measures from all pig herds are even lower than the results presented here.

Still, the large differences between the scores of different farms show that there is a lot of improvement possible in many of the herds. On average, the scores for external biosecurity, which are mainly measures imposed on others (visitors, suppliers, etc) are higher than the score on internal biosecurity which are more related to the work and management strategies of the farmers themselves.

As the results show, there are many biosecurity measures that have become common practice for farmers in Belgium, like providing farm-specific clothing and shoes for visitors to prevent the entry of diseases through visitors. On the other hand, some effective biosecurity measures, like isolate diseased pigs in a separate compartment, should be more frequently practiced. Especially in the internal biosecurity measures still a lot of improvement can be made.

The results show that this biosecurity scoring system is an efficient tool to quantify the biosecurity on a farm. It elucidates out strong and weak points of the herd and may help to set priorities for improving and monitoring the biosecurity status. As an objective score is given, it's easier to see improvement in time and to compare with other herds. The latter can motivate farmers to improve or to maintain their biosecurity score.

REFERENCES

1. Ribbens S., Vangroenweghe F., Maes D., Vandersmissen T., Dewulf J. A scoring system for biosecurity status in pig herds. In: *Proceedings of the 20th IPVS Congress*, Durban, South Africa, 22-26 June 2008

RISK FACTORS ASSOCIATED WITH ACQUIRED CEFTIOFUR RESISTANCE IN *E. COLI* FROM BROILER CHICKENS

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INTRODUCTION

Cephalosporins are an important group of antimicrobials in veterinary as well as in human medicine. In a recent survey on antimicrobial resistance in *E.coli* from broiler chickens, a remarkably high level of resistance to ceftiofur, a cephalosporin of the third generation, was found. Resistance levels in 32 Belgian broiler farms (each of them visited and sampled twice with one production round in between) varied between 0 and 100% with on average 39.1% of resistant isolates on farm level (Persoons et al., 2009).

These levels are much higher than what was recorded a few years ago (Verloo et al., 2003), and what is reported in other countries. It is linked to the emergence of Extended Spectrum Beta Lactamase genes (Smet et al., 2008), the genes encoding cephalosporin resistance.

Since no cephalosporins are registered for use in poultry in Belgium since 2001 this sudden increase is unexpected and worrying. Therefore it is crucial to try to identify factors that drive the emergence and spread of cephalosporin resistance in the broiler gut flora.

MATERIAL & METHODS

32 broiler farms were randomly selected for antimicrobial resistance determination in *E. coli*. Resistance to ceftiofur was tested by means of the Kirby-Bauer disk diffusion method according to CLSI guidelines for inoculums standardization and incubation. Neosensitab (Rosco, Denmark) antibiotic disks were used and zone diameters were read and interpreted according to the manufacturer's guidelines.

In the same 32 farms a large questionnaire was conducted at the moment of sampling. 75 factors were taken along in the questionnaire, including general management factors, animal health, hygiene, environmental factors, antibiotic treatments, etc.

A linear multivariate regression model including farm as a random factor was built to identify risk factors for acquiring ceftiofur resistance.

RESULTS

The factors retained in the multivariate model ($p < 0.05$) were hatchery, amoxicillin administration, flumequine administration and trimethoprim/sulfonamide administration. No significant interactions were found between the factors. Table 1 shows the factors, their coefficients and corresponding p- value.

Table 1. Factors included in the multivariate linear regression model.

Factor	Coefficient (β)	p- value
Amocycillin treatment	0.23	0.002
Flumequine treatment	0.53	0.002
Trimethoprim-sulfa treatment	0.16	0.026
Hatchery		
A	<i>Ref.^a</i>	
B	0.13	0.248
C	0.08	0.660
D	0.21	0.214
E	- 0.19	0.206
F	0.14	0.475
G	0.01	0.108
H	0.77	< 0.001

^a Ref indicates the reference category for that variable.

DISCUSSION

The results indicate that part of the ceftiofur resistance is probably already present in the chicks when they arrive on farm and may depend on the hatchery of origin. Whether this is the result of improper use of ceftiofur in the hatchery or originates from somewhere earlier in the production flow, like the mother birds, needs to be further examined. Treatment during production, with other antimicrobials than ceftiofur, may add to the expected level of ceftiofur resistance. For amoxycillin, also a beta lactam antibiotic, cross-resistance with ceftiofur has been described and this may explain the link between amoxicillin use and ceftiofur resistance. There is no cross-resistance between ceftiofur and trimethoprim/sulfonamide or flumequine and therefore it is not easily explainable why ceftiofur resistance rises when these antimicrobials are used. Maybe these results are an indication of new developing cross-resistance or the result of other, unmeasured, underlying factors.

The high level of ceftiofur resistance found in Belgian broiler farms thus seems to be greatly dependant of some well defined factors that need careful consideration on whether they are imperative to broiler farming, or could be made obsolete to avoid a further rise of cephalosporin resistance levels in *E.coli* from broilers.

ACKNOWLEDGEMENTS

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REFERENCES

- Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M, Martel A, Catry B, Butaye P, Haesebrouck F. 2009. Prevalence and persistence of antimicrobial resistance in *E.coli* from broiler chickens. *Micr Drug Res*, article in press.
- Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, Herman L, Haesebrouck F, Butaye P. 2008. Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* Isolates in Belgian broiler farms. *Antimicrob Agents Chemother*. 52:1238-43.

Verloo D, Butaye P, Dierick K, Imbrechts H. 2003. Descriptive epidemiology of the resistance observed in *Escherichia coli* isolated from healthy cattle, pigs and broilers, their meat and meat products. Proceedings of the Flemish Society for Veterinary Epidemiology and Economics, 11th December 2003, pp. 67.

SEROSURVEY OF FOUR ‘EMERGING’ CATTLE DISEASES (Q-FEVER, NEOSPOROSIS, LEPTOSPIROSIS AND SALMONELLOSIS) IN NORTHERN-BELGIAN DAIRY HERDS USING BULK-MILK SAMPLES

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INTRODUCTION

A survey was conducted to estimate the seroprevalence of 4 potentially ‘emerging’ infectious cattle diseases in Northern-Belgian dairy herds. Diseases included were Q-fever (*Coxiella burnetii*), Leptospirosis (*Leptospira hardjo*), Salmonellosis (*Salmonella dublin* and *Salmonella typhimurium*) and finally Neosporosis (*Neospora caninum*). All infections primarily have a negative repercussion on fertility in cattle (*e.g.* through abortions or metritis), although other clinical appearances (*e.g.* (subclinical) mastitis, general illness and mortality etc.) are also possible. *Coxiella*, *Leptospira* and *Salmonella* moreover have a zoonotic importance. Therefore, accurate knowledge on the distribution of these infections is necessary. This study was organized by ‘VEEPEILER Rund’ and funded by the Sanitary Fund of Belgium.

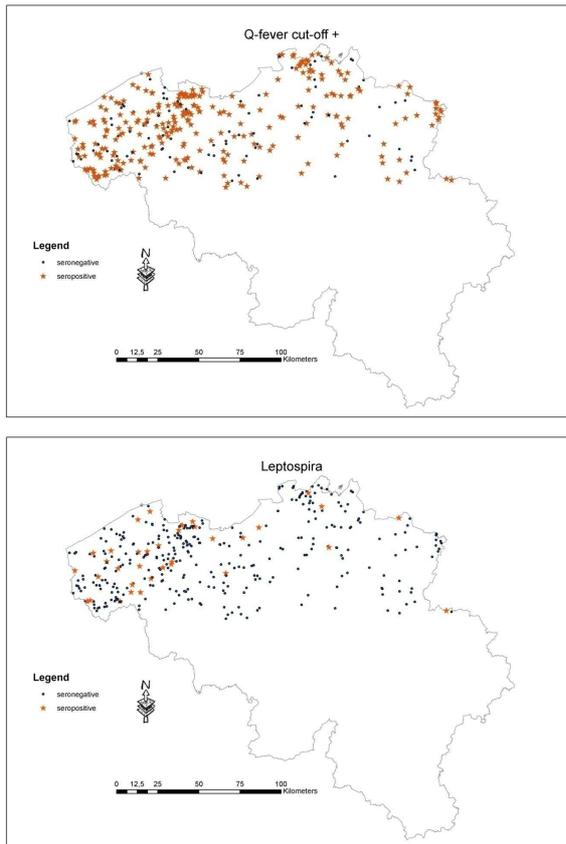
MATERIALS AND METHODS

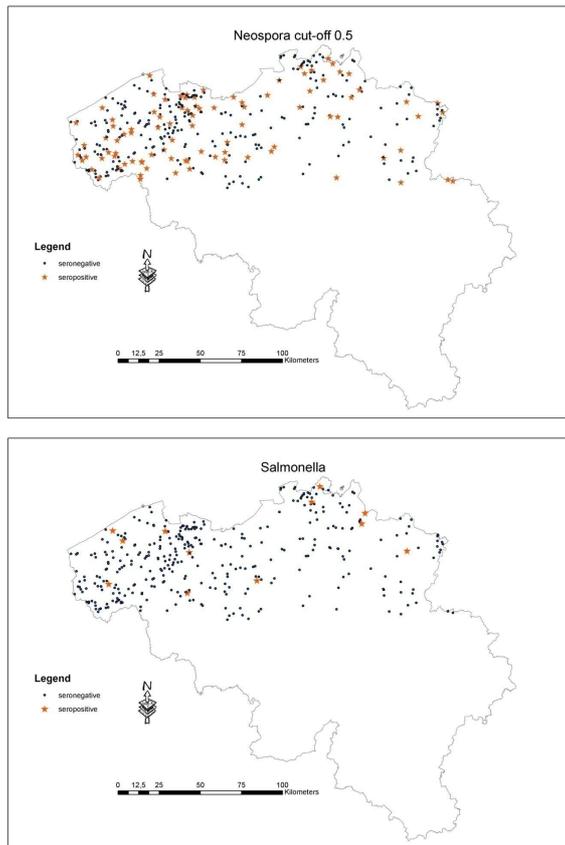
The sampling frame consisted of all producing dairy cattle herds registered in the regional dairy control system database of Northern-Belgium (MCC-Vlaanderen, 2008). A total of 6,287 dairy herds represented the study population. A sample of 363 herds was calculated to be necessary to estimate a seroprevalence of 50% (assuming no *prior* knowledge) with a desired precision of 5% and a 95% confidence level. Herds were randomly chosen and the sample was stratified by province. For each selected herd, bulk-milk samples were collected on-farm (August 2008) through the routine milk collection services of MCC-Vlaanderen. Samples were investigated for antibodies against the different infections using four commercial indirect ELISAs (Ruminants Milk/Serum Q fever test, LSIVET; PrioCHECK L. hardjo Ab, CEDI; HerdChek Neospora, IDEXX). Sample analysis was performed by MCC Vlaanderen except for the Salmonella ELISA (Gezondheidsdienst voor dieren, Deventer, The Netherlands). Provincial herd seroprevalence and some basic demographic data was analyzed with a Chi² test for two-dimensional contingency tables. Serostatus was plotted using ArcMAP 9.2 (ESRI, Redlands, CA, USA).

RESULTS

The estimated between-herd seroprevalence was 74.3% (95%CI: 69.93-78.67) for Q-fever, 9.7% (95%CI: 6.74-12.66) for Leptospirosis, 25.4% (95%CI: 21.05-29.75) for Neosporosis and 3.3% (95%CI: 1.51-5.09) for Salmonellosis. No clear regional difference in distribution could be attributed. In Figure 1, serostatus of the studied infections is plotted.

Figure 1: Serostatus of 4 ‘emerging’ infectious diseases in a random sample of dairy cattle herds Northern-Belgium.





DISCUSSION

With this study, we have a first indication that antibodies and consequently Q-fever might be largely present in dairy herds of Northern-Belgium (*i.e.* almost three fourth are bulk-milk seropositive). Further studies are needed to elaborate on the number of infected or excreting cows present at these herds. This is a prerequisite to make an accurate judgment on the potential zoonotic risk of cattle or unpasteurized milk, or the importance of this bacteria in provoking bovine abortions. The seroprevalence for *Neospora caninum* of ~25% confirms the significance of this parasite as one of the most important bovine infectious abortion agents. Cut-off level of the Neospora ELISA was set to a level related with a within-herd seroprevalence $\geq 15\%$ (Bartels, 2008). The level of herd seroprevalence against *Leptospira hardjo* and *Salmonella spp.* in Northern-Belgium was respectively higher and lower compared to a similar study in the Netherlands. In Belgium, no control programs against the latter infections exist. In some other European countries, such programs are already initiated. The lower Salmonella seroprevalence compared to the Netherlands might be an underestimation of the actual herd prevalence, as this study did not include young stock. More elaborate studies are necessary to determine whether regional differences or clustering between herds is present. This might be the case for *Leptospira hardjo* and *Salmonella sp.*

CONCLUSIONS

This study provided some necessary data about the distribution of four ‘emerging’ cattle diseases in Northern-Belgian dairy herds through bulk-milk analysis. Compared with individual serum samples, the collection of bulk-milk samples (*i.e.* which may be considered as a pooled sample of all lactating cows in the herd), is a noninvasive, convenient and economical way of sampling. Therefore, bulk-milk sampling is becoming a routinely used

tool and has several perspectives to offer in (official) certification programs, provided it is used at regular testing intervals. Before this is possible, further analysis of the test characteristics and the relation of infection at the individual level (as is already known for the Neospora ELISA) is necessary.

ACKNOWLEDGEMENTS

The study was funded by VEEPEILER Rund.

SUCSESSES AND CHALLENGES IN VETERINARY EPIDEMIOLOGY

Jan Slingenbergh - FAO, Rome

Abstract

Whereas in the past much of the attention by classical epidemiologists went to studying disease dynamics in time and in space, mainly to support progressive disease containment of rather static pathogens and disease complexes, today's challenges are far more complex and require insight in disease emergence and its drivers, extending into disease ecology and pathogen genetic evolution. One possible avenue to advance in disease ecology is to look at disease flare-up as an invasion process involving a novel host ecological vacuum. During the initial epidemic or colonisation phase the pathogens selected for are the more invasive ones. For a subsequent endemic or consolidation phase, pathogen persistence relies on a sustainable pathogen-host relationship. This entails a shift from r to K selection in the pathogen ecological strategy. Pathogens prone to invasion may also be characterised in r and K terms, paving the way for pro-active surveillance to detect potential disease emergence on a real time basis.

Introduction

When it comes to enlisting achievements in veterinary medicine to which epidemiologists made a significant contribution perhaps a mentioning should be made of the FAO Global Rinderpest Eradication Programme (GREP). GREP is scheduled to become concluded in 2010. Improvements in the identification and monitoring of different rinderpest virus lineages enabled epidemiologists to model the required vaccination coverage in different agro-pastoral settings across Africa, Middle East and Asia. This, together with innovative participatory surveillance and early warning, has been vital to the success attained by GREP.

Likewise, have epidemiologists played a major role in recent years in Europe in countering foot and mouth disease (FMD) and highly pathogen avian influenza (HPAI) incursions. In most countries in the European Union new systems have been put in place to strengthen early warning, early detection and early response, to prevent and redress any transboundary animal disease or emerging vector-borne zoonotic pathogen invasions. These innovations of the

public veterinary services rely heavily on the inputs by epidemiologists. There is no time for complacency as the nature of the job keeps changing.

Past successes in the elimination of livestock diseases in Europe

In retrospect, Sweden, in 1700, was the first country in Europe to declare freedom from rinderpest. At that time, there was already the basic notion among farmers and traders of disease transmission, of direct host-to-host passage of disease within a herd or flock and, also, of the possibility to disrupt the transmission between herds and individuals through avoidance of contact, quarantine and stamping out measures. In the course of the last three centuries, an increasing number of infectious livestock diseases in Europe have progressively been brought under control (Neuteboom & Slingenbergh, 2006). From the World Organisation for Animal Health (OIE) records, it appears that countries in Europe have all been working from the same priority list. Geography played an important role; mostly, disease freedom was first claimed in Scandinavia and the British Isles, next encompassing Baltic and central European countries, and only thereafter expanding into western and, to a lesser extent, eastern and Mediterranean Europe. The list of diseases comprised rinderpest, contagious bovine pleuropneumonia, sheep and goat pox, glanders, foot and mouth disease, bovine brucellosis, Newcastle disease, classical swine fever, anthrax, rabies, bovine tuberculosis, trichinellosis, Aujeszky's disease, infectious bovine rhino-tracheitis and bovine leucosis. Remarkably, the countries in Europe all tend to eliminate these diseases more or less in the same sequence. As a result, Europe turned gradually but progressively free from a growing number of infectious livestock diseases, paving the way for ever more large scale animal agriculture development.

The challenges of emerging diseases

In recent years there have been set backs in disease control and prevention, comprising flare-up of FMD and HPAI in western Europe, sheep and goat pox, brucellosis and peste des petits (PPR) ruminants in the eastern Mediterranean basin, and a growing list of vector borne, mostly zoonotic disease agents encroaching Europe from eastern, south-eastern and southern directions. Blue tongue virus (BTV), tick borne encephalitis (TBE), Crimean Congo haemorrhagic fever (CCHF), Hanta virus, Chikungunya, dengue, and West Nile (WNV) viruses are among the many concerns of medical and veterinary concern. These vector borne diseases pose new challenges for epidemiologists. It is all too easy to subscribe disease

emergence to just climate change and globalisation. For example, the diversity of host species, disease vectors and pathogenic agents tends to increase as we move towards the equator. Hence, a complex of ecological factors along with human and livestock demographics, land use, farming systems, recreation plus also economic and societal dynamics, presumably all contribute to explain the progressive increase in disease flare up. In Europe, the EDEN project (Emerging Diseases in a changing European Environment), extending also into northern and western Africa, has made a major contribution to the clarification of these emerging vector borne disease complexes. Slowly but progressively epidemiologists are closing on disease emergence (Sumilo *et al.*, 2007).

Invasion dynamics portrayed as r- and K-selected pathogen evolution

Perhaps there is one aspect of disease emergence that has so far received relatively little attention; the notion that disease emergence is actually on the increase with highly flexible pathogens continually and rapidly evolving to accommodate today's major landscape dynamics. Disease emergence is conveniently defined here in very broad terms as an increase in the incidence of a disease. The latter may concern an enhancement of transmission rate, ensuing directly from the host contact dynamics, or extend to more profound host ecological changes and parasite or pathogen genetic evolution, involving more complex transmission-virulence trade-offs and/or adjustment of host specificity.

Invasion dynamics and associated shifts in pathogen ecological strategy may conveniently be portrayed against the backdrop of r- and K-selection described for the population dynamics of invading species (Southwood *et al.*, 1974; Villareal *et al.*, 2000; Sakai *et al.*, 2001). The terms r- and K-strategies are taken from the logistic equation $dN/dt = r (1 - N/K) N$ where growth rate r and carrying capacity K determine the pattern of change in population size (N) in time (t). A K-strategist is expected to stay around the level of carrying capacity of its habitat, avoiding mortality rather than balancing it by replication. In contrast, an r-strategist associates with unstable habitats and conspicuously fluctuating populations; exposed to selection pressures at all population levels with a premium for rapid growth particularly at very low densities. Compared to the specialist K-strategist, an opportunistic, generalist r-type species is smaller in size, faster in reproduction, short lived, a less effective resource exploitant, less competitive, and less persisting; an r-strategist fits a dynamic environment.

Following the successful introduction of a pathogen into the new host environment (Antia *et al.*, 2003) early colonisation is facilitated by the relative abundance of hosts available,

resulting in a rapid increase in the number of hosts infected. However, eventually, with the new host resource becoming less available to the invading pathogen, the epidemic will curb. A less predictable host environment and a fiercer pathogen-host confrontation may translate in boom-and-bust disease dynamics and oscillation in the number of infected hosts. In situations where pathogen-host interactions persist, new patterns will emerge, tuned to the new situation and evolving into a replicable disease cycle and consolidation of new pathogen features. Thus, the emerging disease dynamics entail a shift from r to K selection, with the epicurve reflecting the corresponding stages in disease ecology and pathogen evolution (Fig. 1).

Practical evidence supporting the emergence of more flexible pathogens

To test the above semi-quantitative framework for invasion and host radiation, we carried out an exhaustive review of relatively recent outbreaks reported by FAO, OIE and/or the World Health Organization (WHO) as events of major veterinary and/or medical importance, mostly with a sub-continental scale distribution and lasting several decennia. We included pathogens circulating in wildlife, food and agriculture and/or solely in humans as hosts. All precursor pathogens are of animal origin. Work in progress (Slingenbergh & Engering, *in prep.*) suggests that temporo-spatial disease invasion dynamics and pathogen evolution are aligned. In fact, the pathogens selected for during the rapid spread of disease in a new host environment are r-selected whilst the eventual persistence of the invading agent relies on the adoption of K-selected properties. Hence, in the early stages of disease colonisation of a novel host ecological landscape, host population or host body type, fitness is with swiftly spreading aggressive pathogens with an opportunistic host range, readily spilling over to novel species. During the subsequent consolidation phase, the prevailing pathogen fitness context shifts in nature, favouring in particular pathogens entering into sustainable and more lasting pathogen-host interactions, involving less aggression and a move towards endemicity, with a fixed, well demarcated host range.

Apart from the above evidence suggesting an r to K shift in pathogen selection during invasion, it is also possible to rank progenitor invasive pathogens in r and K terms. As shown in Table 1, pathogens which have performed a species jump all pertain to the group of single stranded RNA viruses, except for monkey pox, a double stranded DNA virus. The pathogens capable of a virulence jump are viruses and also two bacteriae. The pathogens showing

changes in transmission ecology, clearly the largest group, comprise, in order of importance, viruses, bacteriae, and macroparasites. It appears that indeed pathogen flexibility decreases with size and genomic complexity, with RNA viruses as prominent r-selected strategists being the first to exploit any novel host ecological vacuum. When considering the pathogen size, type and transmission mode as shown in Table 1 we note that the smaller, generalist viruses either feature a direct transmission mode or are being transferred by haematophagous insects. Swift, successful transfer of the pathogen between hosts, including replication in a biological vector, would secure uninterrupted reproduction, supporting an r-selected strategy. For emerging diseases caused by bacteria we noted a more prominent role for the outside-the-host pathogen stage, be it through food and other forms of contamination or persistence in soil or in water. This indicates a slight shift towards a more K-type profile, given the greater importance going to pathogen persistence and geographic location. The shift becomes even more distinct when considering the macro-parasites, featuring complex transmission details and all lacking the ability to significantly adjust the level of virulence or perform a host species jump, other than through expanding upon an already broad, opportunistic host range. Horizontal gene transfer (HGT) tends to feature particularly prominently among the r-selected pathogens.

Conclusion

Epidemiological investigation in disease emergence taking a disease ecology perspective and involving real time virus monitoring and phylo-geographic analysis (Archie *et al.*, 2009) may pave the way for preventive risk management. A comprehensive, ecology based global analysis of influenza A virus encroachment of humans and domestic animals is becoming an issue of growing importance. Disease ecology would assist us in predicting where we are heading in terms of pandemic risk. In addition, the clarification of disease behaviour in time and in space against the backdrop of the dynamic farming landscape makes it possible to arrest the drivers of disease emergence and explore the options available to reverse the flare-up of disease in food and agriculture.

References

1. Neuteboom, O., & Slingenbergh, J. The development of disease free areas across Europe. *Journal of Food, Agriculture & Environment*, **4**(2) (2006)
2. Southwood, T., May, R., Hassell, M. & Conway, G. Ecological strategies and population parameters. *American Naturalist*, 791-804 (1974).
3. Sumilo, D., Asokliene, L., Bormane, A., Vasilenko, V., Golovljova, I. & Randolph, S. Climate change cannot explain the upsurge of tick-borne encephalitis in the Baltics. *PLoS ONE* **2**(6) (2007).
4. Villarreal, L. P., Defilippis, V. R. & Gottlieb, K. A. Acute and persistent viral life strategies and their relationship to emerging diseases. *Virology* **272**, 1-6 (2000).
5. Sakai, A. K. *et al.* The Population Biology of Invasive Species. *Annu. Rev. Ecol. Syst.* **32**, 305-332 (2001).
6. Antia, R., Regoes, R. R., Koella, J. C. & Bergstrom, C. T. The role of evolution in the emergence of infectious diseases. *Nature* **426**, 658-661 (2003).
7. Archie, E. A., Luikart, G. & Ezenwa, V. O. Infecting epidemiology with genetics: a new frontier in disease ecology. *Trends Ecol Evol* **24**, 21-30 (2009).

Figure 1

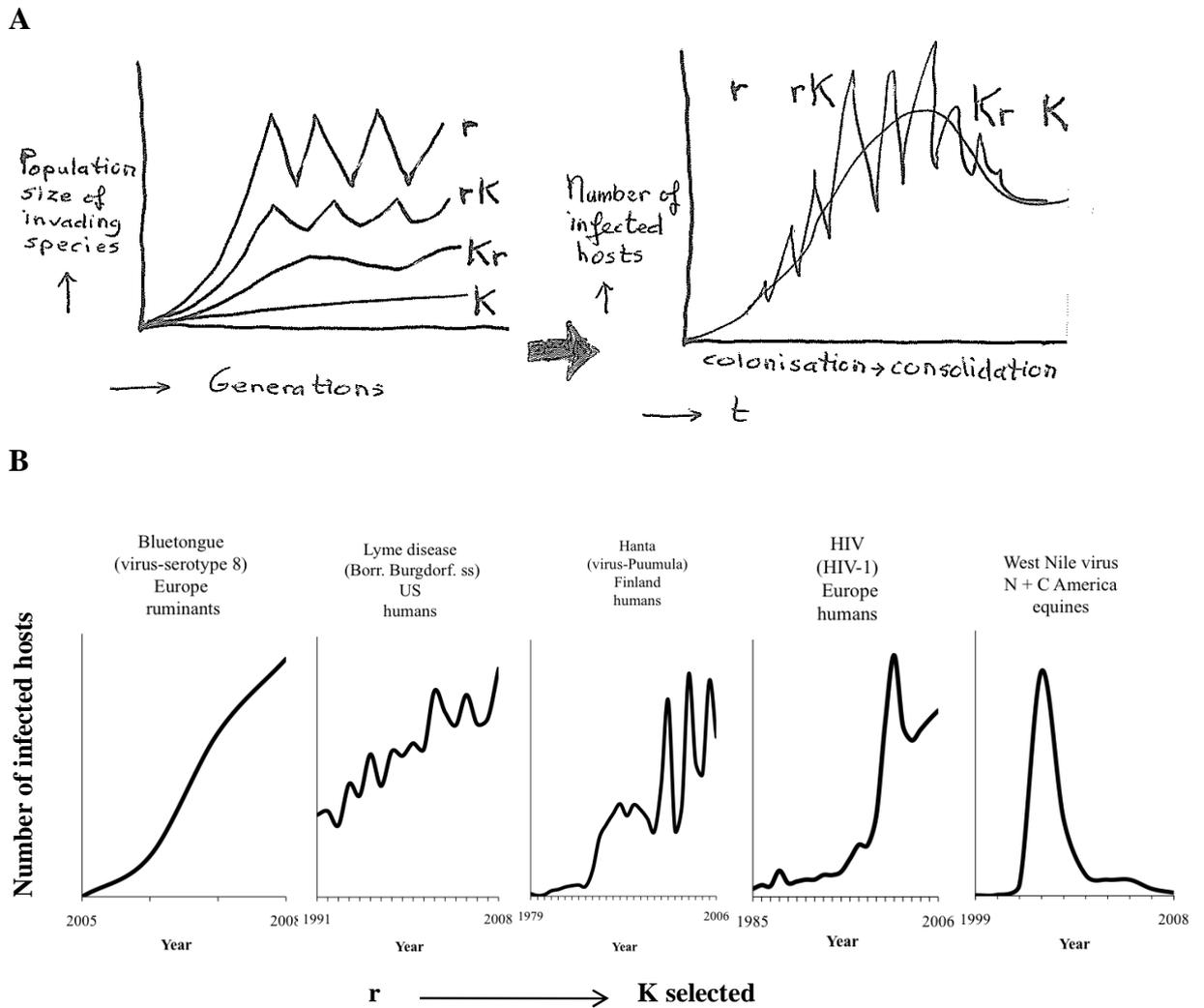


Fig. 1. The epicurve of an emerging disease pathogen reflects the population dynamics of an invading species changing its ecological strategy and also its identity during the invasion process. Emerging pathogens typically switch from an r- to K-selected strategy, in four successive steps (A). Empirical data support these hypothesised stages in the emerging pathogen invasion trajectory (B).

Table 1. Progenitor pathogens ranked according to size and other r-K features.

pathogen	type	genome size Kb(p)	genetic selection Hor/Vert	transmission mode
Infectious Bursal Disease Virus/Gumboro	dsRNA virus	6 (s)	H/V	direct oral
Norovirus	ssRNA virus	7.5	H/V	food, aerosol
Foot and mouth disease virus	ssRNA virus	7.8	H/V	direct contact
Porcine Teschovirus	ssRNA virus	8	H/V	direct oral, nasal, environment
Simian and Human immunodeficiency virus (SIV/HIV)	ssRNA virus	9.75	H/V	direct sex, body fluids
Venezuelan equine encephalomyelitis virus	ssRNA virus	10	V	vector-borne disease
Dengue virus	ssRNA virus	11	H/V	vector-borne disease
Japanese encephalitis virus	ssRNA virus	11	H/V	vector-borne disease
Lassa virus	ssRNA virus	11 (s)	V	complex
Tick-borne encephalitis virus	ssRNA virus	11	V	vector-borne disease
West Nile virus	ssRNA virus	11	H/V	vector-borne disease
Rift Valley fever virus	ssRNA virus	11.3 (s)	H/V	vector-borne disease
Chikungunya virus	ssRNA virus	11.8	V	vector-borne disease
Rabies virus	ssRNA virus	13	V	direct bite
Influenza virus	ssRNA virus	13.5 (s)	H/V	direct respiratory
Porcine circo virus (PRRS)	ssRNA virus	14	H/V	complex
Newcastle disease virus	ssRNA virus	15	H?/V	direct respiratory
Peste des petits ruminants virus	ssRNA virus	15	V	complex
Hantavirus	ssRNA virus	16 (s)	H/V	aerosols, bite
Nipah virus	ssRNA virus	18	V	complex
Ebola virus	ssRNA virus	19	H/V	direct body fluids
Bluetongue virus	dsRNA virus	19.2 (s)	H/V	vector-borne disease
Crimean-Congo haemorrhagic fever virus	ssRNA virus	19.2 (s)	V	vector-borne disease, direct
African horse sickness virus	dsRNA virus	19.5 (s)	H/V	vector-borne disease
Severe acute respiratory syndrome Corona virus	ssRNA virus	29	H/V	complex
Capripox virus (sheep and goat pox)	dsDNA virus	150	H/V	complex
Capripox virus (Lumpy skin disease)	dsDNA virus	150	H/V	mechanical vector
African swine fever virus	dsDNA virus	170	V	vector-borne disease, direct
Monkeypox virus	dsDNA virus	200	V	direct contact
Lyme (<i>Borrelia burgorferi</i>)	bacteria	1,443	V	vector-borne disease
MRSA <i>Staphylococcus aureus</i>	bacteria	2,839	H/V	direct contact
<i>Brucella abortus</i> and <i>B. melitensis</i>	bacteria	3,294	V	complex
Leptospirosis	bacteria	4,600	V	direct contact, environment
Salmonella (non-thypoid)	bacteria	4,857	H/V	food, oral

Anthrax	bacteria	5,454	H/V	environment
<i>Escherichia coli</i>	bacteria	5,528	H/V	food, oral
Leishmaniasis (<i>Leishmania donovani</i>)	protozoa	35,000	V	vector-borne disease
Trypanosomiasis (<i>Trypanosoma brucei rhodesiense</i>)	protozoa	35,000	H/V	vector-borne disease
Trichinellosis	helminths	56,800	V	food
<i>Echinococcus granulosus</i> and <i>E. multilocularis</i>	helminths	150,000	V	food
Old World Screwworm fly (<i>Chrysomya bezziana</i>)	Diptera	1500,000	V	wound infestation

ds: double stranded; ss: single-stranded; H: horizontal; V: vertical; s: segmented.

Table 1; supporting references

1. Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M. & Kawaoka, Y. Evolution and ecology of influenza A viruses. *Microbiol Rev* **56**, 152-179 (1992).
2. Spickler, A. R. Influenza. www.cfsph.iastate.edu/Factsheets/pdfs/influenza.pdf Last updated: August 2007 (2007).
3. Brown, I. H. The epidemiology and evolution of influenza viruses in pigs. *Vet Microbiol* **74**, 29-46 (2000).
4. Tumova, B. Equine influenza--a segment in influenza virus ecology. *Comp Immunol Microbiol Infect Dis* **3**, 45-59 (1980).
5. Daly, J. M., Newton, J. R. & Mumford, J. A. Current perspectives on control of equine influenza. *Vet Res* **35**, 411-423 (2004).
6. Crawford, P. C. *et al.* Transmission of equine influenza virus to dogs. *Science* **310**, 482-485 (2005).
7. Cohen, M. S., Hellmann, N., Levy, J. A., DeCock, K. & Lange, J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J Clin Invest* **118**, 1244-1254 (2008).
8. Cadogan, M. & Dalgleish, A. G. HIV immunopathogenesis and strategies for intervention. *Lancet Infect Dis* **8**, 675-684 (2008).
9. Sharp, P. M. Origins of human virus diversity. *Cell* **108**, 305-312 (2002).
10. Rezza, G. *et al.* Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* **370**, 1840-1846 (2007).
11. Chhabra, M., Mittal, V., Bhattacharya, D., Rana, U. & Lal, S. Chikungunya fever: a re-emerging viral infection. *Indian J Med Microbiol* **26**, 5-12 (2008).
12. Chevillon, C., Briant, L., Renaud, F. & Devaux, C. The Chikungunya threat: an ecological and evolutionary perspective. *Trends Microbiol* **16**, 80-88 (2008).
13. Tssetsarkin, K. A., Vanlandingham, D. L., McGee, C. E. & Higgs, S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* **3**, e201 (2007).
14. Santhosh, S. R. *et al.* Comparative full genome analysis revealed E1: A226V shift in 2007 Indian Chikungunya virus isolates. *Virus Res* **135**, 36-41 (2008).
15. Dubovi, E. J. & Njaa, B. L. Canine influenza. *Vet Clin North Am Small Anim Pract* **38**, 827-835 (2008).
16. Yoon, K. J. *et al.* Influenza virus infection in racing greyhounds. *Emerg Infect Dis* **11**, 1974-1976 (2005).
17. Weaver, S. C. & Barrett, A. D. Transmission cycles, host range, evolution and emergence of arboviral disease. *Nat Rev Microbiol* **2**, 789-801 (2004).
18. Walton, T. E., Holbrook, F. R., Bolivar-Raya, R., Ferrer-Romero, J. & Ortega, M. D. Venezuelan equine encephalomyelitis and African horse sickness. Current status and review. *Ann N Y Acad Sci* **653**, 217-227 (1992).
19. Weaver, S. C., Ferro, C., Barrera, R., Boshell, J. & Navarro, J. C. Venezuelan equine encephalitis. *Annu Rev Entomol* **49**, 141-174 (2004).
20. Eaton, B. T., Broder, C. C. & Wang, L. F. Hendra and Nipah viruses: pathogenesis and therapeutics. *Curr Mol Med* **5**, 805-816 (2005).
21. Groseth, A., Feldmann, H. & Strong, J. E. The ecology of Ebola virus. *Trends Microbiol* **15**, 408-416 (2007).
22. Hoenen, T., Groseth, A., Falzarano, D. & Feldmann, H. Ebola virus: unravelling pathogenesis to combat a deadly disease. *Trends Mol Med* **12**, 206-215 (2006).
23. Zampieri, C. A., Sullivan, N. J. & Nabel, G. J. Immunopathology of highly virulent pathogens: insights from Ebola virus. *Nat Immunol* **8**, 1159-1164 (2007).

24. Lahm, S. A., Kombila, M., Swanepoel, R. & Barnes, R. F. Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994-2003. *Trans R Soc Trop Med Hyg* **101**, 64-78 (2007).
25. Luby, S. P. *et al.* Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis* **12**, 1888-1894 (2006).
26. Person-to-person transmission of Nipah virus during outbreak in Faridpur District, 2004. *Health and Science Bulletin* **2**, 5-9 (2004).
27. Chen, J. & Subbarao, K. The Immunobiology of SARS. *Annu Rev Immunol* **25**, 443-472 (2007).
28. Feng, Y. & Gao, G. F. Towards our understanding of SARS-CoV, an emerging and devastating but quickly conquered virus. *Comp Immunol Microbiol Infect Dis* **30**, 309-327 (2007).
29. Parker, S., Nuara, A., Buller, R. M. & Schultz, D. A. Human monkeypox: an emerging zoonotic disease. *Future Microbiol* **2**, 17-34 (2007).
30. Chen, N. *et al.* Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. *Virology* **340**, 46-63 (2005).
31. Likos, A. M. *et al.* A tale of two clades: monkeypox viruses. *J Gen Virol* **86**, 2661-2672 (2005).
32. <http://www.gumboro.com>.
33. Muller, H., Islam, M. R. & Raue, R. Research on infectious bursal disease--the past, the present and the future. *Vet Microbiol* **97**, 153-165 (2003).
34. Saif, Y. M. Infectious bursal disease and hemorrhagic enteritis. *Poult Sci* **77**, 1186-1189 (1998).
35. Oladele, O. A., Adene, D. F., Obi, T. U. & Nottidge, H. O. Comparative susceptibility of chickens, turkeys and ducks to infectious bursal disease virus using immunohistochemistry. *Vet Res Commun* **33**, 111-121 (2009).
36. Jeon, W. J. *et al.* Very virulent infectious bursal disease virus isolated from wild birds in Korea: epidemiological implications. *Virus Res* **137**, 153-156 (2008).
37. Kobasa, D. & Kawaoka, Y. Emerging influenza viruses: past and present. *Curr Mol Med* **5**, 791-803 (2005).
38. Albina, E. Epidemiology of porcine reproductive and respiratory syndrome (PRRS): an overview. *Vet Microbiol* **55**, 309-316 (1997).
39. Mateu, E. & Diaz, I. The challenge of PRRS immunology. *Vet J* **177**, 345-351 (2008).
40. http://www.oie.int/wahis/public.php?page=disease_timelines.
41. Cho, J. G. & Dee, S. A. Porcine reproductive and respiratory syndrome virus. *Theriogenology* **66**, 655-662 (2006).
42. Blaha, T. The "colorful" epidemiology of PRRS. *Vet Res* **31**, 77-83 (2000).
43. <http://www.thepigsite.com/diseaseinfo/97/porcine-reproductive-respiratory-syndrome-prrs>.
44. Greger, M. The human/animal interface: emergence and resurgence of zoonotic infectious diseases. *Crit Rev Microbiol* **33**, 243-299 (2007).
45. Data from: Summary of Notifiable diseases 2006, MMWR March 21, 2008, Volume 55, no 53, CDC and from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5651md.htm> and <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5752md.htm>.
46. Yoon, J. W. & Hovde, C. J. All blood, no stool: enterohemorrhagic Escherichia coli O157:H7 infection. *J Vet Sci* **9**, 219-231 (2008).
47. Gyles, C. L. Shiga toxin-producing Escherichia coli: an overview. *J Anim Sci* **85**, E45-62 (2007).
48. Corriere, M. D. & Decker, C. F. MRSA: an evolving pathogen. *Dis Mon* **54**, 751-755 (2008).
49. Chavez, T. T. & Decker, C. F. Health care-associated MRSA versus community-associated MRSA. *Dis Mon* **54**, 763-768 (2008).
50. Grundmann, H., Aires-de-Sousa, M., Boyce, J. & Tiemersma, E. Emergence and resurgence of methicillin-resistant Staphylococcus aureus as a public-health threat. *Lancet* **368**, 874-885 (2006).
51. Navarro, M. B., Huttner, B. & Harbarth, S. Methicillin-resistant Staphylococcus aureus control in the 21st century: beyond the acute care hospital. *Curr Opin Infect Dis* **21**, 372-379 (2008).
52. Data from DengueNet: <http://www.who.int/globalatlas/dataQuery/default.asp>.
53. Holmes, E. C. & Twiddy, S. S. The origin, emergence and evolutionary genetics of dengue virus. *Infect Genet Evol* **3**, 19-28 (2003).
54. Cologna, R., Armstrong, P. M. & Rico-Hesse, R. Selection for virulent dengue viruses occurs in humans and mosquitoes. *J Virol* **79**, 853-859 (2005).
55. Twiddy, S. S. *et al.* Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. *Virology* **298**, 63-72 (2002).
56. Schwartz-Cornil, I. *et al.* Bluetongue virus: virology, pathogenesis and immunity. *Vet Res* **39**, 46 (2008).
57. Meiswinkel, R. *et al.* The 2006 outbreak of bluetongue in northern Europe--the entomological perspective. *Prev Vet Med* **87**, 55-63 (2008).

58. Rodeia, S. P., Deluyker, H., Pfeiffer, D. U. & Salman, M. D. The bluetongue outbreak in North-West Europe: the outcome from the epidemiological investigation coordinated by the European Food Safety Authorities (EFSA). *Prev Vet Med* **87**, 1-3 (2008).
59. Elbers, A. R., Backx, A., Ekker, H. M., van der Spek, A. N. & van Rijn, P. A. Performance of clinical signs to detect bluetongue virus serotype 8 outbreaks in cattle and sheep during the 2006-epidemic in The Netherlands. *Vet Microbiol* **129**, 156-162 (2008).
60. Maan, S. *et al.* Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. *Virology* **377**, 308-318 (2008).
61. Talbi, C. *et al.* Evolutionary history and dynamics of dog rabies virus in western and central Africa. *J Gen Virol* **90**, 783-791 (2009).
62. Nel, L. H. & Markotter, W. Lyssaviruses. *Crit Rev Microbiol* **33**, 301-324 (2007).
63. Biek, R., Henderson, J. C., Waller, L. A., Rupprecht, C. E. & Real, L. A. A high-resolution genetic signature of demographic and spatial expansion in epizootic rabies virus. *Proc Natl Acad Sci U S A* **104**, 7993-7998 (2007).
64. Capua, I. & Alexander, D. J. Human health implications of avian influenza viruses and paramyxoviruses. *Eur J Clin Microbiol Infect Dis* **23**, 1-6 (2004).
65. Seal, B. S., King, D. J. & Sellers, H. S. The avian response to Newcastle disease virus. *Dev Comp Immunol* **24**, 257-268 (2000).
66. Diallo, A. *et al.* The threat of peste des petits ruminants: progress in vaccine development for disease control. *Vaccine* **25**, 5591-5597 (2007).
67. Kwiatek, O. *et al.* Peste des petits ruminants (PPR) outbreak in Tajikistan. *J Comp Pathol* **136**, 111-119 (2007).
68. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008, OIE.* www.oie.int.
69. *Data US from:* <http://www.cdc.org> and <http://diseasemaps.usgs.gov>.
70. Blitvich, B. J. Transmission dynamics and changing epidemiology of West Nile virus. *Anim Health Res Rev* **9**, 71-86 (2008).
71. Hayes, E. B. & Gubler, D. J. West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annu Rev Med* **57**, 181-194 (2006).
72. Beasley, D. W. Recent advances in the molecular biology of west nile virus. *Curr Mol Med* **5**, 835-850 (2005).
73. Demby, A. H. *et al.* Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne Zoonotic Dis* **1**, 283-297 (2001).
74. <http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm>.
75. <http://www.who.int/mediacentre/factsheets/fs179/en/print.html>.
76. Khan, S. H. *et al.* New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res* **78**, 103-115 (2008).
77. *Data US from Summary of Notifiable diseases 2006, MMWR March 21, 2008, Volume 55, no 53, CDC.*
78. Foley, S. L., Lynne, A. M. & Nayak, R. Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *J Anim Sci* **86**, E149-162 (2008).
79. Boyen, F. *et al.* Non-typhoidal Salmonella infections in pigs: a closer look at epidemiology, pathogenesis and control. *Vet Microbiol* **130**, 1-19 (2008).
80. Tulman, E. R., Delhon, G. A., Ku, B. K. & Rock, D. L. African swine fever virus. *Curr Top Microbiol Immunol* **328**, 43-87 (2009).
81. Tulman, E. R. & Rock, D. L. Novel virulence and host range genes of African swine fever virus. *Curr Opin Microbiol* **4**, 456-461 (2001).
82. http://www.vet.uga.edu/vpp/gray_book02/fad/asf.php.
83. Babiuk, S., Bowden, T. R., Boyle, D. B., Wallace, D. B. & Kitching, R. P. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. *Transbound Emerg Dis* **55**, 263-272 (2008).
84. Heyman, P. & Vaheri, A. Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. *Euro Surveill* **13** (2008).
85. Khaiboullina, S. F., Morzunov, S. P. & St Jeor, S. C. Hantaviruses: molecular biology, evolution and pathogenesis. *Curr Mol Med* **5**, 773-790 (2005).
86. Klein, S. L. & Calisher, C. H. Emergence and persistence of hantaviruses. *Curr Top Microbiol Immunol* **315**, 217-252 (2007).
87. Vijayachari, P., Sugunan, A. P. & Shriram, A. N. Leptospirosis: an emerging global public health problem. *J Biosci* **33**, 557-569 (2008).
88. *Data from* http://www.cdc.gov/ncidod/dvbid/lyme/ld_statistics.htm.
89. Kurtenbach, K. *et al.* Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol* **4**, 660-669 (2006).

90. Simarro, P. P., Jannin, J. & Cattand, P. Eliminating human African trypanosomiasis: where do we stand and what comes next? *PLoS Med* **5**, e55 (2008).
91. Mackenzie, J. S. Emerging zoonotic encephalitis viruses: lessons from Southeast Asia and Oceania. *J Neurovirol* **11**, 434-440 (2005).
92. Diagana, M., Preux, P. M. & Dumas, M. Japanese encephalitis revisited. *J Neurol Sci* **262**, 165-170 (2007).
93. Mellor, P. S. & Hamblin, C. African horse sickness. *Vet Res* **35**, 445-466 (2004).
94. Bhanuprakash, V., Indrani, B. K., Hosamani, M. & Singh, R. K. The current status of sheep pox disease. *Comp Immunol Microbiol Infect Dis* **29**, 27-60 (2006).
95. Ergonul, O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis* **6**, 203-214 (2006).
96. Flick, R. & Whitehouse, C. A. Crimean-Congo hemorrhagic fever virus. *Curr Mol Med* **5**, 753-760 (2005).
97. Annual OIE/FAO FMD Reference Laboratory Network Report. January – December 2007. http://www.wrlfmd.org/ref_labs/fmd_ref_lab_reports.htm.
98. Knowles, N. J. & Samuel, A. R. Molecular epidemiology of foot-and-mouth disease virus. *Virus Res* **91**, 65-80 (2003).
99. Sellers, R. & Gloster, J. Foot-and-mouth disease: a review of intranasal infection of cattle, sheep and pigs. *Vet J* **177**, 159-168 (2008).
100. Lopman, B. *et al.* Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet* **363**, 682-688 (2004).
101. Said, M. A., Perl, T. M. & Sears, C. L. Healthcare epidemiology: gastrointestinal flu: norovirus in health care and long-term care facilities. *Clin Infect Dis* **47**, 1202-1208 (2008).
102. Bull, R. A., Tu, E. T., McIver, C. J., Rawlinson, W. D. & White, P. A. Emergence of a new norovirus genotype II.4 variant associated with global outbreaks of gastroenteritis. *J Clin Microbiol* **44**, 327-333 (2006).
103. Widdowson, M. A. *et al.* Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus--United States, 2002. *J Infect Dis* **190**, 27-36 (2004).
104. Nayak, M. K. *et al.* A new variant of Norovirus GII.4/2007 and inter-genotype recombinant strains of NVGII causing acute watery diarrhoea among children in Kolkata, India. *J Clin Virol* **45**, 223-229 (2009).
105. Patel, M. M., Hall, A. J., Vinje, J. & Parashar, U. D. Noroviruses: a comprehensive review. *J Clin Virol* **44**, 1-8 (2009).
106. Soldan, S. S. & Gonzalez-Scarano, F. Emerging infectious diseases: the Bunyaviridae. *J Neurovirol* **11**, 412-423 (2005).
107. Randolph, S. E. Tick-borne encephalitis virus, ticks and humans: short-term and long-term dynamics. *Curr Opin Infect Dis* **21**, 462-467 (2008).
108. Suss, J. Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. *Euro Surveill* **13** (2008).
109. Kostoff, R. N., Morse, S. A. & Oncu, S. The seminal literature of anthrax research. *Crit Rev Microbiol* **33**, 171-181 (2007).
110. Passalacqua, K. D. & Bergman, N. H. Bacillus anthracis: interactions with the host and establishment of inhalational anthrax. *Future Microbiol* **1**, 397-415 (2006).
111. Cutler, S. J., Whatmore, A. M. & Commander, N. J. Brucellosis--new aspects of an old disease. *J Appl Microbiol* **98**, 1270-1281 (2005).
112. Godfroid, J. *et al.* From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Vet Res* **36**, 313-326 (2005).
113. Guerra, H. The brucellae and their success as pathogens. *Crit Rev Microbiol* **33**, 325-331 (2007).
114. Dujardin, J. C. *et al.* Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. *Emerg Infect Dis* **14**, 1013-1018 (2008).
115. Schriefer, A., Wilson, M. E. & Carvalho, E. M. Recent developments leading toward a paradigm switch in the diagnostic and therapeutic approach to human leishmaniasis. *Curr Opin Infect Dis* **21**, 483-488 (2008).
116. Eckert, J., Conraths, F. J. & Tackmann, K. Echinococcosis: an emerging or re-emerging zoonosis? *Int J Parasitol* **30**, 1283-1294 (2000).
117. Garcia, H. H., Moro, P. L. & Schantz, P. M. Zoonotic helminth infections of humans: echinococcosis, cysticercosis and fascioliasis. *Curr Opin Infect Dis* **20**, 489-494 (2007).
118. Gottstein, B., Pozio, E. & Nockler, K. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clin Microbiol Rev* **22**, 127-145 (2009).
119. Siddig, A. *et al.* Seasonality of Old World screwworm myiasis in the Mesopotamia valley in Iraq. *Med Vet Entomol* **19**, 140-150 (2005).

DETERMINATION OF THE PREVALENCE AND IDENTIFICATION OF RISK FACTORS FOR *SALMONELLA* INFECTIONS IN LAYING HENS HOUSED IN CONVENTIONAL AND ALTERNATIVE SYSTEMS

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1. Introduction

The 1st of January 2012 it will be forbidden in the EU to house laying hens in conventional battery cages. From that day onwards the housing will be restricted to enriched cages and non-cage systems such as aviary systems, floor-raised and free-range systems. The shift to these alternative systems was inspired by animal welfare. If this ban of battery cages will also have an impact on the prevalence of several pathogenic and zoonotic agents is not yet thoroughly documented. In the case of *Salmonella*, the EU called for a specific targeted research project: the Safehouse project. It is within the framework of this project that the underneath described study has been carried out.

The aim of this cross-sectional field study is to determine the prevalence and risk factors for *Salmonella* infections in commercial laying hens housed in conventional and alternative housing systems in 4 EU member states.

2. Material and methods

Selection of the farms

Farms were selected from the national Identification & Registration databases. Only farms with more than 1000 laying hens, being in the last month of the production cycle were selected. The composition of the subset of sampled farms that was aimed at was ¼ conventional battery cages and ¾ non-cage systems. Participation was voluntary. In total 192 laying hen farms were sampled (59 conventional battery cage flocks, 58 floor-raised flocks, 53 free-range flocks and 22 organic flocks) in Belgium, Germany, Greece and Italy. Only one flock per farm was sampled.

Sample type and bacteriological analysis

The following samples were collected: 5 pooled faeces samples, 1 dust sample and cloacal swabs of 40 randomly selected hens. All samples were analyzed using ISO 6579:2002_Amd1:2007, as recommended by the Community Reference Laboratory for *Salmonella* in Bilthoven, The Netherlands.

Questionnaire design and data analysis

A questionnaire was filled in during an on-farm interview. Questions related to general farm and flock characteristics and biosecurity measures. The potential relationship between risk factors and *Salmonella* status of the sampled farm was evaluated by means of multivariate

logistic regression model with the *Salmonella* status of the sampled flock as a binary outcome variable. All 2-way interactions between significant main effects were tested.

3. Results

Salmonella Enteritidis and/or Typhimurium could be detected in 22 out of the 192 laying hen flocks. Fourteen flocks were found positive only in the pooled faeces, 6 were positive both in the pooled faeces and the cloacal swabs and 2 were only positive in the cloacal swabs. The within flock prevalence based on the cloacal swabs never exceeded 9.70%.

In the final model the absence of dry cleaning in between production cycles ($P < 0.01$), sampling in the wintertime ($P = 0.01$), the housing in conventional battery cages ($P = 0.01$) and the absence of vaccination against *Salmonella* ($P = 0.04$) turned out to be risk factors for a *Salmonella*-infection (Table 1).

4. Discussion

Compared with the results of the EFSA baseline study of 2005 (EFSA, 2007), the prevalence of *Salmonella* Enteritidis in Belgium and Greece was lower in this study. However, it should be kept in mind that both the sampling method and the distribution of sampled housing types in this study are different than that of the EFSA study.

The estimates of the within flock prevalence based on the cloacal swabs were usually relatively low indicating that in general only a small percentage of birds in the positive flocks were shedding the bacterium. It needs to be stressed that this is not necessarily an accurate indication of the number of birds infected with *Salmonella*.

The housing in conventional battery cages turned out to be a significant risk factor, which is in accordance with the results of the baseline study, both at the EU level (EFSA, 2007) and at the level of the individual member states (Methner et al., 2006; Namata et al., 2008; Huneau-Salaün et al., 2009). This is most likely due to a combination of factors such as the bigger flock size in battery cage systems and the higher age of the infrastructure than in non-cage systems. This effect of age of the infrastructure can be explained by the fact that the older the infrastructure, the more difficult it gets to achieve sufficient standards of cleaning. The importance of cleaning is also demonstrated by the observation that the absence of dry cleaning in between production rounds turned out to be a significant risk factor. The seasonal effect could be explained by the fact that in outdoor systems the hens are kept inside due to cold and wet weather conditions or by the lower air quality in wintertime. Vaccination against *Salmonella* could be identified as a protective factor. However, it should be kept in mind that *Salmonella* can still be found in the intestines of a fairly large proportion of vaccinated hens (Davies and Breslin, 2004) which implies the risk of a renewed shedding of the bacterium.

The results of this study illustrate that the prevalence of *Salmonella* Enteritidis in European laying hens is still substantial. Despite the fact that in alternative housing systems the chance of oro-faecal transmission of *Salmonella* is much higher than in conventional battery cages, no higher prevalence of *Salmonella* could be observed in flocks housed in these alternative systems.

5. Acknowledgments

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Table 1. Results of the univariable and multivariate analysis for the identification of risk factors for *Salmonella* Enteritidis or Typhimurium infection on 192 European laying hen farms.

Categorical variable	n	Univariate analysis		Multivariable analysis		
		OR	P-value			
Dry cleaning						
No	44	11.24	< 0.01	2.68	1.21-13.94	< 0.01
Yes (ref)	148	-	-	-	-	-
Vaccination status against <i>Salmonella</i>						
No	111	5.37	< 0.01	5.83	1.13-30.04	0.04
Yes (ref)	81	-	-	-	-	-
Type of housing			0.01			0.01
Conventional battery (ref)	59	-	-	-	-	-
Floor-raised	58	0.18	0.01	0.06	0.01-0.34	< 0.01
Free-range	53	0.26	0.03	0.24	0.06-1.02	0.05
Organic	22	0.15	0.08	0.20	0.02-2.12	0.18
Season of sampling			0.02			0.01
Winter (ref)	34	-	-	-	-	-
Spring	59	0.26	0.03	0.01	0.00-0.56	< 0.01
Summer	52	0.11	0.01	0.03	0.00-0.27	< 0.01
Autumn	47	0.41	0.12	0.38	0.09-1.65	0.20

6. References

- Davies R. and Breslin M., 2004. Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used. Av. Path. 33(2), 133-144
- EFSA, 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*. The EFSA Journal 97, 84 pp
- Huneau-Salatin A., Chemaly M., Le Bouquin S., Lalande F., Petetin I., Rouxel S., Michel V., Fravallo P. and Rose N., 2009. Risk factors for *Salmonella enterica* subsp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period. Prev. Vet. Med. 89(1-2), 51-58
- Methner U., Diller R., Reiche R. and Böhlend K., 2006. Occurrence of *Salmonellae* in laying hens in different housing systems and conclusion for the control. Münch. Tierarz. Wochenschr. 119, 467-473
- Namata H., E. Méroc, M. Aerts, C. Faes, J. Cortinas Abrahantes, H. Imberechts and K. Mintiens, 2008. *Salmonella* in Belgian laying hens: an identification of risk factors. Prev. Vet. Med. 83, 323-336

EPIDEMIOLOGY AND POLICY: THE PAST AND THE FUTURE

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1) Introduction and history of Veterinary Epidemiology and Policy in Belgium

Veterinary epidemiology is rather young discipline in veterinary science. The first textbooks on veterinary epidemiology were published in the late 1970's. Although the term epidemiology was already used in many courses, the discipline was formally introduced in the veterinary curriculum in Belgium by the creation of the Chair in Veterinary Epidemiology at Ghent University in 1992, held by Prof. Hubert Deluyker. Prof. Deluyker's work primarily focused on mastitis in dairy herds but the Belgian outbreaks of classical swine fever in 1990 and 1993-94 provided new opportunities for the application of epidemiological methods to support policy makers in controlling animal disease epidemics. Throughout the years, the Veterinary Epidemiology unit of Ghent University has extended its critical mass through participation in national and international research projects on the control of emerging animal diseases and anti-microbial resistance (Laevens et al. 1998a, 1998b, 1999; Mintiens et al. 2001, 2005; Dewulf et al. 2000a; 2000b, 2001a, 2001b, 2001c, 2002; 2004; 2005).

On the other hand, the Veterinary Services of the Belgian Ministry of Agriculture have been involved in official animal disease control programmes for many decades. Eradication programmes for endemic diseases as brucellosis, leucosis, and tuberculosis have been implemented throughout the 20th century. Towards the end of that century it became obvious that complete eradication of these diseases was not straightforward and needed additional efforts. One of the reasons for this failure was found in the insufficient quality and standardization of the diagnostic tools that were used in these programmes. In addition, the Veterinary Services found in the 1990's new challenges in the control of economically important diseases as Aujeszky's disease in swine and IBR, BVD, ParaTb in cattle. In 1996, the Coordination Centre for Veterinary Diagnostics (CCVD) was founded at the Veterinary and Agrochemical Research. The primary role of the CCVD was to support the Veterinary Services in improving and evaluating the official disease control programmes by coordinating the standardisation and quality enhancement of the diagnostic tools that were used (quality assurance of diagnostic assays). It is of utmost importance that a correct judgment about the precision and accuracy can be done of the diagnostic assays used in control programmes. Boelaert and colleagues designed large-scale surveys (cross-sectional studies) in Belgium for three pathogens of the former B list of the OIE's International Animal Health Code: pseudorabies (Aujeszky's disease), bovine herpes virus 1 (BoHV-1, infectious bovine rhinotracheitis virus) and *Mycobacterium avium* subsp. *Paratuberculosis* (Map) (Boelaert *et al.* 1999, 2000a, 2000b) and *Salmonella* in pigs (Van Vlaanderen and Biront *et al.*, 2000, Laevens *et al.*, 2003). These surveys were designed to estimate the herd (animal) seroprevalences in Belgium and to assess relevant risk factors. The aim of these studies was to provide guidance to the eradication programmes and was repeated, if possible, for many years (Figure 1 is an example: Aujeszky disease). Due to these surveys, information about the analysis and interpretation of surveys regarding became available and helped the debate about the accuracy of the information of these surveys provided and their design. Important policy issues and questions as 'Do these surveys provide animal health managers with adequate and accurate information to argue substantial animal health trade-related decisions' became more and more important. The premise of this paper is that veterinary epidemiology and animal

health policy go hand in hand and that epidemiology can contribute to better policy making and ultimately better animal (population) health.

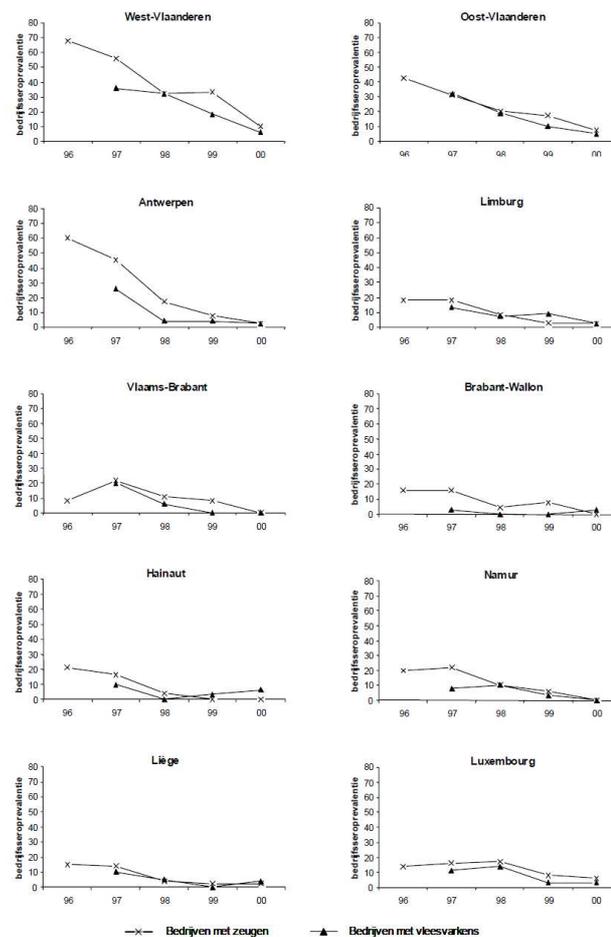


Figure 1: Herd seroprevalence of Aujeszky disease from 1996 until 2000.

2) The role of policy in Veterinary Epidemiology in Belgium

Nowadays, the Federal Public Services (Health, Food Chain Safety and Environment) and the Federal Agency for Safety of the Food Chain (FASFC) are responsible for the legislation, the implementation and the evaluation of animal diseases control in Belgium. Since 1993, the European market enlarged and trade in animals and products of animal origin between different European Union countries has grown and become liberal. This globalization of trade of animals and animal products exerts a strong pressure on animal disease management. This sets out the basic rules for food safety, and animal health standards. In order to limit the health risks inherent to this trade to acceptable levels, the policy and the regulations fixed by national and international authorities (World Trade Organisation Sanitary and Phytosanitary Agreement (SPS Agreement)) must be respected. Animal Health policy should be formulated based on values, ideology, political pressures and evidence. In many languages, the words “politics” and “policy” have the same meaning. Although many of us believe that policy should be largely or entirely evidence-based, there is widespread agreement that evidence (at least, scientific evidence) plays a relatively minor role in policy making. Epidemiology contributes to the evidence. An epidemiological key notion is contained in the SPS agreement is risk analysis. Risk analysis is the cornerstone of which is risk assessment that generates data by comprehensive surveillance systems with a solid epidemiological design (Zepeda et al., 2001). Therefore, precise and up-to-date epidemiological knowledge and information about the status of the major diseases is of utmost importance. In addition, the implementation

of restrictions on trade is only allowed based on epidemiological reliable data (evidence based surveillance data). Epidemiological surveillance is essential to protect animals against new (exotic) diseases as well as the implementation and evaluation of disease control programmes and enables the collection of data on zoonoses proving its value to protecting public health.

3) How veterinary epidemiology can help produce better health policy?

Veterinary epidemiology can contribute to each stage of the ‘policy cycle’ and includes 5 steps: i) assessment of animal population health (surveys, observational studies (case-control, cohort, cross sectional) , transmission studies, etc) ii) assessment of potential interventions to improve animal health (directly, models, etc) iii) policy choices iv) policy implementation and iv) policy evaluation.

The first step is to describe the target population and understand its demographic trends (mapping and using the Sanitel/Sanitrace databases). Descriptive epidemiology can then measure the health of this population, identify trends and patterns in health, and assess the population’s health risks and health needs. This will help to identify risk factors, health problems and population groups that might be priority targets for policy development. For some diseases it is particularly important to identify and quantify inequalities in risk and/or animal health (target surveillance). Analytical epidemiology can determine the causes of health problems, identifying both individual-level and population-level factors. Secondly, epidemiological research and models from risk assessment can identify potential policy interventions, synthesize existing knowledge regarding their effectiveness, contribute relevant new research, and assess the potential of each approach. Clinical epidemiologists have become very good at synthesizing existing knowledge in their development of systematic reviews and meta-analysis. A meta-analysis has been defined as: “the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings (Glass, 1976). This process is however not so easy (lack of randomization) for the population-level interventions that are most often used in policy making. The application of meta-analysis to observational studies is very relevant to policy-oriented epidemiology. Outputs of stochastic and statistical models can inform (examples are strategies to reduce the risk for human salmonellosis within the pork production chain (Bollaerts et al., 2008, figure 2) and the spread of Blue Tongue Virus serotype 8 via the wind (Hendrickx et al., 2008)) can inform decision makers by providing projections of the impact of potential interventions on the health of a specific population and/or the spread of a specific disease.

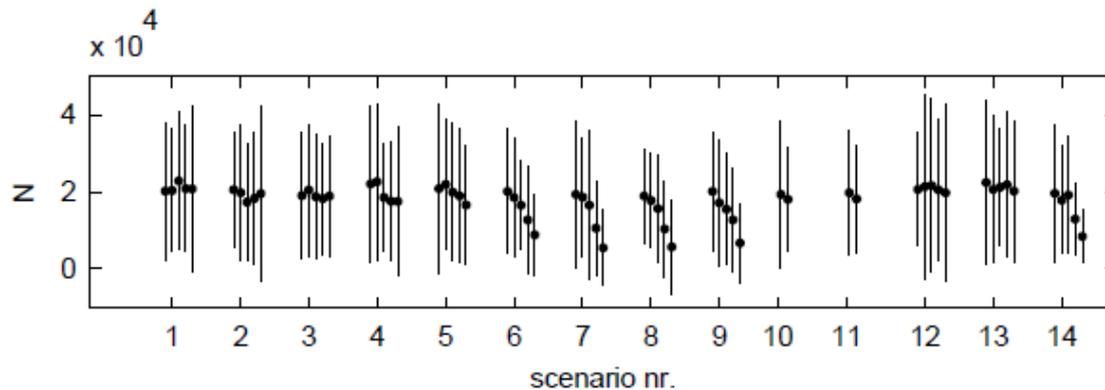


Figure 2: Scenario-analysis (what if) showing a graphical summary of the ‘Metzoon’ model (Bollaerts et al., 2008) representing the average number of annual cases ($\pm 2 \times$ standard error) of human salmonellosis through consumption of fresh minced pork according the QMRA-Metzoon model (Bollaerts et al., 2008).

Computer simulations of health and disease are very useful here, given their ability to superimpose the epidemiologic processes upon the underlying population dynamics, dealing with far more variables than we can manage, and considering both beneficial and adverse effects. In addition, they can provide answers to “What if” questions like “What would be the effect of a compulsory versus voluntary vaccination against Blue tongue Virus?”. The interventions can be compared for their impact and provide the basis for the economic analyses that will help policy-makers choose among them. Epidemiology can also assist the process of consensus development for selection of a particular policy, using priority-setting techniques borrowed from the social sciences. In addition, epidemiology can help to set targets for the chosen policies, ensuring that the targets are realistic and internally consistent (disease models are again relevant here) and can also inform needs-based resource allocation for animal health services and guide the development of information systems (Vetgeotools, GIS applications, MOSS website). Epidemiology can assess the impacts of policies and can use surveillance methods to monitor the future health, which starts the cycle again.

4) Should epidemiology have a larger influence on veterinary health policy?

Policy decisions are too often made more on the basis of political ideology, cost savings, pressure from interest groups and media attention than research evidence. Epidemiology is as much affected by this problem as any other scientific discipline. Many epidemiologists have preferred to confine their role to “the science”, avoiding the grime of policy-making. There is no doubt that policy-oriented epidemiology is distinctly practical (pragmatic) in nature and can sometimes appear to lack of rigour. However, imperfect estimates that have the best available empirical basis are usually better than wild guesses. On the other hand, policy-makers tend to come from very different backgrounds from those of epidemiologists which make communication sometimes difficult. Policy-makers want “the answer” and not a range of possibilities presented with a bunch of qualifications—and they want it immediately, while epidemiologists are trained to be sceptical (emphasizing possible sources of error rather than providing the unqualified advice that policy-makers want) and cautious (which tends to mean slow).

Epidemiology can play a bigger role in policy-making if it is evidence-based policy, specifically policy based on epidemiologic evidence. As a discipline, we must *broaden our expertise*, to include a greater knowledge of policy and its formation on the one hand, and of appropriate epidemiologic methods and tools on the other. The latter include a rehabilitation

of descriptive epidemiology, better use of animal health data (including administrative data !), emphasis on the population dynamics and disease dynamics, social determinants (farmers behavior) of animal health (since these are what government policies can try to influence directly) and disease modelling. We need to import several techniques from the social sciences, including geographical information systems and multilevel modelling. Demography is particularly important: since policy is implemented in real populations, the underlying population in the denominator is as important to population health as the epidemiologic events in the numerator. Borrowing from economics is already well underway, by way of economic analyses and methods for determining the utilities of various animal health states.

CONCLUSION

There are a lot of challenges, but we believe that policy-relevant epidemiology and decision analysis models are important tools for efficient risk management and to assist decision makers to choose the right strategy to control and/or evaluate (re)emerging animal diseases. Greater emphasis on policy-relevant topics will allow veterinary epidemiology and surveillance systems to make an even greater contribution to the general animal health. And besides that, it's fun!

REFERENCES

- Boelaert, F., P. Biront, B. Soumare, M. Dispas, E. Vanopdenbosch, J. P. Vermeersch, A. Raskin, J. Dufey, D. Berkvens, And P. Kerkhofs (2000a). "Prevalence Of Bovine Herpesvirus-1 In The Belgian Cattle Population." *Prev.Vet.Med.* 45(3-4): 285-295.
- Boelaert, F., H. Deluyker, D. Maes, J. Godfroid, A. Raskin, H. Varewijck, M. Pensaert, H. Nauwynck, F. Castryck, C. Miry, J. M. Robijns, B. Hoet, E. Segers, V. Van, I. A. Robert, And F. Koenen (1999). "Prevalence Of Herds With Young Sows Seropositive To Pseudorabies (Aujeszky's Disease) In Northern Belgium." *Prev.Vet.Med.* 41(4): 239-255.
- Boelaert, F., K. Walravens, P. Biront, J. P. Vermeersch, D. Berkvens, And J. Godfroid (2000b). "Prevalence Of Paratuberculosis (Johne's Disease) In The Belgian Cattle Population." *Vet.Microbiol.* 77(3-4): 269-281.
- Bollaerts, K.E., Messens, W., Delhalle, L., Aerts, M., Van Der Stede, Y., Dewulf, J., Quoilin, S., Maes, D., Mintiens, K., Grijspeerdt, K. 2009. Development Of A Quantitative Microbial Risk Assessment For Human Salmonellosis Through Household Consumption Of Fresh Minced Pork Meat In Belgium. *Risk Anal.* 29(6):820-40.
- Dewulf, J., F. Koenen, S. Ribbens, A. Haegeman, H. Laevens, and A. De Kruif (2005). "Evaluation of the epidemiological importance of classical swine fever infected, E2 sub-unit marker vaccinated animals with RT-nPCR positive blood samples." *J Vet Med B Infect Dis Vet Public Health* 52(9): 367-71.
- Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2000a). "Airborne transmission of classical swine fever virus under experimental conditions." *The Veterinary Record* 147: 735-738.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2001a). "An E2 sub-unit marker vaccine does not prevent horizontal or vertical transmission of classical swine fever virus." *Vaccine* 20(1-2): 86-91.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2001b). "Evaluation of the potential of dogs, cats and rats to spread classical swine fever virus." *Vet.Rec.* 149(7): 212-213.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2001c). "An experimental infection with classical swine fever virus in pregnant sows: transmission of the virus, course of the disease, antibody response and effect on gestation." *Journal of Veterinary Medicine Series B* 48(8): 583-591.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2002). "An experimental infection to investigate the indirect transmission of classical swine fever virus by excretions of infected pigs." *Journal of Veterinary Medicine Series B* 49(9): 452-456.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2004). "Efficacy of E2-sub-unit marker and C-strain vaccines in reducing horizontal transmission of classical swine fever virus in weaner pigs." *Prev Vet Med* 65(3-4): 121-33.

Dewulf, J., H. Laevens, F. Koenen, H. Vanderhallen, K. Mintiens, H. Deluyker, and A. de Kruif (2000b). "An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs [In Process Citation]." *Vaccine* 19(4-5): 475-482.

Hendrickx G, Gilbert M, Staubach C, Elbers A, Mintiens K, Gerbier G, Ducheyne E. 2008. A Wind Density Model To Quantify The Airborne Spread Of Culicoides Species During North-Western Europe Bluetongue Epidemic, 2006. *Prev Vet Med.* 87(1-2):162-81. Epub 2008 Jul 17.

Laevens, H., H. Deluyker, F. Koenen, G. Van Caenegem, J. P. Vermeersch, and A. de Kruif (1998a). "An experimental infection with a classical swine fever virus in weaner pigs. II. The use of serological data to estimate the day of virus introduction in natural outbreaks." *Vet.Quart.* 20(2): 46-49.

Laevens, H., F. Koenen, H. Deluyker, and D. Berkvens (1998b). "An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response." *Vet.Quart.* 20(2): 41-45.

Laevens, H., F. Koenen, H. Deluyker, and A. de Kruif (1999). "Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response." *The Veterinary Record* 145(9): 243-248.

Laevens, H., Mintiens, K. 2002. Een Bewakingsprogramma Ter Reductie Van De Salmonella Prevalentie In Belgische Varkensbedrijven. Rapport Ccdd Voor Favv:2002:1-9.

Mintiens, K., H. Deluyker, H. Laevens, F. Koenen, J. Dewulf, And A. De Kruif (2001). "Descriptive Epidemiology Of A Classical Swine Fever Outbreak In The Limburg Province Of Belgium In 1997." *J.Vet.Med.B* 48(2): 143-149.

Mintiens K, Verloo D, Venot E, Laevens H, Dufey J, Dewulf J, Boelaert F, Kerkhofs P, Koenen F. 2005. Estimating The Probability Of Freedom Of Classical Swine Fever Virus Of The East-Belgium Wild-Boar Population. *Prev Vet Med.* 70(3-4):211-22.

Catry, B., Dewulf, J., De Kruif, A., Vanrobaeys, M., Haesebrouck, F., Decostere, A. 2007. Accuracy Of Susceptibility Testing Of *Pasteurella Multocida* And *Mannheimia Haemolytica*. *Microb Drug Resist.*13(3):204-11.

Persoons, D., Van Hoorebeke, S., Hermans, K., Butaye, P., De Kruif, A., Haesebrouck, F., Dewulf, J. 2009. Methicillin-Resistant *Staphylococcus Aureus* In Poultry. *Emerg Infect Dis.*15(3):452-3.

Ribbens, S., Dewulf, J., Koenen, F., Maes, D., De Kruif, A. 2007. Evidence Of Indirect Transmission Of Classical Swine Fever Virus Through Contacts With People. *Vet Rec.* 160(20):687-90.

Zepeda, C., Salman, M., Ruppanner, R., 2001. International Trade, Animal Health And Veterinary Epidemiology: Challenges And Opportunities. *Prev. Vet. Med.* 48:261-271.

RISK APPROACH MODELS TO ESTIMATE THE SENSITIVITY FOR DIFFERENT SURVEILLANCE COMPONENTS: BLUETONGUE IN BELGIUM A CASE STUDY

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Introduction

Bluetongue (BT) is an arthropod-borne viral disease of both wild and domestic ruminants. The distribution of the virus is dependant on environmental and climatic conditions which allows the vector to accomplish its transmission cycle. In August 2006, from the original focus in the area where Belgium, the Netherlands and Germany share borders, an epidemic of Bluetongue Virus (BTV) serotype 8 gradually disseminated throughout the North-Western European countries, causing the most severe outbreak of this disease ever recorded (Méroci et al., 2008). The EU Legislation 1266/2007 modified by 1108/2008 prescribes the implementation of passive clinical surveillance and sentinel surveillance and a combination of serological and/or virological surveillance, as well as a targeted risk based monitoring. Each country is recommended to adapt its surveillance system in order to meet the objectives and prove the efficacy of its system.

The study has been done in this context for Belgium and three major components characterize the Belgian BTV surveillance, monthly sentinel (sero) surveillance, yearly cross sectional serological survey ('winter screening'), clinical passive surveillance. The aim of this study was to evaluate these three major components of BTV surveillance in Belgium.

Material and Methods

The scenario trees as illustrated by Martin et al. (2007) were used to conduct this study. A scenario tree for each surveillance component was designed in different Excel spread sheets. All factors interfering with the probability of infection or detection were taken in account. In this study it was assumed the components were all independent.

The first node identified was an infection node "Country status" to which the design prevalence (DP) was attributed.

The following nodes were the category nodes "Zone", "Vector activity", and "Specie", the major factors retained in the tree influencing the risk of infection. Relative risks (RR) and respective population proportions (PPr) as well as sampled population proportion (SPr) were attributed to each of these category nodes.

The infection node "Herd status" for each combination of category nodes "Zone" and "Vector activity" above was obtained. The parameters RR and PPr entered above enabled the calculation of the adjusted risk of infection (AR) for each herd type combination, which in turn would provide the herd effective risk of infection (HEPI). The same was done within each herd taking in account the "Specie" category node, which provided an "Animal status" node with its respective animal effective risk of infection (AEPI).

At the end of each limb of the tree, effective probability of detection (EPD) for each limb of the tree were calculated, with the multiplication of the SPr in each risk group and the effective probabilities of infection obtained above as well as the herd and animal sensitivities (HSe and ASe respectively). The animal sensitivity and the herd sensitivity took in account the sampling probability as well as the expected prevalence and the diagnostic test properties. These were computed in EpiTools (AusVet©). A range of different expected prevalence, and sample sizes were simulated to identify the minimum, most likely and maximum HSe and ASe. The diagnostic test characteristics were based on the competitive Elisa test from Vandenbussche, et al. (2008).

The computation of each probability of detection for each risk group provided a unit sensitivity (USE), which is the probability of detecting the disease given the country is infected by randomly sampling one unit in the whole population, and a component sensitivity (GSe) which is the probability of detecting the disease given the country is infected by sampling all units sampled in that surveillance component.

The DP's were obtained from the regulation E.C.1266/2007. The SPr's and PPr's were obtained from national databases. The RR's were obtained following empirical statistical methods (Faes, 2009), through literature review, as well as expert opinion. In order to account for the uncertainty and variability of the different parameters, appropriate distributions were fitted.

The monthly surveillance data/component enabled as well the computation of the posterior probability of freedom (PFree) for that given month as well as the probability of infection (PInf) for that given month, taking in account the probability of introduction (PIntro). The latter was set to 0 from January till March, the vector free period, and to 0.5 from April to December onwards the vector activity period (in accordance to the Belgian definition of vector activity period).

Results

Table I: Results obtained for the unit sensitivity, per component after a full year of surveillance. The mean (Mean USE), the minimum (Min USE), maximum (Max USE) and standard deviation (Std Dev) are shown.

Component	Mean USE	Max USE	Min USE	Std Dev
Winterscreening	0,000273	0,000407	0,000195	0,0000361
Sentinel	0,000387	0,000557	0,000206	0,0000506
Outbreak	0,000336	0,000338	0,000129	0,0000431

Discussion

The scenario trees as illustrated by Martin et al. (2007), used to conduct this study, have proven to be very useful tools in evaluating disease surveillance programs, as all ready seen in the passed (Hadorn et al., 2009; Martin et al., 2007; Welby et al., submitted).

Out of this study, it appears that the passive clinical surveillance provides good estimation of the current disease status in the country. Nevertheless its minimum value can be very low in comparison to the other components. Thus this might underline that the efficiency of this surveillance component is strongly dependant on the level of disease awareness.

The simulations done per month could enable policy makers to have a clear insight on the uncertainty around this probability freedom for each month. Interestingly we observe that the sentinel surveillance though seemingly provides less evidence towards freedom probability, it is the first component to detect the disease. Here again, this illustrates the importance of the level of disease awareness, as proven in other countries too (Elbers et al., 2009; Hadorn et al., 2009).

It is evident that the output of this study is strongly dependant on the assumptions, fitting distributions around most of the parameters taking in account the uncertainty and variability around them, allowed to have a good insight on the different surveillance systems running. The simulations done in Epitools (AusVet©) taking in account the possible ranges of herd and

animal sensitivity depending on the disease awareness, expected prevalence, and sampling probability enabled a more appropriate representation of the uncertainty, and variability. But having empirical data on those parameters would of course bring added value, as all these current simulations were based on assumptions. The input parameters could be improved as well regarding the different relative risks.

Similar simulations would have to be run regarding an early detection system. Also it would be interesting to run a cost benefit analysis as this is a key element guiding decision makers in their choices for the design of a surveillance system in many countries.

As a main conclusion, this study has enabled to underline important elements to quantify the sensitivity of whole surveillance system taking in account all the components as well as risk factors, sampling probability, and expected prevalence, which is a useful tool to meet the international standards when implementing disease surveillance in a country.

References

- Elbers A.R., van der Spek A.N., van Rijn P.A., 2009, Epidemiologic characteristics of bluetongue virus serotype 8 laboratory-confirmed outbreaks in The Netherlands in 2007 and a comparison with the situation in 2006. *Prev Vet Med* 92, 1-8.
- Faes C., 2009, Spatial risk analysis for bluetongue in Northern Europe. Appendix 2 Attachment 2 of Work package 6.6 EPIZONE Epidemiology and Surveillance of BTV report.
- Hadorn D.C., Racloz V., Schwermer H., Stark K.D., 2009, Establishing a cost-effective national surveillance system for Bluetongue using scenario tree modelling. *Vet Res* 40, 57.
- Martin P.A., Cameron A.R., Greiner M., 2007, Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. *Prev. Vet. Med.* 79, 71-97.
- Méroc E., Faes C., Herr C., Staubach C., Verheyden B., Vanbinst T., Vandebussche F., Hooyberghs J., Aerts M., De Clercq K., Mintiens K., 2008, Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium. *Vet Microbiol* 131, 133-144.
- Vandebussche F., Vanbinst T., Verheyden B., Van Dessel W., Demeestere L., Houdart P., Bertels G., Praet N., Berkvens D., Mintiens K., Goris N., De Clercq K., 2008, Evaluation of antibody-ELISA and real-time RT-PCR for the diagnosis and profiling of bluetongue virus serotype 8 during the epidemic in Belgium in 2006. *Vet Microbiol* 129, 15-27.
- Welby S, van den Berg T., Marché S., Houdart P., Hooyberghs J., Mintiens K., Redesigning the serological surveillance programme for notifiable Avian Influenza in Belgian professional poultry holdings (submitted).

EVALUATION OF SCENARIOS FOR REDUCING HUMAN SALMONELLOSIS THROUGH HOUSEHOLD CONSUMPTION OF FRESH MINCED PORK MEAT

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1. INTRODUCTION

Despite its declining incidence, Salmonellosis is the second most frequently reported zoonotic disease in the European Union (EU) and its control and prevention is prioritized by the EU and its member states. For Belgium, reducing the *Salmonella* contamination in the pork production might be a good human Salmonellosis mitigation strategy. Indeed, following EU baseline surveys on the prevalence of *Salmonella* in slaughter pigs in 2006-2007, higher prevalences are observed for Belgium compared to the other EU member states and a similar result holds for the *Salmonella* contamination at post-processing. In the literature, several strategies aiming to reduce the *Salmonella* contamination in the pork production have been suggested, e.g. the use of acidified feed for slaughter pigs and improved slaughter hygiene. In order to evaluate the effectiveness of such potential mitigation strategies, policy makers are increasingly using scenario-analysis (or 'what-if'-analysis) as a tool to aid decision making. In the current study, a scenario analysis is carried out using the METZOON-model, being a modular 'farm-to-fork' risk model recently developed following the Codex Alimentarius Principles. The METZOON-model is introduced and described in detail in [1.].

2. MATERIALS AND METHODS

2.1. Selected Mitigation Strategies

Several strategies aiming to reduce the number of human salmonellosis cases due to home consumption of fresh minced pork meat are investigated. In total, an exhaustive list of 14 *Salmonella* mitigation strategies, that can be evaluation without substantially changing the METZOON-model, is considered. The strategies are implemented at different stages of the minced pork meat production and consumption. The description of the strategies are given in Table 1.

2.2. Scenario-analysis and experimental design

A scenario-analysis can be thought off as an scientific experiment, hence involving a careful consideration on experimental design. In the context of scenario-analysis, two types of designs are particularly applicable, i.e. the completely randomized (CR) design and the randomized complete block (RCB) design. In a CR design, the effect of a factor is evaluated by systematically changing that factor without controlling for potential nuisance factors. In a scenario-analysis, this comes down to repeatedly running independent iterations of the risk model while systematically changing the variable corresponding to the mitigation strategy of interest, typically over a finite set of values $P = \{p_1, p_2, \dots, p_n\}$. However, although its commonly done, this is not a powerful design if, given a specific value p_i , the results of the iterations are still highly variable. In this case, the RCB design could remedy. In such a design, the effect of the variable of interest is investigated while known nuisance

factors are controlled for. In a scenario-analysis, this translates to running an iteration of the model for each value p_i from $P = \{p_1, p_2, \dots, p_n\}$ in turn while fixing nuisance factors, like the input variables in a risk model. As such, one homogeneous 'block' of risk outcomes is generated. This process is then repeated several times, each time fixing the 'blocked' input parameters to other randomly selected values, yielding independent 'blocks' of dependent iterations.

2. 1. Evaluating Scenarios using Effect Size

Typically, the results of a scenario-analysis are analyzed using t-tests comparing the effectiveness of a particular mitigation strategy with the baseline. However, in simulation studies, the effect size can be chosen arbitrarily large, rendering even negligible differences significant. An other common measure to present the results of a scenario-analysis is the relative reduction, which is only based on differences in means, and as such, ignores the variability in the outcome variable. An alternative measure that does not suffer from above mentioned shortcomings, is the effect size expressed as a standardized difference in means or $ES = (\mu_{scenario} - \mu_{baseline}) / \sigma_{baseline}$, indicating whether the observed difference is large enough to be of substantial interest. Corresponding on the design used (CR or RCB), the corresponding confidence intervals are to be calculated differently. Details can be found in [2.]

Table 1. Description of the Salmonella mitigation strategies evaluated using the METZOON-model.

Stage	Nb.	Description scenario
Primary production	1.	Reducing the probability that pigs are seropositive at primary production with 10%, 25%, 50% and 75%.
Transport-lairage	2.	Reducing the probability that pigs are internally infected at lairage with 10%, 25%, 50% and 75%.
	3.	Reducing the probability that pigs are externally infected at lairage with 10%, 25%, 50% and 75%.
Slaughterhouse	4.	Reducing the probability that a carcass is contaminated after killing with 10%, 25%, 50% and 75%.
	5.	Reducing the probability that a carcass is contaminated after singeing with 10%, 25%, 50% and 75%.
	6.	Reducing the probability that a carcass is contaminated after polishing with 10%, 25%, 50% and 75%.
	7.	Reducing the probability that a carcass is contaminated after evisceration with 10%, 25%, 50% and 75%.
	8.	Reducing the probability that a carcass is contaminated after chilling with 10%, 25%, 50% and 75%.
Post-processing	9.	Reducing the number of <i>Salmonella</i> CFUs in a meat mix with 10%, 25%, 50% and 75%.
Distribution-storage	10.	Avoiding microbial growth due to temperature abuse during transport from retail to home.
	11.	Avoiding microbial growth due to temperature abuse during storage at home.
Preparation-consumption	12.	Reducing the probability of not hand washing during cooking with 10%, 25%, 50% and 75%.
	13.	Reducing the probability that the same cutting board is used after meat handling with 10%, 25%, 50% and 75%.
	14.	Reducing the probability of undercooking with 10%, 25%, 50% and 75%.

3. RESULTS

In the METZOON-model, uncertainties were modeled by using uncertainty distributions for the input parameters rather than ignoring them by arbitrarily restricting the input parameter space. However, this resulted in a huge variability between iterations of the model when adopting the CR-design and as such, no meaningful results could be obtained within reasonable computation time. Therefore, we opt to use the RCB design creating homogeneous 'blocks' by fixing all input parameters. The different scenarios given in Table 1 are evaluated using several outcome variables, calculated using $R = 1000$ iterations of the model, which is repeated $B = 100$ times in order to obtain a distribution for each of the outcome variables. The results of the 10 most effective scenarios are summarized in Table 2.

Table 2. Summary of the predicted number of annual cases for the 5 most effect scenarios: Average number of annual cases (95% confidence intervals) and effect size (95% confidence interval).

Nb.	Short description	Annual cases	Effect Size
6.	Decreasing contamination after polishing with 75%.	08861, [03071, 20509]	-1.395, [-1.716,-1.074]
7.	Decreasing contamination after evisceration with 75%.	05536, [00602, 12038]	-1.436, [-1.778,-1.094]
8.	Decreasing contamination after chilling with 50%.	10374, [03704, 23816]	-1.388, [-1.714,-1.061]
8.	Decreasing contamination after chilling with 75%.	05655, [01187, 13175]	-2.151, [-2.561,-1.740]
9.	Reducing <i>Salmonella</i> CFUs in meat mix with 75%	06768, [02620, 12335]	-1.727, [-2.100,-1.353]

4. DISCUSSION

The results of this study indicate that the most effective scenarios are the ones taken at the end of the slaughter line and during post-processing. Improving consumer awareness is found to be effective as well. The METZOON-model and the results obtained from the scenario-analysis may help policy makers formulate new regulations. Indeed, the output of the different scenarios can provide realistic microbiological targets (criteria) to be implemented. Due to the modular approach in the METZOON-model the criteria can be set, if opted by the decision makers, at each level of the pork production and consumption chain.

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6. REFERENCES

- [1.] Bollaerts, K., Mesens, W., Delhalle, L., Aerts, M., Van der Stede, Y., Dewulf, J., Quoilin, S., Maes, D., Mintiens, K. and Grijspeerdt, K. (2009). Development of a Quantitative Microbial Risk Assessment for human salmonellosis through Household consumption of fresh minced pork meat in Belgium. *Risk Analysis*, 29, 821-840.
- [2.] Bollaerts, K., Mesens, W., Aerts, M., Dewulf, J., Maes, D., Grijspeerdt, K. and Van der Stede, Y. (2009). Evaluation of scenarios for reducing human salmonellosis through Household consumption of fresh minced pork meat. *Risk Analysis*, submitted.

EPIDEMIOLOGICAL ANALYSIS OF THE TRICHINELLA INFECTION SITUATION IN BELGIUM

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As part of a request from Belgium to be officially recognised by the European Commission as a region where the risk of *Trichinella* in domestic swine is negligible, the Scientific Committee is asked to perform an epidemiological study of the Belgian *Trichinella* situation and to propose a risk-based determination of the number of domestic swine (slaughter pigs raised under controlled housing conditions and pigs at risk; this latter category comprises outdoorreared pigs and breeding pigs) and indicator animals (foxes) to be tested annually in the case the recognition is attributed, in accordance with Regulation (EC) No 2075/2005.

Based on official data obtained with the digestion method, the real prevalence of *Trichinella* in domestic swine in Belgium is estimated at 0% (IC 95% : 0% - 0%, n = 136.311.723, exact binomial distribution) for the period from 1992 to 2008. This is less than one case per million pigs, which constitutes a negligible risk. The prevalence in horses is estimated at 0% (IC 95% : 0% - 0,0014%; n = 208.717) for the period from 1993 to 2008. The prevalence in wild boars is estimated at 0,0025% (IC 95% : 0,0003% - 0,0089% ; n = 81.042) for the period from 2001 to 2008. The prevalence in foxes is estimated at 0,2% (IC 95% : 0,0051% - 1,11% ; n=499) for the period from 2003 to 2009. In other domestic and/or wild animal species, the prevalence is zero. In man, the last case of trichinellosis caused by consumption of pork dated from 1893, and the last case caused by consumption of wild boar meat dated from 1978.

The sensitivity of the current surveillance system is higher than 99%, and the results of the ring tests do not lower this sensitivity under these 99%.

The Scientific Committee has quantitatively determined the risk level of *Trichinella* in domestic swine in Belgium, with two methods. The methodology described by Alban et al. (2008) has been used to compare the situation in Belgium and in Denmark, which obtained in 2007 the official recognition status as region with negligible risk of *Trichinella* based on this method. Based on this method, it was determined that the probability that the Belgian domestic swine population is free of *Trichinella*, based on the current surveillance program (testing all the pigs from all the categories), amounted to 98,91% (IC 95% : (98,69% – 99,1%). This can be considered as a negligible risk. Based on the risk-based surveillance program (testing only the swine population at risk, the probability amounted to 97,50% (97,13% - 97,82), what can also be considered as a negligible risk. However, the Scientific Committee makes comments on the methodology described by Alban et al. (2008) and proposes an alternative method based on scenario analyses. Based on this method, the probability that Belgium is currently free of *Trichinella* is 98,5%, which can also be considered as a negligible risk.

This indicates that an alleviated surveillance program aimed at the pig categories at risk can be proposed. The Scientific Committee recommends to continue to systematically test all domestic swine at risk (337.973 pigs, in accordance to estimations of 2008), all wild boars (cfr. wild fauna and cases in 2004 and 2007) and all horses (cfr. import risk), which is statutory layed down for the latter species. The scenarioanalysis method allows to evaluate the probability of detection of an eventual introduction of *Trichinella* in the population in function

of different testing options of the slaughter pigs raised under controlled housing conditions. These options inform the risk managers on the choices to be made for the monitoring of this risk.

Concerning the wild fauna, the Committee recommends to test annually 2.922 foxes, also rats captured during other monitoring programs, and approximately fifty samples from other wild carnivores. The Scientific Committee underlines also the importance of the strict respect of the biosecurity measures, notably concerning the feeding of pigs, and concerning the measures aimed at avoiding introduction of the parasite in pig farms from outside and from the wild fauna.

PREDICTING THE SPREAD OF BTV1 BY WIND FROM SOUTHERN FRANCE IN 2009

Els Ducheyne, Yves van der Stede, Estelle Meroc and Guy Hendrickx

Introduction

During the BTV8 epidemic in 2006 a wind model has been developed which showed a strong link between spatial spread patterns of the epidemic and the occurrence of potentially infective wind events originating from pixels with infected farms depicted as weekly cumulative wind density maps (Hendrickx *et al*, 2008). This model whilst being descriptive, correctly predicted the exponential spread of the epidemic in 2007. Based on extracted spatial spread parameters a third-generation predictive simulation modelling approach has now been developed (Ducheyne *et al*, submitted). This predictive model will now be applied to evaluate the risk of natural introduction of bluetongue in Belgium and the possible impact of vaccination on the total number of cases and the spatial extent of the epidemic.

Material and methods

A full model description is given in Ducheyne *et al* (submitted). The model is briefly described in the following paragraph. The number of cattle and ruminant farms per municipality was obtained from AFSSA. This was spatially joined to the spatial data layer of the French municipalities. The spatial denominator layer was overlaid with the CORINE land cover (JRC, 2000). The frequency of farms per land cover class as derived from the CORINE data set was determined and land cover classes without farms were eliminated. The farms were then randomly distributed within each municipality and within the valid land cover classes to obtain the individual estimated location of the farm. The cases per municipality were then randomly selected from the farms. When the impact of vaccination is included, farms were randomly selected according to the percentage of vaccination at department level. Three scenario's were included: (i) random select per department, (ii) select the farms that were infected then redistribute the vaccine over the other farms (minimum level) and (iii) assume that the farms that were infected are immune and thus do not require vaccination and redistribute the vaccines over the other farms within the department.

In order to identify different spatio-temporal clusters, a retrospective space-time permutation model at municipality level was used to analyse the data (Kulldorff *et al*, 2005). Incidence was derived from the epidemiological curve and fitted using a least square estimator to the Pearl-Verhulst growth function. The distinction between short (8km), medium (40km) and long distance (> 40 km) spread was estimated using the nearest infected farm procedure as described in Hendrickx *et al* (2008). The local infection probability is determined as the ratio of number of infected farms within the radius of the short distance spread over the total number of farms. Finally, the wind data, obtained for 2008 from the ECMWF, was used to derive the wind probability for each farm within the medium distance spread.

The model was initially seeded using the cases prior to July 28, 2008 (13 cases). The cases for week *t* were then selected using a Monte Carlo Markov Chain procedure, for the local (50%) and the medium distance (45%) spread separately.

Results

Modelling the 2008 epidemic

South-west France

Three distinct spatio-temporal clusters are identified within the dataset. The initial cluster ($p=0.001$) starting on July 17 and finishing on August 20, 2008 had a relative risk ratio of

5.02. The second cluster starts on August 14 and ends on September 3 had a relative RR of 1.88. The final cluster starts on September 11 and ends on December 3. While the epidemic curves in the first and second cluster follows a near-Gaussian distribution, the third cluster has a peak in the beginning of September.

When this clustering is taken into account, the model predicts an outbreak similar to the observed data (Fig 1). In the predicted output, there is a high density of cases in the initial zone of introduction, a second high-density area starting from South-central France along the Pyrenees and thirdly a cloud of cases northwards of the first two zones. There is a large gap of cases in the Landes. Also there is higher density of cases at the bottom of the Massif Central. From Fig. 1 also follows that when the surveillance zone would be delineated starting from the predicted cases, the spatial extent is 100 km more northwards than the actual observed zone.

Brittany and Normandy

In Brittany, three cases were observed during the autumn of 2008. Because they arose later in the year these presumably did not give rise to a recorded spread though non-reported other clinical cases have been observed (Lancelot, personal communication). To simulate spread should the initial case have occurred at the beginning of the 'bluetongue season' the model was ran starting in July. If the cases had started earlier, the entire peninsula of Bretagne would have been covered. The simulation was repeated in Normandy. In this case a seed was selected near the border with Brittany. The predicted spread is mainly along the coast, consistent with the wind patterns. It can also be observed that in this case the restriction zone would extend towards Belgium, thus suggesting that Belgian farms near the French border could have been at risk.

Modelling the 2009 epidemic using vaccination data

South-west France

Given the vaccination status in May 2009 and the current known locations of cases, the number of cases in 2009 will be maximum 2500. The spatial extent could increase further northwards over a distance of 300 km. No cases will be found in the Massif Central even though there is a high density of bovine and ovine in that area. The distance from the restriction zone to the Belgian border will be at least 300km.

Modelling cases from 2009

On September 2009, a new case was found in Alliers. Although this was a non-symptomatic case, we run the model given this seed and the vaccination coverage. The model predicts 220 cases. This may be due to the high amount of bovine vaccination coverage in the departments surrounding Alliers and the high amount of vaccination coverage within the department of Alliers. Given this there was no imminent threat to Belgium.

Discussion

This model is the first to predict the spread of bluetongue before the 'bluetongue season'. Other models such as the atmospheric dispersion models by Gloster *et al* (2006, 2007) are used to analyse in retrospect the possible introduction of the disease within a previously disease-free region. However, this is not used operationally for spread of disease modelling after the initial introduction.

The predicted outcome of the model with three clusters for 2008 correctly identified the entire area that was covered by the disease. In the counties of Haute-Pyrénées and Gers however, the model predicts a high density of cases, which were not observed. This may be explained by the fact that at the onset of the epidemic vaccination was conducted in

these areas (Hooyberghs, personal Gloster et al. Will bluetongue come on the wind to the United Kingdom in 2007?. The Veterinary Record (2007) vol. 160 pp. 422-426communication), thus explaining why there is a gap in the observed data.

In the prediction there is a higher density of cases around the Massif Central, whilst at the same time no cases are predicted within the Massif Central. This is linked to the impact of slope on the spread of the disease (Bishop *et al.*, 2004; Hendrickx *et al.*, 2008) The higher density of infected *Culicoides* in combination with the presence of hosts can lead to higher density of cases in those areas.

In the first simulation exercise in Brittany, we simulated a scenario where the disease started in July instead of in late autumn. It can be seen from the obtained output that under these conditions there is a uniform spread across the peninsula. It remains uncertain how long the infected cattle was ill before they were diagnosed (oral comm. Lancelot, 2009), thus potentially having caused other, as yet undiagnosed, infected cattle that may initiate a new outbreak in 2009.

In retrospect, it seems that the model is overestimating the actual number of cases in 2009. Up until now, no clinical cases are observed. This may be due to the vaccination effort. It seems that vaccination can prevent build up from a few cases but will not hamper once the disease is established. Earlier analysis of the effect of vaccination in the BTV8 epidemic (Ducheyne *et al.*, submitted) indicated that in order to reduce the geographical extent of bluetongue the level of vaccination should be at least 80%.

References

- Ducheyne, E., De Clercq, E. M., Goossens, E. and Hendrickx G., A stochastic predictive model to predict neighbourhood and wind spread of BTV8. Submitted to Plos One
- Hendrickx, G., Gilbert, M., Staubach, C., Elbers, A., Mintiens, K., Ducheyne, E., 2008, A wind density model to quantify the airborne spread of *Culicoides* species, *Prev. Vet. Med.*, 87, 162-18
- Gloster, J., Mellor, P.S., Burgin, L. Sanders, C., Carpenter, S., 2006, Will bluetongue come on the wind to the United Kingdom in 2007? *The Veterinary Record*, 160, 422-426
- Kulldorff, M., Heffernan, R., Hartman, J., Assunção, R., Mostashari, F., 2005, A space-time permutation scan statistic for disease outbreak detection. *PLoS Medicine*, 2(3): e59

SPATIAL RISK FACTOR ANALYSIS FOR BLUETONGUE IN NORTHERN EUROPE

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1. Introduction

Bluetongue serotype 8 (BTV-8) was introduced into North-West Europe in 2006 and spread to several European countries. The 2006 BTV-8 outbreak in Northern Europe affected cattle and sheep farms in large parts of Belgium, the Netherlands, Germany and Luxemburg. It is important to understand why farms in some areas are more affected as compared to other areas. It is clear that there is not a single risk factor that determines the risk for farms in a region to be infected with BTV-8, but that it is an interplay between many factors. This presentation focuses on a spatial and spatio-temporal multivariable risk factor analysis of the 2006 BTV-8 outbreak in Northern Europe.

2. Material

The risk factors that are considered in the analysis are climate, land use, farm-and animal-density, altitude, movement of animals (from area of first infection) and wind. Analysis is performed at the municipality level. In the geographical analysis we concentrate on the differences among areas, without accounting for the time trend.

In a geographical analysis, the risk for a farm to be infected with BTV-8 during the year 2006 can be estimated as the proportion of the number of infected farms with the total number of farms per municipality. In this analysis, both sheep and cattle farms are considered (together). Since however the total number of sheep farms in Germany is not available at the municipality level, but only at the district level, the total number of sheep farms at municipality level is approximated by a proportion of the total number of sheep farms in the district, accounting for the size of the municipality.

The information on the climate is given as daily mean temperature, daily altitude-adjusted temperature and daily precipitation at 198 weather stations in the study area. From these measurements, averages from May 1 to November 30 are calculated per weather station. Since the risk factor analysis will be performed at the municipality level, a prediction model is used to estimate the average temperature and precipitation per municipality. The centroids of the municipality are used as a representation of the municipality. A tensor product spline model is used as prediction model, based on all weather stations with no missing values in the period May 1 to November 30. Weather stations that do not have temperature or precipitation measurements during some days or weeks in the study area were not included in the analysis, since a simple average over the available measurements could yield biased estimates.

Environmental information per municipality is available as the proportion of forest, crop, pasture and urban areas per municipality. Since these variables are (almost) linearly related, using all these variables in a multivariable analysis will yield problems with multicollinearity. A univariate analysis suggests no effect of the proportion of crop areas on the risk for farms in a municipality to be infected with the BTV-virus. Therefore, the forest, crop and pasture variables are used in the multivariable risk factor analysis, with the proportion of crop as baseline category. Other environmental information per municipality is the altitude, farm-density and animal-density. However, all these variables are highly correlated with the land coverage variable, and thus, contain the same type of information on the

environmental differences among regions. Therefore, these variables are discarded from the multivariable risk factor analysis.

Both transport and wind are possible risk factors for the spread of BTV-8. The cumulative number of transported animals from the area of first infection in the period 2006, and the cumulative number of wind events from an infected farm are available, and are used in the multivariable analysis.

3. Methods

Some methods for the statistical analysis of counts of infectious diseases in small areas have been proposed in literature (Held et al. 2005, Knorr-Held and Richardson 2003, Mugglin et al. 2002, Paul et al. 2008). We model the number of infected farms Y_i in municipality i as a binomial probability with the number of farms n_i as the number of events

$$Y_i \sim \text{Binomial}(n_i, \pi_i),$$

and π_i the probability for a farm in municipality i to get infected in 2006. The probability to get infected is modeled as

$$\text{logit}(\pi_i) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \lambda \sum_{j \in \mathfrak{N}_i} y_j,$$

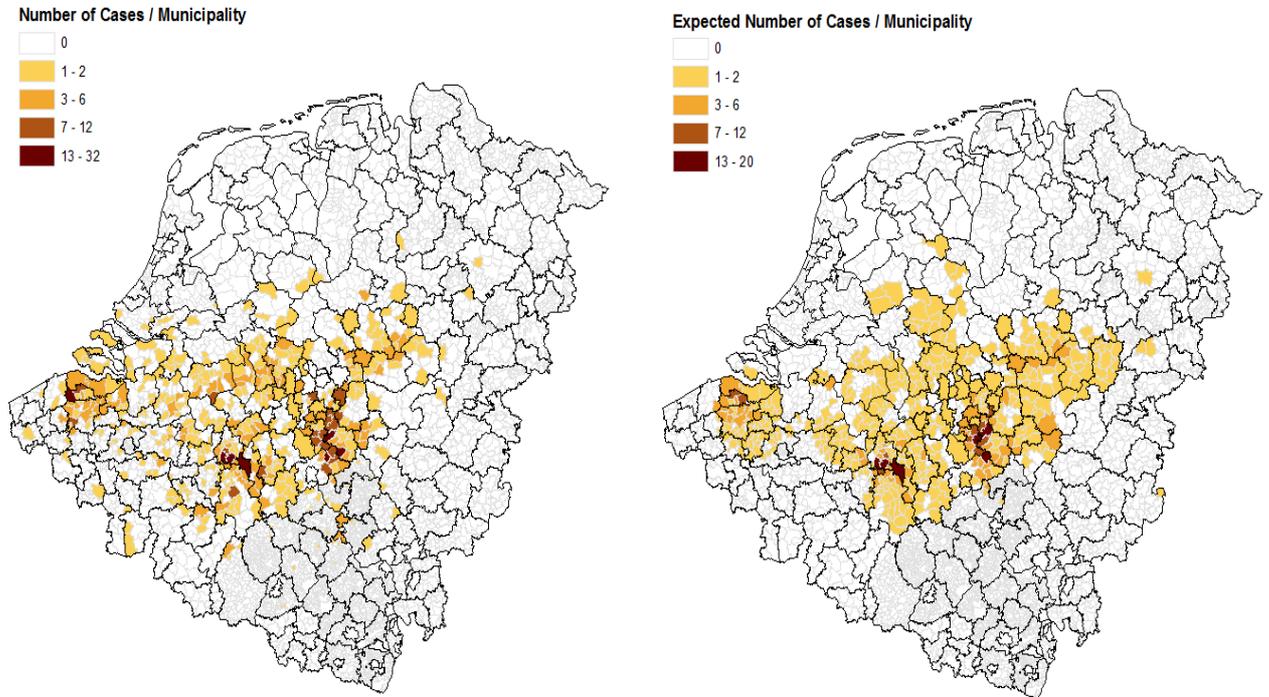
with \mathfrak{N}_i the set of neighboring municipalities of municipality i . A pair of municipalities is said to be neighbors if the distance between the centroids of the municipalities is smaller than 10 km. The disease probability is thus separated in two parts: a risk factor component $\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots$ containing all risk factors (and pairwise interactions) and an epidemic component $\lambda \sum_{j \in \mathfrak{N}_i} y_j$, a dependence on the number of outbreaks in neighboring municipalities, describing the local spread of the disease (within 10 km distance). Since more variability is expected as assumed under a binomial model, an overdispersion factor is accounted for into this model.

All the previously described risk factors are considered as main effects in the model, together with all possible pairwise interactions. Both backward and forward model selection methods were used to select the best model based on a Bayesian Information Criterion (BIC). The BIC penalizes the likelihood with the number of parameters, accounting for the number of observations in the data.

4. Conclusions and discussion

From the final model we can conclude that the land use in a municipality is a very important risk factor. It is the combination of different land types (especially forests together with pasture) which makes an area to be a high-risk area for infection. Also a large precipitation increases the risk of pasture areas. The local spread, reflected by the epidemic component, shows a significant increasing effect corresponding to an increase of cases in the neighborhood of the municipality. There is a significantly increasing effect of the risk with the number of wind events from infected areas, although areas with mainly forests are less sensitive to the spread due to wind events. The spread due to animal movements is also significant, with an odds ratio of 1.011 corresponding to 10 extra animals transported from the area of first infection. Also high temperature together with a high precipitation enforces the risk of infection. A risk map based on the results has been made, together with the

predicted number of events accounting for the number of farms in a municipality. The predicted number of events seems to match quite well the observed outbreak, meaning that the risk factor model describes most of the variability.



Vet-geoTools, a new spatial decision support system to manage disease outbreaks more rapidly and efficiently

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Introduction

Infectious and vector-borne disease outbreaks cause a heavy burden on an already stressed livestock sector. Therefore the rapid control of animal disease outbreaks is essential to limit the amount of direct and indirect economic losses caused by such diseases. In some cases it may also be essential to limit their potential impact on public health. During the last decade, the economic impact of both contagious diseases such as Classical Swine Fever, Foot-and-Mouth Disease and Avian Influenza (Dewulf *et al*, 2005), as well as (emerging) vector-borne diseases such as bluetongue had an enormous economic impact. The direct cost of even a small outbreak such as the 1997 CSF outbreak, which was limited to 8 infected farms and 64 contacts farms, was estimated at 11M€ (Mintiens *et al*, 2001). Bluetongue spread in three years over a total area 2.25 million km² within temperate Europe (Hendrickx, 2009). The spread of zoonoses such as the highly pathogenic H5N1 Avian Influenza and more recently Mexican Flu is furthermore increasing the fear of a pandemic with a major public health impact. Once a disease has been detected, a key factor to limit the disease impact will be how efficiently high quality data in general, and geo-referenced data in particular, are acquired, processed and analyzed as part of the decision making process. In this paper we describe a series of key functionalities of Vet-geoTools, a newly developed spatial decision support system (SDSS) that aims at managing infectious disease outbreaks more rapidly and more efficiently. Vet-geoTools is a server based software package which includes a fully operational GIS engine, an EU certified "from farm to fork" traceability database and a set of functionalities to assist with all spatial aspects of disease monitoring and control.

Adopted approach

Spatial Decision Support Systems

A Spatial Decision Support System (SDSS) is a system of hardware, software and procedures to facilitate the management, manipulation, analysis, modelling, representation and display of geo-referenced data to solve complex problems regarding planning and management of resources (NCGIA¹, 1990).

The power of a SDSS comes from the ability to relate information in a spatial context and to reach a conclusion about this relationship. Infectious diseases spreading from one farm to another, either through direct contact, wind, import via transport networks, or dissemination by arthropod vectors are prime candidates for such a spatial analysis approach.

Defining the functional requirements

A performing SDSS is dependent on high quality input data and the availability of tailor-made functionalities. Data quality largely depends on the livestock system and available resources. Western European high input animal husbandry systems usually feature a wealth of information contained in multiple databases. It is essential to identify these data sources and to link this information within a centralized database management system (DBMS). To

¹ National Center for Geographic Information and Analysis, US

identify additional data needs and functional requirements of Vet-geoTools a stepwise approach was followed. First, a series of available Belgian and European contingency plans and guidelines for the control of infectious diseases was consulted and all information related to spatial data needs and analysis requirements were listed. Then the acquired information was grouped by operational functionality and a series of use cases were designed. At various stages throughout this one year process, expert meetings were organized in Belgium with representatives of the Food Safety Agency (FAVV-AFSCA), the Veterinary and Agrochemical Research Centre (CODA-CERVA), Dierengezondheidszorg Vlaanderen (DGZ) and the Epidemiology group of the Veterinary Faculty at Ghent University (UGent).

Developing the system

As part of the preparatory phase described above a three month KMO *Innovatiestudie* was conducted with support from the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT). The aim of this preliminary study was to make key ICT choices related to the core architecture of Vet-geoTools. As outcome “Intertrace TM” from PAN-Livestock Services², Reading University, UK and “Orbit-GIS TM” from Eurotronics³, Lokeren, Belgium were selected as core DBMS and GIS engine respectively.

Finally Vet-geoTools was developed as part of a two year KMO *Innovatieproject* (IWT) in collaboration with Eurotronics. Based on the identified requirements a series of ten work packages were designed:

1. *Systems analysis* to further refine the systems analysis and set the final list of functionality priorities.
2. *InterTrace extension* to include the required spatial related tables to the Intertrace central database.
3. *OrbitGIS extension* to include raster tools and other additional functionalities to the central GIS engine.
4. *Zonation tool*: discussed in more detail below.
5. *Tracing tool*: discussed in more detail below.
6. *Logistics tool*: discussed in more detail below.
7. *Premises tool*: to digitize and include as part of the system maps of premises.
8. *Spatial epidemiology tool*: discussed in more detail below.
9. *Data tools en control panels* to establish the link between the system components in a user friendly way.
10. *Validation*: beta testing stage with representatives from the user community (FAVV, CODA UG).

Functionalities

The functionalities of Vet-geoTools include four key modules who are discussed here and which have a major impact on the improved spatial management of infectious disease outbreaks in high input animal husbandry systems: the zonation, tracing, logistics and spatial epidemiology module.

It is important to recall that systems such as Vet-geoTools are highly dependent on the quality of the input data. In case high quality data are not available, *e.g.* in low input extensive animal husbandry systems in developing countries, a spatial disease management system should focus first on acquiring denominator and other relevant spatial data sets in the most cost efficient way. Such a Vet-geoTools “extensive” package is currently being developed by Avia-GIS in South Africa as part of the EPISTIS research project by the Belgian Science

² <http://www.panlivestock.com/AboutITSystems/InterTrace.htm>

³ <http://www.eurotronics.com/?c=software/orbit.htm>

Policy Office (Belspo) and in six African countries starting in Uganda as part of the ICONZ RTD project funded by the European Commission (FP7).

Zonation module

The zonation module enables to generate complex sequential disease management risk zones (e.g. quarantine zone, protection zone, restriction zone...). These zones can automatically be adapted to landscape infrastructure such as roads, railways, rivers, forest edges, to ease field operations. Automated reports can be generated as required on either of those, e.g.: farm lists per zone with status, cost evaluation stamping out, crossroad lists for warning signs, etc.

Tracing module

The tracing module enables to store and analyse retro-actively farm forward tracing data related to direct and indirect disease contamination risk. The main objective here is to rapidly include information gathered by official vets regarding the spatial risk of spread of the epidemic. The various risk zones can be automatically adapted to this new information.

Logistics module

The logistic module enables to manage lowest risk routing of a variety of interveners between the different risk zones to mitigate as much as possible contamination risk. Practical examples include: weekly visit routes for official vets, planning delivery of animal feed, planning of milk collection and the planning of pick-up of carcasses. The tool also manages complex logistic questions such as: how to collect bulk milk in a given area with a known number of farms and a given volume of the milk tanks. Minimum risk routes can be calculated centrally and GPS compatible files (*.gpx) can be sent by e-mail to drivers.

Spatial epidemiology module

The spatial epidemiology module enables to analyse disease data and produce epidemiological reports according to a series of national and international formats. As a standard the weekly Bluetongue newsletter developed as part of the EFSA BTV epidemiology study (EFSA, 2007) was used. Depending upon requirements (e.g. OIE, ADNS, EFSA...) templates can be designed which enable automated reporting using the latest available epidemiological data.

The module also includes specific tools which enable to calculate required sample sizes, denominator kernel density and epidemiological curves, as well as to conduct spatio-temporal cluster analysis (SaTScan™). In peacetime the tool can be used to analyse epidemiological data of previous epidemics and simulate various control options.

Discussion

To our knowledge no other systems similar to Vet-geoTools are currently available. Whilst several of the proposed functionalities can be conducted by a skilled GIS operator using standard GIS software and scripts, the strength of the proposed system is to (i) integrate both data and functionalities in the same environment, and (ii) propose a complete series of tailored tools specifically adapted to the objective of more rapid and efficient disease outbreak management. The main added value is that during a crisis operators at various operational levels and from various disciplines have access to the same data in the same software environment and therefore can focus more rapidly on solving problems and proposing solutions.

At this stage Vet-geoTools was successfully beta tested in collaboration with the epidemiology groups of UG and CODA using a set of exercises developed with data from the CSF outbreak of 1997 in Belgium.

References

- Dewulf, J., Koenen, F., De Clercq, K., Van Den Berg, T., Ribbens, S., De Kruif, A (2005) Uitbraken en bestrijding van klassieke varkenspest, mond-en klauwzeer en hoog pathogene aviaire influenza in de Europese unie. *Vlaams Diergeneeskundig Tijdschrift* 74: (2) 103-116
- EFSA (2007) Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe: pp. 42, 9 annexes.
- Hendrickx, G. (2009) The spread of bluetongue in Europe. *Small Ruminant Research* (in press)
- Mellor, P.S. and Wittmann, E.J. (2002) Bluetongue virus in the Mediterranean basin, 1998 – 2001. *Veterinary Journal* 164: 20-37.
- Mintiens, K., Deluyker, H., Laevens, H. Koenen, F., Dewulf, J., De Kruif, A. (2001) Descriptive epidemiology of a Classical Swine Fever outbreak in the Limburg Province of Belgium in 1997. *Journal of Veterinary Medicine series B-Infectious Diseases and Veterinary Public Health* 48: (2) 143-149.
- Thiry, E., Saegerman, C., Guyot, H., Kirten, P., Losson, B., Rollin, F., Bodmer, M., Czaplicki, G., Toussaint, J.F., DE Clercq, K., Dochy, J.M., Dufey, J., Gillemans, J.L., Messeman, K. (2006) Bluetongue in Northern Europe. *Veterinary Record* 159: 327.

WELKE EPIDEMIOLOGISCHE TOOLS HEEFT HET BELEID NODIG?

Hooyberghs J, Houdart Ph.

Het Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV) is verantwoordelijk voor de organisatie en de uitvoering van de officiële controles in de ganse voedselketen, met inbegrip van de dierenziekten. Waar dierenziektebestrijding in het verleden vooral empirisch werd aangepakt, wordt nu zowel op Belgisch als op Europees niveau in toenemende mate beroep gedaan op epidemiologische hulpmiddelen. Ook het controleprogramma van het FAVV is gebaseerd op epidemiologische principes.

In het verleden werden vooral prevalentiestudies georganiseerd. Een voorbeeld hiervan waren de jaarlijkse prevalentiestudies voor de ziekte van Aujeszky. Met behulp van steekproefsgewijs serologisch onderzoek kan de vordering van een eradicatieprogramma op een betrouwbare wijze gevolgd worden.

Op basis van deze prevalentiestudies en gegevens beschikbaar in bestaande gegevensbanken of uit specifieke questionnaires worden risicofactorenanalyses uitgevoerd. Op die manier kunnen nieuwe interventiestrategieën uitgewerkt worden. De voorbije jaren werden op Europees niveau een reeks base line studies uitgevoerd voor Salmonella, Campylobacter en MRSA. Voor België werden deze surveys uitgevoerd door het FAVV. Naast een prevalentieschatting, nodig voor het vastleggen van Europese doelstellingen voor de bestrijding van Salmonella in de primaire sector, heeft EFSA ook risicofactorenanalyses uitgevoerd. Mede op basis hiervan werd bijvoorbeeld de vaccinatie van leghennen tegen Salmonella enteritidis verplicht in alle Europese lidstaten met een hoge prevalentie.

Voor bepaalde ziekten, zoals aviaire influenza, moet volgens internationale normen de afwezigheid aangetoond worden. Na het voltooiën van eradicatieprogramma's (varkenspest, Aujeszky, blauwtong, ...) moet eveneens de afwezigheid van de ziekte met voldoende betrouwbaarheid aangetoond worden. Hiervoor is een specifieke aanpak vereist.

De voorbije jaren werd Europa geconfronteerd met een aantal nieuwe ziekten (blauwtong, hoog pathogene aviaire influenza, ...). Om deze risico's beter te kunnen voorspellen en beheren zijn er aangepaste hulpmiddelen nodig, zowel om de risico's op introductie van nieuwe ziekten zo goed mogelijk te controleren als om bij introductie nieuwe ziekten zo snel mogelijk vast te stellen (early warning).

Om bestrijdings- en bewakingsprogramma's voor dierenziekten te evalueren en te optimaliseren kunnen risicomodellen uitgewerkt worden. Voor aviaire influenza, *Brucella abortus* en enzoötische runderleucose heeft dit al aanleiding gegeven tot min of meer drastische aanpassingen teneinde deze programma's efficiënter te maken. In de toekomst zullen alle belangrijke bestrijdings- en bewakingsprogramma's op een gelijkaardige manier geëvalueerd worden en zo nodig bijgestuurd worden.

De bestrijding en bewaking van dierenziekten en andere risico's in de voedselketen kunnen aanzienlijke financiële inspanningen vergen. Vooraleer programma's opgestart worden moeten dan ook de kosten en baten tegen elkaar afgewogen worden. Het draagvlak van programma's is mede afhankelijk van een goede kosten baten verhouding. Bij het uitwerken en eventueel bijsturen van programma's zijn ook hier betrouwbare gegevens noodzakelijk.

Bij risicomanagement is tijd een belangrijke factor. Vaak is onvoldoende tijd beschikbaar om een gevaar op al zijn aspecten te onderzoeken. Het is dan essentieel dat de beschikbare gegevens op korte tijd geanalyseerd worden, zodat snel een advies kan gegeven worden. Het FAVV doet hiervoor beroep op het Wetenschappelijk Comité en financiert het Centrum voor Coördinatie van de Diergeneeskundige Diagnostiek (CCDD) van het CODA waar meerdere epidemiologen studies voor het FAVV uitvoeren.

QUANTIFICATION OF BIOSECURITY STATUS IN BELGIAN PIG HERDS USING AN ONLINE SCORING SYSTEM

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INTRODUCTION

Biosecurity gains importance for the health management of pig farms. In order to quantify the biosecurity situation on pig farms, a scoring system was developed by the Veterinary Epidemiology Unit of the faculty of Veterinary Medicine, Ghent University and incorporated in a free online application (www.biocheck.ugent.be) (Ribbens et al., 2008).

MATERIALS AND METHODS

The scoring system takes both external (preventing pathogens from entering the herd) and internal biosecurity measures (reducing within herd spread of the infection) into account. Both parts are divided into 6 subcategories each consisting of 2 to 13 questions. The different subcategories in external biosecurity are: purchase of animals and sperm, removal of manure and dead animals, feed and water supply, personnel and visitors, vermin and bird control and environment & region. Internal biosecurity is divided in: disease management, suckling period, nursery unit, fattening unit, measures between compartments and working lines, material management and cleaning and disinfection.

Each question in a subcategory and each subcategory on its own receive a weight based on literature on pathogen transmission and general knowledge of infection risks (Ribbens et al., 2008). To calculate the total score for external or internal biosecurity, the scores for the subcategories are added up. A score between 0 and 100 is obtained for both external and internal biosecurity, with zero being the worst possible situation and 100 being the best possible situation. The mean of both scores gives the overall biosecurity score. The scoring system is adapted to be appropriate for every type of pig unit (fattening herd, breeding herd, mixed herd, etc). The questionnaire is initially developed in Dutch but will soon be translated into French.

RESULTS

From December 2008 until August 2009, 99 herds (i.e. 12 breeding herds, 5 fattening herds and 82 mixed herds) had voluntarily filled in the questionnaire. The distribution of these herds between the different provinces matched the distribution of pig herds in Belgium. On 27% of the farms, other animals were kept for professional use (of which 65% has cattle). The average score for external biosecurity was 65 (min 29; max 95). The score for internal biosecurity was lower in most farms with an average of 50 (min 18; max 89). The overall biosecurity score was on average 58 (min 28; max 84).

Some selected results relating to external biosecurity showed that 82% of the herds purchasing new breeding animals use quarantine facilities for an average period of 36 days and that 71% of these farms performed all-in all-out in the quarantine stable. Sperm is purchased on 90% of the mixed and breeding farms and 66% knows the health status of the

farm of origin. Farm-specific clothing and footwear is provided to visitors in 95%. Carcass removal can be done from the road on 77% of the farms, but only 53% of the farmers regularly perform cleaning and disinfection of the carcass storage. In 29%, the farmers never clean and disinfect hands after handling carcasses or wears gloves when handling carcasses. A sanitary transition zone isn't available or used in 23% although it's legally obliged and only 36% makes visitors wash and disinfect their hands. Although most farmers are very strict concerning hygienic measures taken before entering the stables for visitors, only in 50% of the herds, the farmer and/or personnel carry out these hygienic measures themselves before entering the stables. On 47% of the farms, cats and dogs are allowed in the stables. On 10% of the farms, the transporter of live animals has entrance to the stables and in 60% of these cases, the driver didn't wear farm-specific clothing. Loading of the animals is done directly from the stable or central corridor in 81%. Only half of the farmers examine the quality of the drinking water used for the pigs every year.

Concerning internal biosecurity, all-in all-out management is practiced in 85% of the herds in the nursery unit, 71% of the herds in the fattening unit. From all the herds, 88% mostly cleans and disinfects every stable after a production round, but only very few (5) verifies the efficiency of these measures. Only in 34% of the herds diseased animals are housed in separate hospital pens and 50% manipulates the diseased animals after the healthy ones. Suckling piglets are transferred between sows on 99% of the herds, of which 29% does this more than once and 44% keeps on performing this operation after 4 days post partum. In 73%, the farmer never changes clothing and 74% never washes hands between the different compartments. Only 58% of the farmers always works from the younger to the older pigs. In spite of the use of disposable needles, the needle is only changed after 101 animals on average. Although most farmers practice all-in all-out management, 24% mixes pigs of different ages in order to obtain pens with pigs of similar weight in the nursery and/or fattening unit. On 58% of the farms, a sanitary stand empty period is applied after each production round. Only 36% of the herds have a foot bath with disinfectant at the entry of the farm, although this is also legally obliged.

In general there is a positive correlation between the scores for external and internal biosecurity (figure 1). The correlation between the overall biosecurity and herd size is slightly positive in relation to the number of sows and slightly negative in relation to the number of fattening pigs.

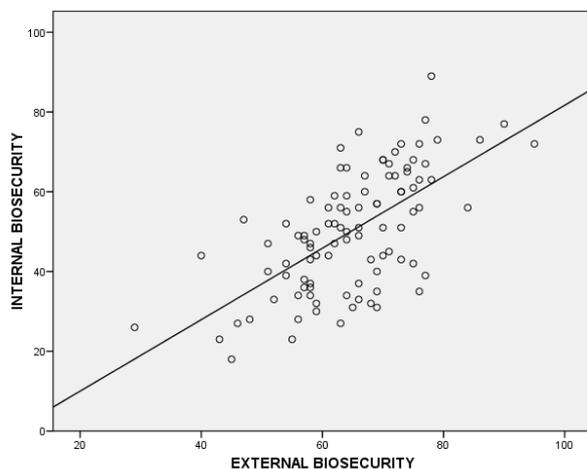


Figure 1. The correlation between the scores for external and internal biosecurity on Belgian pig farms.

DISCUSSION AND CONCLUSIONS

It needs to be emphasized that these 99 farms voluntarily filled in the questionnaire and scoring system and therefore it is to be expected that these herds are not a representative sample from the entire population but rather the herds with an interest for biosecurity. Therefore it is likely that the biosecurity measures from all pig herds are even lower than the results presented here.

Still, the large differences between the scores of different farms show that there is a lot of improvement possible in many of the herds. On average, the scores for external biosecurity, which are mainly measures imposed on others (visitors, suppliers, etc) are higher than the score on internal biosecurity which are more related to the work and management strategies of the farmers themselves.

As the results show, there are many biosecurity measures that have become common practice for farmers in Belgium, like providing farm-specific clothing and shoes for visitors to prevent the entry of diseases through visitors. On the other hand, some effective biosecurity measures, like isolate diseased pigs in a separate compartment, should be more frequently practiced. Especially in the internal biosecurity measures still a lot of improvement can be made.

The results show that this biosecurity scoring system is an efficient tool to quantify the biosecurity on a farm. It elucidates out strong and weak points of the herd and may help to set priorities for improving and monitoring the biosecurity status. As an objective score is given, it's easier to see improvement in time and to compare with other herds. The latter can motivate farmers to improve or to maintain their biosecurity score.

REFERENCES

1. Ribbens S., Vangroenweghe F., Maes D., Vandersmissen T., Dewulf J. A scoring system for biosecurity status in pig herds. In: *Proceedings of the 20th IPVS Congress*, Durban, South Africa, 22-26 June 2008

RISK FACTORS ASSOCIATED WITH ACQUIRED CEFTIOFUR RESISTANCE IN *E. COLI* FROM BROILER CHICKENS

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INTRODUCTION

Cephalosporins are an important group of antimicrobials in veterinary as well as in human medicine. In a recent survey on antimicrobial resistance in *E.coli* from broiler chickens, a remarkably high level of resistance to ceftiofur, a cephalosporin of the third generation, was found. Resistance levels in 32 Belgian broiler farms (each of them visited and sampled twice with one production round in between) varied between 0 and 100% with on average 39.1% of resistant isolates on farm level (Persoons et al., 2009).

These levels are much higher than what was recorded a few years ago (Verloo et al., 2003), and what is reported in other countries. It is linked to the emergence of Extended Spectrum Beta Lactamase genes (Smet et al., 2008), the genes encoding cephalosporin resistance.

Since no cephalosporins are registered for use in poultry in Belgium since 2001 this sudden increase is unexpected and worrying. Therefore it is crucial to try to identify factors that drive the emergence and spread of cephalosporin resistance in the broiler gut flora.

MATERIAL & METHODS

32 broiler farms were randomly selected for antimicrobial resistance determination in *E. coli*. Resistance to ceftiofur was tested by means of the Kirby-Bauer disk diffusion method according to CLSI guidelines for inoculums standardization and incubation. Neosensitab (Rosco, Denmark) antibiotic disks were used and zone diameters were read and interpreted according to the manufacturer's guidelines.

In the same 32 farms a large questionnaire was conducted at the moment of sampling. 75 factors were taken along in the questionnaire, including general management factors, animal health, hygiene, environmental factors, antibiotic treatments, etc.

A linear multivariate regression model including farm as a random factor was built to identify risk factors for acquiring ceftiofur resistance.

RESULTS

The factors retained in the multivariate model ($p < 0.05$) were hatchery, amoxicillin administration, flumequine administration and trimethoprim/sulfonamide administration. No significant interactions were found between the factors. Table 1 shows the factors, their coefficients and corresponding p- value.

Table 1. Factors included in the multivariate linear regression model.

Factor	Coefficient (β)	p- value
Amocycillin treatment	0.23	0.002
Flumequine treatment	0.53	0.002
Trimethoprim-sulfa treatment	0.16	0.026
Hatchery		
A	<i>Ref.^a</i>	
B	0.13	0.248
C	0.08	0.660
D	0.21	0.214
E	- 0.19	0.206
F	0.14	0.475
G	0.01	0.108
H	0.77	< 0.001

^a Ref indicates the reference category for that variable.

DISCUSSION

The results indicate that part of the ceftiofur resistance is probably already present in the chicks when they arrive on farm and may depend on the hatchery of origin. Whether this is the result of improper use of ceftiofur in the hatchery or originates from somewhere earlier in the production flow, like the mother birds, needs to be further examined. Treatment during production, with other antimicrobials than ceftiofur, may add to the expected level of ceftiofur resistance. For amoxycillin, also a beta lactam antibiotic, cross-resistance with ceftiofur has been described and this may explain the link between amoxicillin use and ceftiofur resistance. There is no cross-resistance between ceftiofur and trimethoprim/sulfonamide or flumequine and therefore it is not easily explainable why ceftiofur resistance rises when these antimicrobials are used. Maybe these results are an indication of new developing cross-resistance or the result of other, unmeasured, underlying factors.

The high level of ceftiofur resistance found in Belgian broiler farms thus seems to be greatly dependant of some well defined factors that need careful consideration on whether they are imperative to broiler farming, or could be made obsolete to avoid a further rise of cephalosporin resistance levels in *E.coli* from broilers.

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REFERENCES

- Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M, Martel A, Catry B, Butaye P, Haesebrouck F. 2009. Prevalence and persistence of antimicrobial resistance in *E.coli* from broiler chickens. *Micr Drug Res*, article in press.
- Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, Herman L, Haesebrouck F, Butaye P. 2008. Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* Isolates in Belgian broiler farms. *Antimicrob Agents Chemother*. 52:1238-43.

Verloo D, Butaye P, Dierick K, Imbrechts H. 2003. Descriptive epidemiology of the resistance observed in *Escherichia coli* isolated from healthy cattle, pigs and broilers, their meat and meat products. Proceedings of the Flemish Society for Veterinary Epidemiology and Economics, 11th December 2003, pp. 67.

SEROSURVEY OF FOUR ‘EMERGING’ CATTLE DISEASES (Q-FEVER, NEOSPOROSIS, LEPTOSPIROSIS AND SALMONELLOSIS) IN NORTHERN-BELGIAN DAIRY HERDS USING BULK-MILK SAMPLES

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INTRODUCTION

A survey was conducted to estimate the seroprevalence of 4 potentially ‘emerging’ infectious cattle diseases in Northern-Belgian dairy herds. Diseases included were Q-fever (*Coxiella burnetii*), Leptospirosis (*Leptospira hardjo*), Salmonellosis (*Salmonella dublin* and *Salmonella typhimurium*) and finally Neosporosis (*Neospora caninum*). All infections primarily have a negative repercussion on fertility in cattle (*e.g.* through abortions or metritis), although other clinical appearances (*e.g.* (subclinical) mastitis, general illness and mortality etc.) are also possible. *Coxiella*, *Leptospira* and *Salmonella* moreover have a zoonotic importance. Therefore, accurate knowledge on the distribution of these infections is necessary. This study was organized by ‘VEEPEILER Rund’ and funded by the Sanitary Fund of Belgium.

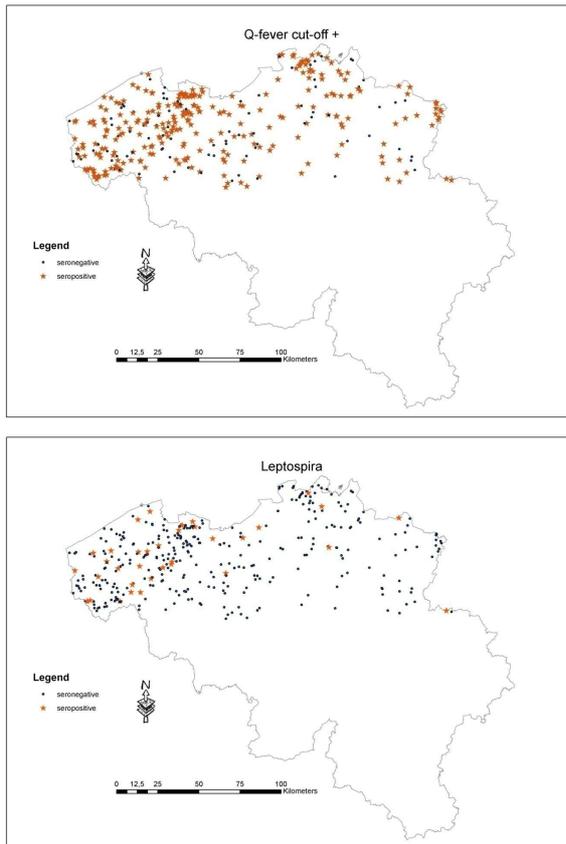
MATERIALS AND METHODS

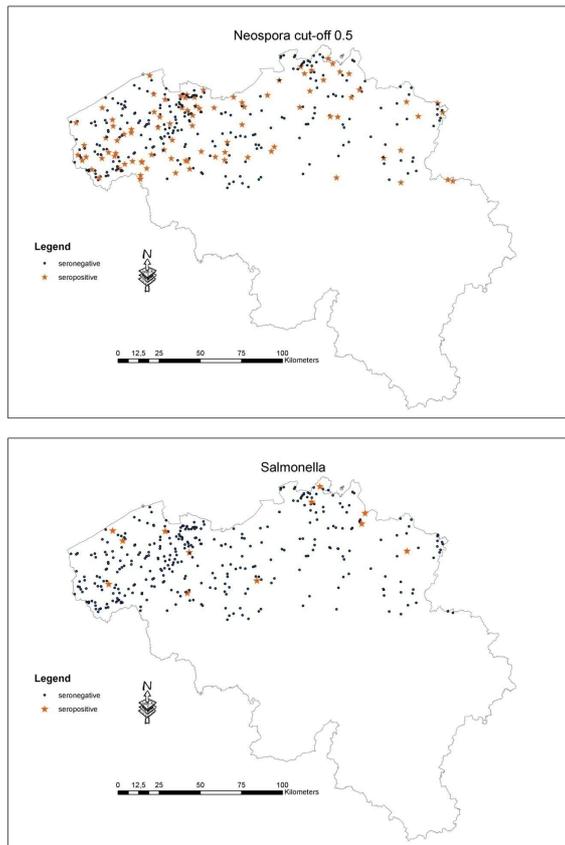
The sampling frame consisted of all producing dairy cattle herds registered in the regional dairy control system database of Northern-Belgium (MCC-Vlaanderen, 2008). A total of 6,287 dairy herds represented the study population. A sample of 363 herds was calculated to be necessary to estimate a seroprevalence of 50% (assuming no *prior* knowledge) with a desired precision of 5% and a 95% confidence level. Herds were randomly chosen and the sample was stratified by province. For each selected herd, bulk-milk samples were collected on-farm (August 2008) through the routine milk collection services of MCC-Vlaanderen. Samples were investigated for antibodies against the different infections using four commercial indirect ELISAs (Ruminants Milk/Serum Q fever test, LSIVET; PrioCHECK L. hardjo Ab, CEDI; HerdChek Neospora, IDEXX). Sample analysis was performed by MCC Vlaanderen except for the Salmonella ELISA (Gezondheidsdienst voor dieren, Deventer, The Netherlands). Provincial herd seroprevalence and some basic demographic data was analyzed with a Chi² test for two-dimensional contingency tables. Serostatus was plotted using ArcMAP 9.2 (ESRI, Redlands, CA, USA).

RESULTS

The estimated between-herd seroprevalence was 74.3% (95%CI: 69.93-78.67) for Q-fever, 9.7% (95%CI: 6.74-12.66) for Leptospirosis, 25.4% (95%CI: 21.05-29.75) for Neosporosis and 3.3% (95%CI: 1.51-5.09) for Salmonellosis. No clear regional difference in distribution could be attributed. In Figure 1, serostatus of the studied infections is plotted.

Figure 1: Serostatus of 4 ‘emerging’ infectious diseases in a random sample of dairy cattle herds Northern-Belgium.





DISCUSSION

With this study, we have a first indication that antibodies and consequently Q-fever might be largely present in dairy herds of Northern-Belgium (*i.e.* almost three fourth are bulk-milk seropositive). Further studies are needed to elaborate on the number of infected or excreting cows present at these herds. This is a prerequisite to make an accurate judgment on the potential zoonotic risk of cattle or unpasteurized milk, or the importance of this bacteria in provoking bovine abortions. The seroprevalence for *Neospora caninum* of ~25% confirms the significance of this parasite as one of the most important bovine infectious abortion agents. Cut-off level of the Neospora ELISA was set to a level related with a within-herd seroprevalence $\geq 15\%$ (Bartels, 2008). The level of herd seroprevalence against *Leptospira hardjo* and *Salmonella spp.* in Northern-Belgium was respectively higher and lower compared to a similar study in the Netherlands. In Belgium, no control programs against the latter infections exist. In some other European countries, such programs are already initiated. The lower Salmonella seroprevalence compared to the Netherlands might be an underestimation of the actual herd prevalence, as this study did not include young stock. More elaborate studies are necessary to determine whether regional differences or clustering between herds is present. This might be the case for *Leptospira hardjo* and *Salmonella sp.*

CONCLUSIONS

This study provided some necessary data about the distribution of four ‘emerging’ cattle diseases in Northern-Belgian dairy herds through bulk-milk analysis. Compared with individual serum samples, the collection of bulk-milk samples (*i.e.* which may be considered as a pooled sample of all lactating cows in the herd), is a noninvasive, convenient and economical way of sampling. Therefore, bulk-milk sampling is becoming a routinely used

tool and has several perspectives to offer in (official) certification programs, provided it is used at regular testing intervals. Before this is possible, further analysis of the test characteristics and the relation of infection at the individual level (as is already known for the Neospora ELISA) is necessary.

ACKNOWLEDGEMENTS

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SUCSESSES AND CHALLENGES IN VETERINARY EPIDEMIOLOGY

Jan Slingenbergh - FAO, Rome

Abstract

Whereas in the past much of the attention by classical epidemiologists went to studying disease dynamics in time and in space, mainly to support progressive disease containment of rather static pathogens and disease complexes, today's challenges are far more complex and require insight in disease emergence and its drivers, extending into disease ecology and pathogen genetic evolution. One possible avenue to advance in disease ecology is to look at disease flare-up as an invasion process involving a novel host ecological vacuum. During the initial epidemic or colonisation phase the pathogens selected for are the more invasive ones. For a subsequent endemic or consolidation phase, pathogen persistence relies on a sustainable pathogen-host relationship. This entails a shift from r to K selection in the pathogen ecological strategy. Pathogens prone to invasion may also be characterised in r and K terms, paving the way for pro-active surveillance to detect potential disease emergence on a real time basis.

Introduction

When it comes to enlisting achievements in veterinary medicine to which epidemiologists made a significant contribution perhaps a mentioning should be made of the FAO Global Rinderpest Eradication Programme (GREP). GREP is scheduled to become concluded in 2010. Improvements in the identification and monitoring of different rinderpest virus lineages enabled epidemiologists to model the required vaccination coverage in different agro-pastoral settings across Africa, Middle East and Asia. This, together with innovative participatory surveillance and early warning, has been vital to the success attained by GREP.

Likewise, have epidemiologists played a major role in recent years in Europe in countering foot and mouth disease (FMD) and highly pathogen avian influenza (HPAI) incursions. In most countries in the European Union new systems have been put in place to strengthen early warning, early detection and early response, to prevent and redress any transboundary animal disease or emerging vector-borne zoonotic pathogen invasions. These innovations of the

public veterinary services rely heavily on the inputs by epidemiologists. There is no time for complacency as the nature of the job keeps changing.

Past successes in the elimination of livestock diseases in Europe

In retrospect, Sweden, in 1700, was the first country in Europe to declare freedom from rinderpest. At that time, there was already the basic notion among farmers and traders of disease transmission, of direct host-to-host passage of disease within a herd or flock and, also, of the possibility to disrupt the transmission between herds and individuals through avoidance of contact, quarantine and stamping out measures. In the course of the last three centuries, an increasing number of infectious livestock diseases in Europe have progressively been brought under control (Neuteboom & Slingenbergh, 2006). From the World Organisation for Animal Health (OIE) records, it appears that countries in Europe have all been working from the same priority list. Geography played an important role; mostly, disease freedom was first claimed in Scandinavia and the British Isles, next encompassing Baltic and central European countries, and only thereafter expanding into western and, to a lesser extent, eastern and Mediterranean Europe. The list of diseases comprised rinderpest, contagious bovine pleuropneumonia, sheep and goat pox, glanders, foot and mouth disease, bovine brucellosis, Newcastle disease, classical swine fever, anthrax, rabies, bovine tuberculosis, trichinellosis, Aujeszky's disease, infectious bovine rhino-tracheitis and bovine leucosis. Remarkably, the countries in Europe all tend to eliminate these diseases more or less in the same sequence. As a result, Europe turned gradually but progressively free from a growing number of infectious livestock diseases, paving the way for ever more large scale animal agriculture development.

The challenges of emerging diseases

In recent years there have been set backs in disease control and prevention, comprising flare-up of FMD and HPAI in western Europe, sheep and goat pox, brucellosis and peste des petits (PPR) ruminants in the eastern Mediterranean basin, and a growing list of vector borne, mostly zoonotic disease agents encroaching Europe from eastern, south-eastern and southern directions. Blue tongue virus (BTV), tick borne encephalitis (TBE), Crimean Congo haemorrhagic fever (CCHF), Hanta virus, Chikungunya, dengue, and West Nile (WNV) viruses are among the many concerns of medical and veterinary concern. These vector borne diseases pose new challenges for epidemiologists. It is all too easy to subscribe disease

emergence to just climate change and globalisation. For example, the diversity of host species, disease vectors and pathogenic agents tends to increase as we move towards the equator. Hence, a complex of ecological factors along with human and livestock demographics, land use, farming systems, recreation plus also economic and societal dynamics, presumably all contribute to explain the progressive increase in disease flare up. In Europe, the EDEN project (Emerging Diseases in a changing European Environment), extending also into northern and western Africa, has made a major contribution to the clarification of these emerging vector borne disease complexes. Slowly but progressively epidemiologists are closing on disease emergence (Sumilo *et al.*, 2007).

Invasion dynamics portrayed as r- and K-selected pathogen evolution

Perhaps there is one aspect of disease emergence that has so far received relatively little attention; the notion that disease emergence is actually on the increase with highly flexible pathogens continually and rapidly evolving to accommodate today's major landscape dynamics. Disease emergence is conveniently defined here in very broad terms as an increase in the incidence of a disease. The latter may concern an enhancement of transmission rate, ensuing directly from the host contact dynamics, or extend to more profound host ecological changes and parasite or pathogen genetic evolution, involving more complex transmission-virulence trade-offs and/or adjustment of host specificity.

Invasion dynamics and associated shifts in pathogen ecological strategy may conveniently be portrayed against the backdrop of r- and K-selection described for the population dynamics of invading species (Southwood *et al.*, 1974; Villareal *et al.*, 2000; Sakai *et al.*, 2001). The terms r- and K-strategies are taken from the logistic equation $dN/dt = r (1 - N/K) N$ where growth rate r and carrying capacity K determine the pattern of change in population size (N) in time (t). A K-strategist is expected to stay around the level of carrying capacity of its habitat, avoiding mortality rather than balancing it by replication. In contrast, an r-strategist associates with unstable habitats and conspicuously fluctuating populations; exposed to selection pressures at all population levels with a premium for rapid growth particularly at very low densities. Compared to the specialist K-strategist, an opportunistic, generalist r-type species is smaller in size, faster in reproduction, short lived, a less effective resource exploitant, less competitive, and less persisting; an r-strategist fits a dynamic environment.

Following the successful introduction of a pathogen into the new host environment (Antia *et al.*, 2003) early colonisation is facilitated by the relative abundance of hosts available,

resulting in a rapid increase in the number of hosts infected. However, eventually, with the new host resource becoming less available to the invading pathogen, the epidemic will curb. A less predictable host environment and a fiercer pathogen-host confrontation may translate in boom-and-bust disease dynamics and oscillation in the number of infected hosts. In situations where pathogen-host interactions persist, new patterns will emerge, tuned to the new situation and evolving into a replicable disease cycle and consolidation of new pathogen features. Thus, the emerging disease dynamics entail a shift from r to K selection, with the epicurve reflecting the corresponding stages in disease ecology and pathogen evolution (Fig. 1).

Practical evidence supporting the emergence of more flexible pathogens

To test the above semi-quantitative framework for invasion and host radiation, we carried out an exhaustive review of relatively recent outbreaks reported by FAO, OIE and/or the World Health Organization (WHO) as events of major veterinary and/or medical importance, mostly with a sub-continental scale distribution and lasting several decennia. We included pathogens circulating in wildlife, food and agriculture and/or solely in humans as hosts. All precursor pathogens are of animal origin. Work in progress (Slingenbergh & Engering, *in prep.*) suggests that temporo-spatial disease invasion dynamics and pathogen evolution are aligned. In fact, the pathogens selected for during the rapid spread of disease in a new host environment are r-selected whilst the eventual persistence of the invading agent relies on the adoption of K-selected properties. Hence, in the early stages of disease colonisation of a novel host ecological landscape, host population or host body type, fitness is with swiftly spreading aggressive pathogens with an opportunistic host range, readily spilling over to novel species. During the subsequent consolidation phase, the prevailing pathogen fitness context shifts in nature, favouring in particular pathogens entering into sustainable and more lasting pathogen-host interactions, involving less aggression and a move towards endemicity, with a fixed, well demarcated host range.

Apart from the above evidence suggesting an r to K shift in pathogen selection during invasion, it is also possible to rank progenitor invasive pathogens in r and K terms. As shown in Table 1, pathogens which have performed a species jump all pertain to the group of single stranded RNA viruses, except for monkey pox, a double stranded DNA virus. The pathogens capable of a virulence jump are viruses and also two bacteriae. The pathogens showing

changes in transmission ecology, clearly the largest group, comprise, in order of importance, viruses, bacteriae, and macroparasites. It appears that indeed pathogen flexibility decreases with size and genomic complexity, with RNA viruses as prominent r-selected strategists being the first to exploit any novel host ecological vacuum. When considering the pathogen size, type and transmission mode as shown in Table 1 we note that the smaller, generalist viruses either feature a direct transmission mode or are being transferred by haematophagous insects. Swift, successful transfer of the pathogen between hosts, including replication in a biological vector, would secure uninterrupted reproduction, supporting an r-selected strategy. For emerging diseases caused by bacteria we noted a more prominent role for the outside-the-host pathogen stage, be it through food and other forms of contamination or persistence in soil or in water. This indicates a slight shift towards a more K-type profile, given the greater importance going to pathogen persistence and geographic location. The shift becomes even more distinct when considering the macro-parasites, featuring complex transmission details and all lacking the ability to significantly adjust the level of virulence or perform a host species jump, other than through expanding upon an already broad, opportunistic host range. Horizontal gene transfer (HGT) tends to feature particularly prominently among the r-selected pathogens.

Conclusion

Epidemiological investigation in disease emergence taking a disease ecology perspective and involving real time virus monitoring and phylo-geographic analysis (Archie *et al.*, 2009) may pave the way for preventive risk management. A comprehensive, ecology based global analysis of influenza A virus encroachment of humans and domestic animals is becoming an issue of growing importance. Disease ecology would assist us in predicting where we are heading in terms of pandemic risk. In addition, the clarification of disease behaviour in time and in space against the backdrop of the dynamic farming landscape makes it possible to arrest the drivers of disease emergence and explore the options available to reverse the flare-up of disease in food and agriculture.

References

1. Neuteboom, O., & Slingenbergh, J. The development of disease free areas across Europe. *Journal of Food, Agriculture & Environment*, **4**(2) (2006)
2. Southwood, T., May, R., Hassell, M. & Conway, G. Ecological strategies and population parameters. *American Naturalist*, 791-804 (1974).
3. Sumilo, D., Asokliene, L., Bormane, A., Vasilenko, V., Golovljova, I. & Randolph, S. Climate change cannot explain the upsurge of tick-borne encephalitis in the Baltics. *PLoS ONE* **2**(6) (2007).
4. Villarreal, L. P., Defilippis, V. R. & Gottlieb, K. A. Acute and persistent viral life strategies and their relationship to emerging diseases. *Virology* **272**, 1-6 (2000).
5. Sakai, A. K. *et al.* The Population Biology of Invasive Species. *Annu. Rev. Ecol. Syst.* **32**, 305-332 (2001).
6. Antia, R., Regoes, R. R., Koella, J. C. & Bergstrom, C. T. The role of evolution in the emergence of infectious diseases. *Nature* **426**, 658-661 (2003).
7. Archie, E. A., Luikart, G. & Ezenwa, V. O. Infecting epidemiology with genetics: a new frontier in disease ecology. *Trends Ecol Evol* **24**, 21-30 (2009).

Figure 1

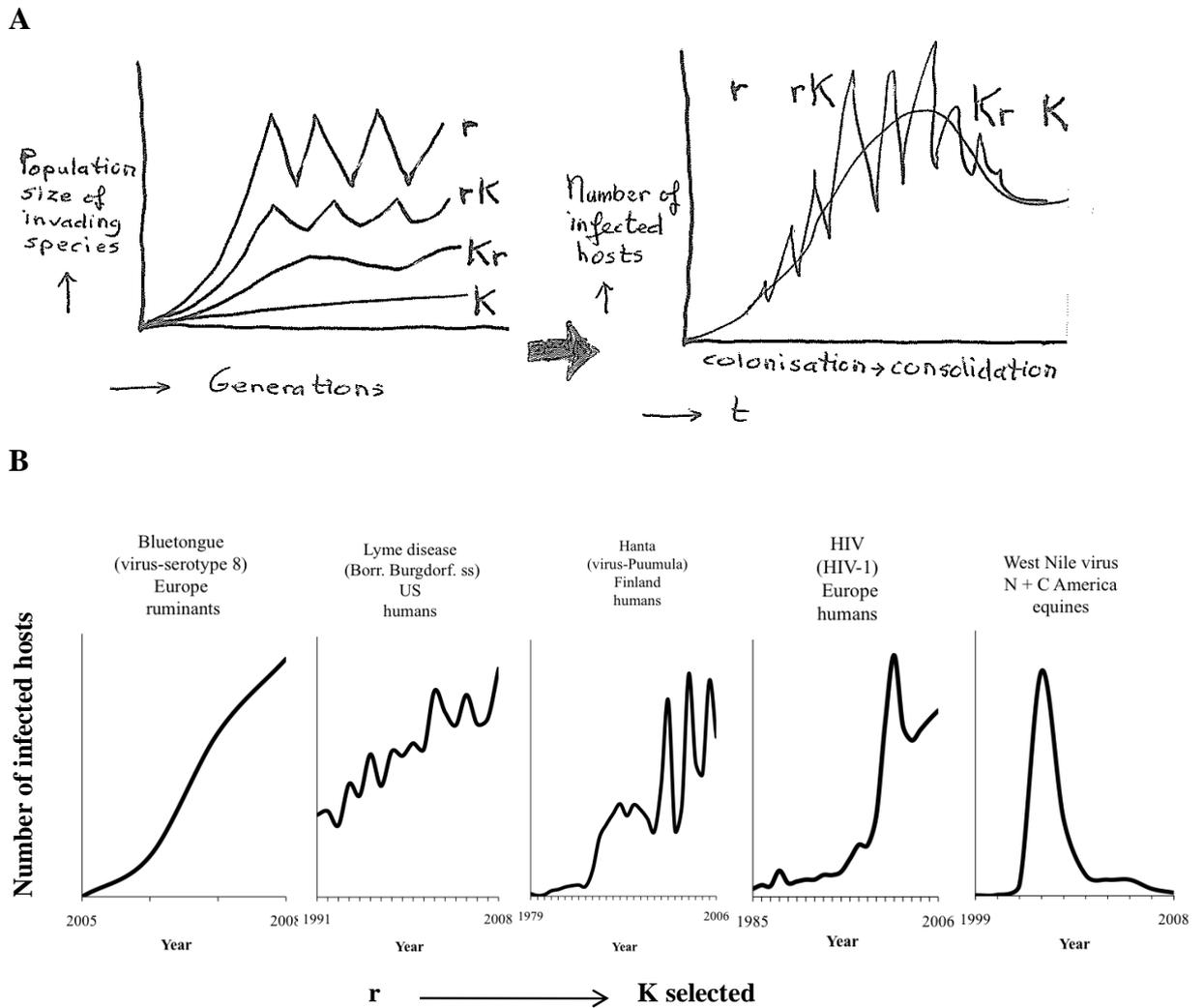


Fig. 1. The epicurve of an emerging disease pathogen reflects the population dynamics of an invading species changing its ecological strategy and also its identity during the invasion process. Emerging pathogens typically switch from an r- to K-selected strategy, in four successive steps (A). Empirical data support these hypothesised stages in the emerging pathogen invasion trajectory (B).

Table 1. Progenitor pathogens ranked according to size and other r-K features.

pathogen	type	genome size Kb(p)	genetic selection Hor/Vert	transmission mode
Infectious Bursal Disease Virus/Gumboro	dsRNA virus	6 (s)	H/V	direct oral
Norovirus	ssRNA virus	7.5	H/V	food, aerosol
Foot and mouth disease virus	ssRNA virus	7.8	H/V	direct contact
Porcine Teschovirus	ssRNA virus	8	H/V	direct oral, nasal, environment
Simian and Human immunodeficiency virus (SIV/HIV)	ssRNA virus	9.75	H/V	direct sex, body fluids
Venezuelan equine encephalomyelitis virus	ssRNA virus	10	V	vector-borne disease
Dengue virus	ssRNA virus	11	H/V	vector-borne disease
Japanese encephalitis virus	ssRNA virus	11	H/V	vector-borne disease
Lassa virus	ssRNA virus	11 (s)	V	complex
Tick-borne encephalitis virus	ssRNA virus	11	V	vector-borne disease
West Nile virus	ssRNA virus	11	H/V	vector-borne disease
Rift Valley fever virus	ssRNA virus	11.3 (s)	H/V	vector-borne disease
Chikungunya virus	ssRNA virus	11.8	V	vector-borne disease
Rabies virus	ssRNA virus	13	V	direct bite
Influenza virus	ssRNA virus	13.5 (s)	H/V	direct respiratory
Porcine circo virus (PRRS)	ssRNA virus	14	H/V	complex
Newcastle disease virus	ssRNA virus	15	H?/V	direct respiratory
Peste des petits ruminants virus	ssRNA virus	15	V	complex
Hantavirus	ssRNA virus	16 (s)	H/V	aerosols, bite
Nipah virus	ssRNA virus	18	V	complex
Ebola virus	ssRNA virus	19	H/V	direct body fluids
Bluetongue virus	dsRNA virus	19.2 (s)	H/V	vector-borne disease
Crimean-Congo haemorrhagic fever virus	ssRNA virus	19.2 (s)	V	vector-borne disease, direct
African horse sickness virus	dsRNA virus	19.5 (s)	H/V	vector-borne disease
Severe acute respiratory syndrome Corona virus	ssRNA virus	29	H/V	complex
Capripox virus (sheep and goat pox)	dsDNA virus	150	H/V	complex
Capripox virus (Lumpy skin disease)	dsDNA virus	150	H/V	mechanical vector
African swine fever virus	dsDNA virus	170	V	vector-borne disease, direct
Monkeypox virus	dsDNA virus	200	V	direct contact
Lyme (<i>Borrelia burgorferi</i>)	bacteria	1,443	V	vector-borne disease
MRSA <i>Staphylococcus aureus</i>	bacteria	2,839	H/V	direct contact
<i>Brucella abortus</i> and <i>B. melitensis</i>	bacteria	3,294	V	complex
Leptospirosis	bacteria	4,600	V	direct contact, environment
Salmonella (non-thypoid)	bacteria	4,857	H/V	food, oral

Anthrax	bacteria	5,454	H/V	environment
<i>Escherichia coli</i>	bacteria	5,528	H/V	food, oral
Leishmaniasis (<i>Leishmania donovani</i>)	protozoa	35,000	V	vector-borne disease
Trypanosomiasis (<i>Trypanosoma brucei rhodesiense</i>)	protozoa	35,000	H/V	vector-borne disease
Trichinellosis	helminths	56,800	V	food
<i>Echinococcus granulosus</i> and <i>E. multilocularis</i>	helminths	150,000	V	food
Old World Screwworm fly (<i>Chrysomya bezziana</i>)	Diptera	1500,000	V	wound infestation

ds: double stranded; ss: single-stranded; H: horizontal; V: vertical; s: segmented.

Table 1; supporting references

1. Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M. & Kawaoka, Y. Evolution and ecology of influenza A viruses. *Microbiol Rev* **56**, 152-179 (1992).
2. Spickler, A. R. Influenza. www.cfsph.iastate.edu/Factsheets/pdfs/influenza.pdf Last updated: August 2007 (2007).
3. Brown, I. H. The epidemiology and evolution of influenza viruses in pigs. *Vet Microbiol* **74**, 29-46 (2000).
4. Tumova, B. Equine influenza--a segment in influenza virus ecology. *Comp Immunol Microbiol Infect Dis* **3**, 45-59 (1980).
5. Daly, J. M., Newton, J. R. & Mumford, J. A. Current perspectives on control of equine influenza. *Vet Res* **35**, 411-423 (2004).
6. Crawford, P. C. *et al.* Transmission of equine influenza virus to dogs. *Science* **310**, 482-485 (2005).
7. Cohen, M. S., Hellmann, N., Levy, J. A., DeCock, K. & Lange, J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J Clin Invest* **118**, 1244-1254 (2008).
8. Cadogan, M. & Dalgleish, A. G. HIV immunopathogenesis and strategies for intervention. *Lancet Infect Dis* **8**, 675-684 (2008).
9. Sharp, P. M. Origins of human virus diversity. *Cell* **108**, 305-312 (2002).
10. Rezza, G. *et al.* Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* **370**, 1840-1846 (2007).
11. Chhabra, M., Mittal, V., Bhattacharya, D., Rana, U. & Lal, S. Chikungunya fever: a re-emerging viral infection. *Indian J Med Microbiol* **26**, 5-12 (2008).
12. Chevillon, C., Briant, L., Renaud, F. & Devaux, C. The Chikungunya threat: an ecological and evolutionary perspective. *Trends Microbiol* **16**, 80-88 (2008).
13. Tssetsarkin, K. A., Vanlandingham, D. L., McGee, C. E. & Higgs, S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. *PLoS Pathog* **3**, e201 (2007).
14. Santhosh, S. R. *et al.* Comparative full genome analysis revealed E1: A226V shift in 2007 Indian Chikungunya virus isolates. *Virus Res* **135**, 36-41 (2008).
15. Dubovi, E. J. & Njaa, B. L. Canine influenza. *Vet Clin North Am Small Anim Pract* **38**, 827-835 (2008).
16. Yoon, K. J. *et al.* Influenza virus infection in racing greyhounds. *Emerg Infect Dis* **11**, 1974-1976 (2005).
17. Weaver, S. C. & Barrett, A. D. Transmission cycles, host range, evolution and emergence of arboviral disease. *Nat Rev Microbiol* **2**, 789-801 (2004).
18. Walton, T. E., Holbrook, F. R., Bolivar-Raya, R., Ferrer-Romero, J. & Ortega, M. D. Venezuelan equine encephalomyelitis and African horse sickness. Current status and review. *Ann N Y Acad Sci* **653**, 217-227 (1992).
19. Weaver, S. C., Ferro, C., Barrera, R., Boshell, J. & Navarro, J. C. Venezuelan equine encephalitis. *Annu Rev Entomol* **49**, 141-174 (2004).
20. Eaton, B. T., Broder, C. C. & Wang, L. F. Hendra and Nipah viruses: pathogenesis and therapeutics. *Curr Mol Med* **5**, 805-816 (2005).
21. Groseth, A., Feldmann, H. & Strong, J. E. The ecology of Ebola virus. *Trends Microbiol* **15**, 408-416 (2007).
22. Hoenen, T., Groseth, A., Falzarano, D. & Feldmann, H. Ebola virus: unravelling pathogenesis to combat a deadly disease. *Trends Mol Med* **12**, 206-215 (2006).
23. Zampieri, C. A., Sullivan, N. J. & Nabel, G. J. Immunopathology of highly virulent pathogens: insights from Ebola virus. *Nat Immunol* **8**, 1159-1164 (2007).

24. Lahm, S. A., Kombila, M., Swanepoel, R. & Barnes, R. F. Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994-2003. *Trans R Soc Trop Med Hyg* **101**, 64-78 (2007).
25. Luby, S. P. *et al.* Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis* **12**, 1888-1894 (2006).
26. Person-to-person transmission of Nipah virus during outbreak in Faridpur District, 2004. *Health and Science Bulletin* **2**, 5-9 (2004).
27. Chen, J. & Subbarao, K. The Immunobiology of SARS. *Annu Rev Immunol* **25**, 443-472 (2007).
28. Feng, Y. & Gao, G. F. Towards our understanding of SARS-CoV, an emerging and devastating but quickly conquered virus. *Comp Immunol Microbiol Infect Dis* **30**, 309-327 (2007).
29. Parker, S., Nuara, A., Buller, R. M. & Schultz, D. A. Human monkeypox: an emerging zoonotic disease. *Future Microbiol* **2**, 17-34 (2007).
30. Chen, N. *et al.* Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. *Virology* **340**, 46-63 (2005).
31. Likos, A. M. *et al.* A tale of two clades: monkeypox viruses. *J Gen Virol* **86**, 2661-2672 (2005).
32. <http://www.gumboro.com>.
33. Muller, H., Islam, M. R. & Raue, R. Research on infectious bursal disease--the past, the present and the future. *Vet Microbiol* **97**, 153-165 (2003).
34. Saif, Y. M. Infectious bursal disease and hemorrhagic enteritis. *Poult Sci* **77**, 1186-1189 (1998).
35. Oladele, O. A., Adene, D. F., Obi, T. U. & Nottidge, H. O. Comparative susceptibility of chickens, turkeys and ducks to infectious bursal disease virus using immunohistochemistry. *Vet Res Commun* **33**, 111-121 (2009).
36. Jeon, W. J. *et al.* Very virulent infectious bursal disease virus isolated from wild birds in Korea: epidemiological implications. *Virus Res* **137**, 153-156 (2008).
37. Kobasa, D. & Kawaoka, Y. Emerging influenza viruses: past and present. *Curr Mol Med* **5**, 791-803 (2005).
38. Albina, E. Epidemiology of porcine reproductive and respiratory syndrome (PRRS): an overview. *Vet Microbiol* **55**, 309-316 (1997).
39. Mateu, E. & Diaz, I. The challenge of PRRS immunology. *Vet J* **177**, 345-351 (2008).
40. http://www.oie.int/wahis/public.php?page=disease_timelines.
41. Cho, J. G. & Dee, S. A. Porcine reproductive and respiratory syndrome virus. *Theriogenology* **66**, 655-662 (2006).
42. Blaha, T. The "colorful" epidemiology of PRRS. *Vet Res* **31**, 77-83 (2000).
43. <http://www.thepigsite.com/diseaseinfo/97/porcine-reproductive-respiratory-syndrome-prrs>.
44. Greger, M. The human/animal interface: emergence and resurgence of zoonotic infectious diseases. *Crit Rev Microbiol* **33**, 243-299 (2007).
45. *Data from: Summary of Notifiable diseases 2006, MMWR March 21, 2008, Volume 55, no 53, CDC and from:* <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5651md.htm> and <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5752md.htm>.
46. Yoon, J. W. & Hovde, C. J. All blood, no stool: enterohemorrhagic Escherichia coli O157:H7 infection. *J Vet Sci* **9**, 219-231 (2008).
47. Gyles, C. L. Shiga toxin-producing Escherichia coli: an overview. *J Anim Sci* **85**, E45-62 (2007).
48. Corriere, M. D. & Decker, C. F. MRSA: an evolving pathogen. *Dis Mon* **54**, 751-755 (2008).
49. Chavez, T. T. & Decker, C. F. Health care-associated MRSA versus community-associated MRSA. *Dis Mon* **54**, 763-768 (2008).
50. Grundmann, H., Aires-de-Sousa, M., Boyce, J. & Tiemersma, E. Emergence and resurgence of methicillin-resistant Staphylococcus aureus as a public-health threat. *Lancet* **368**, 874-885 (2006).
51. Navarro, M. B., Huttner, B. & Harbarth, S. Methicillin-resistant Staphylococcus aureus control in the 21st century: beyond the acute care hospital. *Curr Opin Infect Dis* **21**, 372-379 (2008).
52. *Data from DengueNet:* <http://www.who.int/globalatlas/dataQuery/default.asp>.
53. Holmes, E. C. & Twiddy, S. S. The origin, emergence and evolutionary genetics of dengue virus. *Infect Genet Evol* **3**, 19-28 (2003).
54. Cologna, R., Armstrong, P. M. & Rico-Hesse, R. Selection for virulent dengue viruses occurs in humans and mosquitoes. *J Virol* **79**, 853-859 (2005).
55. Twiddy, S. S. *et al.* Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. *Virology* **298**, 63-72 (2002).
56. Schwartz-Cornil, I. *et al.* Bluetongue virus: virology, pathogenesis and immunity. *Vet Res* **39**, 46 (2008).
57. Meiswinkel, R. *et al.* The 2006 outbreak of bluetongue in northern Europe--the entomological perspective. *Prev Vet Med* **87**, 55-63 (2008).

58. Rodeia, S. P., Deluyker, H., Pfeiffer, D. U. & Salman, M. D. The bluetongue outbreak in North-West Europe: the outcome from the epidemiological investigation coordinated by the European Food Safety Authorities (EFSA). *Prev Vet Med* **87**, 1-3 (2008).
59. Elbers, A. R., Backx, A., Ekker, H. M., van der Spek, A. N. & van Rijn, P. A. Performance of clinical signs to detect bluetongue virus serotype 8 outbreaks in cattle and sheep during the 2006-epidemic in The Netherlands. *Vet Microbiol* **129**, 156-162 (2008).
60. Maan, S. *et al.* Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. *Virology* **377**, 308-318 (2008).
61. Talbi, C. *et al.* Evolutionary history and dynamics of dog rabies virus in western and central Africa. *J Gen Virol* **90**, 783-791 (2009).
62. Nel, L. H. & Markotter, W. Lyssaviruses. *Crit Rev Microbiol* **33**, 301-324 (2007).
63. Biek, R., Henderson, J. C., Waller, L. A., Rupprecht, C. E. & Real, L. A. A high-resolution genetic signature of demographic and spatial expansion in epizootic rabies virus. *Proc Natl Acad Sci U S A* **104**, 7993-7998 (2007).
64. Capua, I. & Alexander, D. J. Human health implications of avian influenza viruses and paramyxoviruses. *Eur J Clin Microbiol Infect Dis* **23**, 1-6 (2004).
65. Seal, B. S., King, D. J. & Sellers, H. S. The avian response to Newcastle disease virus. *Dev Comp Immunol* **24**, 257-268 (2000).
66. Diallo, A. *et al.* The threat of peste des petits ruminants: progress in vaccine development for disease control. *Vaccine* **25**, 5591-5597 (2007).
67. Kwiatek, O. *et al.* Peste des petits ruminants (PPR) outbreak in Tajikistan. *J Comp Pathol* **136**, 111-119 (2007).
68. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008, OIE.* www.oie.int.
69. *Data US from:* <http://www.cdc.org> and <http://diseasemaps.usgs.gov>.
70. Blitvich, B. J. Transmission dynamics and changing epidemiology of West Nile virus. *Anim Health Res Rev* **9**, 71-86 (2008).
71. Hayes, E. B. & Gubler, D. J. West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. *Annu Rev Med* **57**, 181-194 (2006).
72. Beasley, D. W. Recent advances in the molecular biology of west nile virus. *Curr Mol Med* **5**, 835-850 (2005).
73. Demby, A. H. *et al.* Lassa fever in Guinea: II. Distribution and prevalence of Lassa virus infection in small mammals. *Vector Borne Zoonotic Dis* **1**, 283-297 (2001).
74. <http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm>.
75. <http://www.who.int/mediacentre/factsheets/fs179/en/print.html>.
76. Khan, S. H. *et al.* New opportunities for field research on the pathogenesis and treatment of Lassa fever. *Antiviral Res* **78**, 103-115 (2008).
77. *Data US from Summary of Notifiable diseases 2006, MMWR March 21, 2008, Volume 55, no 53, CDC.*
78. Foley, S. L., Lynne, A. M. & Nayak, R. Salmonella challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *J Anim Sci* **86**, E149-162 (2008).
79. Boyen, F. *et al.* Non-typhoidal Salmonella infections in pigs: a closer look at epidemiology, pathogenesis and control. *Vet Microbiol* **130**, 1-19 (2008).
80. Tulman, E. R., Delhon, G. A., Ku, B. K. & Rock, D. L. African swine fever virus. *Curr Top Microbiol Immunol* **328**, 43-87 (2009).
81. Tulman, E. R. & Rock, D. L. Novel virulence and host range genes of African swine fever virus. *Curr Opin Microbiol* **4**, 456-461 (2001).
82. http://www.vet.uga.edu/vpp/gray_book02/fad/asf.php.
83. Babiuk, S., Bowden, T. R., Boyle, D. B., Wallace, D. B. & Kitching, R. P. Capripoxviruses: an emerging worldwide threat to sheep, goats and cattle. *Transbound Emerg Dis* **55**, 263-272 (2008).
84. Heyman, P. & Vaheri, A. Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006. *Euro Surveill* **13** (2008).
85. Khaiboullina, S. F., Morzunov, S. P. & St Jeor, S. C. Hantaviruses: molecular biology, evolution and pathogenesis. *Curr Mol Med* **5**, 773-790 (2005).
86. Klein, S. L. & Calisher, C. H. Emergence and persistence of hantaviruses. *Curr Top Microbiol Immunol* **315**, 217-252 (2007).
87. Vijayachari, P., Sugunan, A. P. & Shriram, A. N. Leptospirosis: an emerging global public health problem. *J Biosci* **33**, 557-569 (2008).
88. *Data from* http://www.cdc.gov/ncidod/dvbid/lyme/ld_statistics.htm.
89. Kurtenbach, K. *et al.* Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol* **4**, 660-669 (2006).

90. Simarro, P. P., Jannin, J. & Cattand, P. Eliminating human African trypanosomiasis: where do we stand and what comes next? *PLoS Med* **5**, e55 (2008).
91. Mackenzie, J. S. Emerging zoonotic encephalitis viruses: lessons from Southeast Asia and Oceania. *J Neurovirol* **11**, 434-440 (2005).
92. Diagana, M., Preux, P. M. & Dumas, M. Japanese encephalitis revisited. *J Neurol Sci* **262**, 165-170 (2007).
93. Mellor, P. S. & Hamblin, C. African horse sickness. *Vet Res* **35**, 445-466 (2004).
94. Bhanuprakash, V., Indrani, B. K., Hosamani, M. & Singh, R. K. The current status of sheep pox disease. *Comp Immunol Microbiol Infect Dis* **29**, 27-60 (2006).
95. Ergonul, O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis* **6**, 203-214 (2006).
96. Flick, R. & Whitehouse, C. A. Crimean-Congo hemorrhagic fever virus. *Curr Mol Med* **5**, 753-760 (2005).
97. Annual OIE/FAO FMD Reference Laboratory Network Report. January – December 2007. http://www.wrlfmd.org/ref_labs/fmd_ref_lab_reports.htm.
98. Knowles, N. J. & Samuel, A. R. Molecular epidemiology of foot-and-mouth disease virus. *Virus Res* **91**, 65-80 (2003).
99. Sellers, R. & Gloster, J. Foot-and-mouth disease: a review of intranasal infection of cattle, sheep and pigs. *Vet J* **177**, 159-168 (2008).
100. Lopman, B. *et al.* Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet* **363**, 682-688 (2004).
101. Said, M. A., Perl, T. M. & Sears, C. L. Healthcare epidemiology: gastrointestinal flu: norovirus in health care and long-term care facilities. *Clin Infect Dis* **47**, 1202-1208 (2008).
102. Bull, R. A., Tu, E. T., McIver, C. J., Rawlinson, W. D. & White, P. A. Emergence of a new norovirus genotype II.4 variant associated with global outbreaks of gastroenteritis. *J Clin Microbiol* **44**, 327-333 (2006).
103. Widdowson, M. A. *et al.* Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus--United States, 2002. *J Infect Dis* **190**, 27-36 (2004).
104. Nayak, M. K. *et al.* A new variant of Norovirus GII.4/2007 and inter-genotype recombinant strains of NVGII causing acute watery diarrhoea among children in Kolkata, India. *J Clin Virol* **45**, 223-229 (2009).
105. Patel, M. M., Hall, A. J., Vinje, J. & Parashar, U. D. Noroviruses: a comprehensive review. *J Clin Virol* **44**, 1-8 (2009).
106. Soldan, S. S. & Gonzalez-Scarano, F. Emerging infectious diseases: the Bunyaviridae. *J Neurovirol* **11**, 412-423 (2005).
107. Randolph, S. E. Tick-borne encephalitis virus, ticks and humans: short-term and long-term dynamics. *Curr Opin Infect Dis* **21**, 462-467 (2008).
108. Suss, J. Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. *Euro Surveill* **13** (2008).
109. Kostoff, R. N., Morse, S. A. & Oncu, S. The seminal literature of anthrax research. *Crit Rev Microbiol* **33**, 171-181 (2007).
110. Passalacqua, K. D. & Bergman, N. H. Bacillus anthracis: interactions with the host and establishment of inhalational anthrax. *Future Microbiol* **1**, 397-415 (2006).
111. Cutler, S. J., Whatmore, A. M. & Commander, N. J. Brucellosis--new aspects of an old disease. *J Appl Microbiol* **98**, 1270-1281 (2005).
112. Godfroid, J. *et al.* From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Vet Res* **36**, 313-326 (2005).
113. Guerra, H. The brucellae and their success as pathogens. *Crit Rev Microbiol* **33**, 325-331 (2007).
114. Dujardin, J. C. *et al.* Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. *Emerg Infect Dis* **14**, 1013-1018 (2008).
115. Schriefer, A., Wilson, M. E. & Carvalho, E. M. Recent developments leading toward a paradigm switch in the diagnostic and therapeutic approach to human leishmaniasis. *Curr Opin Infect Dis* **21**, 483-488 (2008).
116. Eckert, J., Conraths, F. J. & Tackmann, K. Echinococcosis: an emerging or re-emerging zoonosis? *Int J Parasitol* **30**, 1283-1294 (2000).
117. Garcia, H. H., Moro, P. L. & Schantz, P. M. Zoonotic helminth infections of humans: echinococcosis, cysticercosis and fascioliasis. *Curr Opin Infect Dis* **20**, 489-494 (2007).
118. Gottstein, B., Pozio, E. & Nockler, K. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clin Microbiol Rev* **22**, 127-145 (2009).
119. Siddig, A. *et al.* Seasonality of Old World screwworm myiasis in the Mesopotamia valley in Iraq. *Med Vet Entomol* **19**, 140-150 (2005).

DETERMINATION OF THE PREVALENCE AND IDENTIFICATION OF RISK FACTORS FOR *SALMONELLA* INFECTIONS IN LAYING HENS HOUSED IN CONVENTIONAL AND ALTERNATIVE SYSTEMS

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1. Introduction

The 1st of January 2012 it will be forbidden in the EU to house laying hens in conventional battery cages. From that day onwards the housing will be restricted to enriched cages and non-cage systems such as aviary systems, floor-raised and free-range systems. The shift to these alternative systems was inspired by animal welfare. If this ban of battery cages will also have an impact on the prevalence of several pathogenic and zoonotic agents is not yet thoroughly documented. In the case of *Salmonella*, the EU called for a specific targeted research project: the Safehouse project. It is within the framework of this project that the underneath described study has been carried out.

The aim of this cross-sectional field study is to determine the prevalence and risk factors for *Salmonella* infections in commercial laying hens housed in conventional and alternative housing systems in 4 EU member states.

2. Material and methods

Selection of the farms

Farms were selected from the national Identification & Registration databases. Only farms with more than 1000 laying hens, being in the last month of the production cycle were selected. The composition of the subset of sampled farms that was aimed at was ¼ conventional battery cages and ¾ non-cage systems. Participation was voluntary. In total 192 laying hen farms were sampled (59 conventional battery cage flocks, 58 floor-raised flocks, 53 free-range flocks and 22 organic flocks) in Belgium, Germany, Greece and Italy. Only one flock per farm was sampled.

Sample type and bacteriological analysis

The following samples were collected: 5 pooled faeces samples, 1 dust sample and cloacal swabs of 40 randomly selected hens. All samples were analyzed using ISO 6579:2002_Amd1:2007, as recommended by the Community Reference Laboratory for *Salmonella* in Bilthoven, The Netherlands.

Questionnaire design and data analysis

A questionnaire was filled in during an on-farm interview. Questions related to general farm and flock characteristics and biosecurity measures. The potential relationship between risk factors and *Salmonella* status of the sampled farm was evaluated by means of multivariate

logistic regression model with the *Salmonella* status of the sampled flock as a binary outcome variable. All 2-way interactions between significant main effects were tested.

3. Results

Salmonella Enteritidis and/or Typhimurium could be detected in 22 out of the 192 laying hen flocks. Fourteen flocks were found positive only in the pooled faeces, 6 were positive both in the pooled faeces and the cloacal swabs and 2 were only positive in the cloacal swabs. The within flock prevalence based on the cloacal swabs never exceeded 9.70%.

In the final model the absence of dry cleaning in between production cycles ($P < 0.01$), sampling in the wintertime ($P = 0.01$), the housing in conventional battery cages ($P = 0.01$) and the absence of vaccination against *Salmonella* ($P = 0.04$) turned out to be risk factors for a *Salmonella*-infection (Table 1).

4. Discussion

Compared with the results of the EFSA baseline study of 2005 (EFSA, 2007), the prevalence of *Salmonella* Enteritidis in Belgium and Greece was lower in this study. However, it should be kept in mind that both the sampling method and the distribution of sampled housing types in this study are different than that of the EFSA study.

The estimates of the within flock prevalence based on the cloacal swabs were usually relatively low indicating that in general only a small percentage of birds in the positive flocks were shedding the bacterium. It needs to be stressed that this is not necessarily an accurate indication of the number of birds infected with *Salmonella*.

The housing in conventional battery cages turned out to be a significant risk factor, which is in accordance with the results of the baseline study, both at the EU level (EFSA, 2007) and at the level of the individual member states (Methner et al., 2006; Namata et al., 2008; Huneau-Salaün et al., 2009). This is most likely due to a combination of factors such as the bigger flock size in battery cage systems and the higher age of the infrastructure than in non-cage systems. This effect of age of the infrastructure can be explained by the fact that the older the infrastructure, the more difficult it gets to achieve sufficient standards of cleaning. The importance of cleaning is also demonstrated by the observation that the absence of dry cleaning in between production rounds turned out to be a significant risk factor. The seasonal effect could be explained by the fact that in outdoor systems the hens are kept inside due to cold and wet weather conditions or by the lower air quality in wintertime. Vaccination against *Salmonella* could be identified as a protective factor. However, it should be kept in mind that *Salmonella* can still be found in the intestines of a fairly large proportion of vaccinated hens (Davies and Breslin, 2004) which implies the risk of a renewed shedding of the bacterium.

The results of this study illustrate that the prevalence of *Salmonella* Enteritidis in European laying hens is still substantial. Despite the fact that in alternative housing systems the chance of oro-faecal transmission of *Salmonella* is much higher than in conventional battery cages, no higher prevalence of *Salmonella* could be observed in flocks housed in these alternative systems.

5. Acknowledgments

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Table 1. Results of the univariable and multivariate analysis for the identification of risk factors for *Salmonella* Enteritidis or Typhimurium infection on 192 European laying hen farms.

Categorical variable	n	Univariate analysis		Multivariable analysis		
		OR	P-value			
Dry cleaning						
No	44	11.24	< 0.01	2.68	1.21-13.94	< 0.01
Yes (ref)	148	-	-	-	-	-
Vaccination status against <i>Salmonella</i>						
No	111	5.37	< 0.01	5.83	1.13-30.04	0.04
Yes (ref)	81	-	-	-	-	-
Type of housing			0.01			0.01
Conventional battery (ref)	59	-	-	-	-	-
Floor-raised	58	0.18	0.01	0.06	0.01-0.34	< 0.01
Free-range	53	0.26	0.03	0.24	0.06-1.02	0.05
Organic	22	0.15	0.08	0.20	0.02-2.12	0.18
Season of sampling			0.02			0.01
Winter (ref)	34	-	-	-	-	-
Spring	59	0.26	0.03	0.01	0.00-0.56	< 0.01
Summer	52	0.11	0.01	0.03	0.00-0.27	< 0.01
Autumn	47	0.41	0.12	0.38	0.09-1.65	0.20

6. References

- Davies R. and Breslin M., 2004. Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella enterica* serovar Enteritidis had been used. Av. Path. 33(2), 133-144
- EFSA, 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*. The EFSA Journal 97, 84 pp
- Huneau-Salatin A., Chemaly M., Le Bouquin S., Lalande F., Petetin I., Rouxel S., Michel V., Fravallo P. and Rose N., 2009. Risk factors for *Salmonella enterica* subsp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period. Prev. Vet. Med. 89(1-2), 51-58
- Methner U., Diller R., Reiche R. and Böhland K., 2006. Occurrence of *Salmonellae* in laying hens in different housing systems and conclusion for the control. Münch. Tierarz. Wochenschr. 119, 467-473
- Namata H., E. Méroc, M. Aerts, C. Faes, J. Cortinas Abrahantes, H. Imberechts and K. Mintiens, 2008. *Salmonella* in Belgian laying hens: an identification of risk factors. Prev. Vet. Med. 83, 323-336

EPIDEMIOLOGY AND POLICY: THE PAST AND THE FUTURE

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1) Introduction and history of Veterinary Epidemiology and Policy in Belgium

Veterinary epidemiology is rather young discipline in veterinary science. The first textbooks on veterinary epidemiology were published in the late 1970's. Although the term epidemiology was already used in many courses, the discipline was formally introduced in the veterinary curriculum in Belgium by the creation of the Chair in Veterinary Epidemiology at Ghent University in 1992, held by Prof. Hubert Deluyker. Prof. Deluyker's work primarily focused on mastitis in dairy herds but the Belgian outbreaks of classical swine fever in 1990 and 1993-94 provided new opportunities for the application of epidemiological methods to support policy makers in controlling animal disease epidemics. Throughout the years, the Veterinary Epidemiology unit of Ghent University has extended its critical mass through participation in national and international research projects on the control of emerging animal diseases and anti-microbial resistance (Laevens et al. 1998a, 1998b, 1999; Mintiens et al. 2001, 2005; Dewulf et al. 2000a; 2000b, 2001a, 2001b, 2001c, 2002; 2004; 2005).

On the other hand, the Veterinary Services of the Belgian Ministry of Agriculture have been involved in official animal disease control programmes for many decades. Eradication programmes for endemic diseases as brucellosis, leucosis, and tuberculosis have been implemented throughout the 20th century. Towards the end of that century it became obvious that complete eradication of these diseases was not straightforward and needed additional efforts. One of the reasons for this failure was found in the insufficient quality and standardization of the diagnostic tools that were used in these programmes. In addition, the Veterinary Services found in the 1990's new challenges in the control of economically important diseases as Aujeszky's disease in swine and IBR, BVD, ParaTb in cattle. In 1996, the Coordination Centre for Veterinary Diagnostics (CCVD) was founded at the Veterinary and Agrochemical Research. The primary role of the CCVD was to support the Veterinary Services in improving and evaluating the official disease control programmes by coordinating the standardisation and quality enhancement of the diagnostic tools that were used (quality assurance of diagnostic assays). It is of utmost importance that a correct judgment about the precision and accuracy can be done of the diagnostic assays used in control programmes. Boelaert and colleagues designed large-scale surveys (cross-sectional studies) in Belgium for three pathogens of the former B list of the OIE's International Animal Health Code: pseudorabies (Aujeszky's disease), bovine herpes virus 1 (BoHV-1, infectious bovine rhinotracheitis virus) and *Mycobacterium avium* subsp. *Paratuberculosis* (Map) (Boelaert *et al.* 1999, 2000a, 2000b) and *Salmonella* in pigs (Van Vlaanderen and Biront *et al.*, 2000, Laevens *et al.*, 2003). These surveys were designed to estimate the herd (animal) seroprevalences in Belgium and to assess relevant risk factors. The aim of these studies was to provide guidance to the eradication programmes and was repeated, if possible, for many years (Figure 1 is an example: Aujeszky disease). Due to these surveys, information about the analysis and interpretation of surveys regarding became available and helped the debate about the accuracy of the information of these surveys provided and their design. Important policy issues and questions as 'Do these surveys provide animal health managers with adequate and accurate information to argue substantial animal health trade-related decisions' became more and more important. The premise of this paper is that veterinary epidemiology and animal

health policy go hand in hand and that epidemiology can contribute to better policy making and ultimately better animal (population) health.

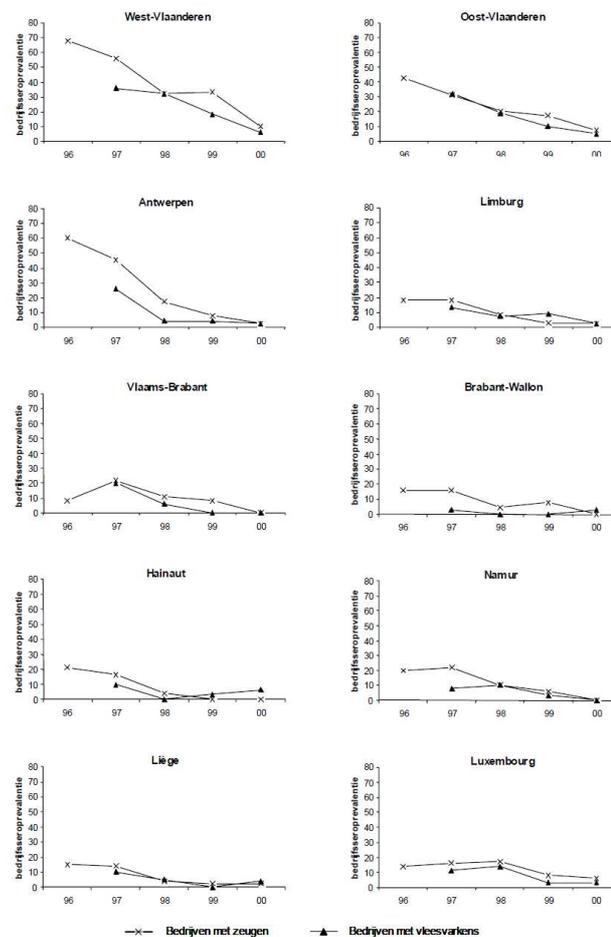


Figure 1: Herd seroprevalence of Aujeszky disease from 1996 until 2000.

2) The role of policy in Veterinary Epidemiology in Belgium

Nowadays, the Federal Public Services (Health, Food Chain Safety and Environment) and the Federal Agency for Safety of the Food Chain (FASFC) are responsible for the legislation, the implementation and the evaluation of animal diseases control in Belgium. Since 1993, the European market enlarged and trade in animals and products of animal origin between different European Union countries has grown and become liberal. This globalization of trade of animals and animal products exerts a strong pressure on animal disease management. This sets out the basic rules for food safety, and animal health standards. In order to limit the health risks inherent to this trade to acceptable levels, the policy and the regulations fixed by national and international authorities (World Trade Organisation Sanitary and Phytosanitary Agreement (SPS Agreement)) must be respected. Animal Health policy should be formulated based on values, ideology, political pressures and evidence. In many languages, the words “politics” and “policy” have the same meaning. Although many of us believe that policy should be largely or entirely evidence-based, there is widespread agreement that evidence (at least, scientific evidence) plays a relatively minor role in policy making. Epidemiology contributes to the evidence. An epidemiological key notion is contained in the SPS agreement is risk analysis. Risk analysis is the cornerstone of which is risk assessment that generates data by comprehensive surveillance systems with a solid epidemiological design (Zepeda et al., 2001). Therefore, precise and up-to-date epidemiological knowledge and information about the status of the major diseases is of utmost importance. In addition, the implementation

of restrictions on trade is only allowed based on epidemiological reliable data (evidence based surveillance data). Epidemiological surveillance is essential to protect animals against new (exotic) diseases as well as the implementation and evaluation of disease control programmes and enables the collection of data on zoonoses proving its value to protecting public health.

3) How veterinary epidemiology can help produce better health policy?

Veterinary epidemiology can contribute to each stage of the ‘policy cycle’ and includes 5 steps: i) assessment of animal population health (surveys, observational studies (case-control, cohort, cross sectional) , transmission studies, etc) ii) assessment of potential interventions to improve animal health (directly, models, etc) iii) policy choices iv) policy implementation and iv) policy evaluation.

The first step is to describe the target population and understand its demographic trends (mapping and using the Sanitel/Sanitrace databases). Descriptive epidemiology can then measure the health of this population, identify trends and patterns in health, and assess the population’s health risks and health needs. This will help to identify risk factors, health problems and population groups that might be priority targets for policy development. For some diseases it is particularly important to identify and quantify inequalities in risk and/or animal health (target surveillance). Analytical epidemiology can determine the causes of health problems, identifying both individual-level and population-level factors. Secondly, epidemiological research and models from risk assessment can identify potential policy interventions, synthesize existing knowledge regarding their effectiveness, contribute relevant new research, and assess the potential of each approach. Clinical epidemiologists have become very good at synthesizing existing knowledge in their development of systematic reviews and meta-analysis. A meta-analysis has been defined as: “the statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings (Glass, 1976). This process is however not so easy (lack of randomization) for the population-level interventions that are most often used in policy making. The application of meta-analysis to observational studies is very relevant to policy-oriented epidemiology. Outputs of stochastic and statistical models can inform (examples are strategies to reduce the risk for human salmonellosis within the pork production chain (Bollaerts et al., 2008, figure 2) and the spread of Blue Tongue Virus serotype 8 via the wind (Hendrickx et al., 2008)) can inform decision makers by providing projections of the impact of potential interventions on the health of a specific population and/or the spread of a specific disease.

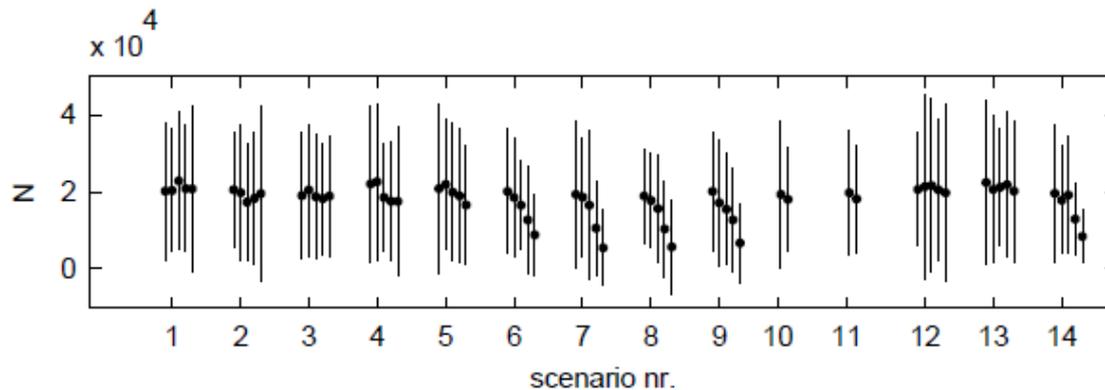


Figure 2: Scenario-analysis (what if) showing a graphical summary of the ‘Metzoon’ model (Bollaerts et al., 2008) representing the average number of annual cases ($\pm 2 \times$ standard error) of human salmonellosis through consumption of fresh minced pork according to the QMRA-Metzoon model (Bollaerts et al., 2008).

Computer simulations of health and disease are very useful here, given their ability to superimpose the epidemiologic processes upon the underlying population dynamics, dealing with far more variables than we can manage, and considering both beneficial and adverse effects. In addition, they can provide answers to “What if” questions like “What would be the effect of a compulsory versus voluntary vaccination against Blue tongue Virus?”. The interventions can be compared for their impact and provide the basis for the economic analyses that will help policy-makers choose among them. Epidemiology can also assist the process of consensus development for selection of a particular policy, using priority-setting techniques borrowed from the social sciences. In addition, epidemiology can help to set targets for the chosen policies, ensuring that the targets are realistic and internally consistent (disease models are again relevant here) and can also inform needs-based resource allocation for animal health services and guide the development of information systems (Vetgeotools, GIS applications, MOSS website). Epidemiology can assess the impacts of policies and can use surveillance methods to monitor the future health, which starts the cycle again.

4) Should epidemiology have a larger influence on veterinary health policy?

Policy decisions are too often made more on the basis of political ideology, cost savings, pressure from interest groups and media attention than research evidence. Epidemiology is as much affected by this problem as any other scientific discipline. Many epidemiologists have preferred to confine their role to “the science”, avoiding the grime of policy-making. There is no doubt that policy-oriented epidemiology is distinctly practical (pragmatic) in nature and can sometimes appear to lack of rigour. However, imperfect estimates that have the best available empirical basis are usually better than wild guesses. On the other hand, policy-makers tend to come from very different backgrounds from those of epidemiologists which make communication sometimes difficult. Policy-makers want “the answer” and not a range of possibilities presented with a bunch of qualifications—and they want it immediately, while epidemiologists are trained to be sceptical (emphasizing possible sources of error rather than providing the unqualified advice that policy-makers want) and cautious (which tends to mean slow).

Epidemiology can play a bigger role in policy-making if it is evidence-based policy, specifically policy based on epidemiologic evidence. As a discipline, we must *broaden our expertise*, to include a greater knowledge of policy and its formation on the one hand, and of appropriate epidemiologic methods and tools on the other. The latter include a rehabilitation

of descriptive epidemiology, better use of animal health data (including administrative data !), emphasis on the population dynamics and disease dynamics, social determinants (farmers behavior) of animal health (since these are what government policies can try to influence directly) and disease modelling. We need to import several techniques from the social sciences, including geographical information systems and multilevel modelling. Demography is particularly important: since policy is implemented in real populations, the underlying population in the denominator is as important to population health as the epidemiologic events in the numerator. Borrowing from economics is already well underway, by way of economic analyses and methods for determining the utilities of various animal health states.

CONCLUSION

There are a lot of challenges, but we believe that policy-relevant epidemiology and decision analysis models are important tools for efficient risk management and to assist decision makers to choose the right strategy to control and/or evaluate (re)emerging animal diseases. Greater emphasis on policy-relevant topics will allow veterinary epidemiology and surveillance systems to make an even greater contribution to the general animal health. And besides that, it's fun!

REFERENCES

- Boelaert, F., P. Biront, B. Soumare, M. Dispas, E. Vanopdenbosch, J. P. Vermeersch, A. Raskin, J. Dufey, D. Berkvens, And P. Kerkhofs (2000a). "Prevalence Of Bovine Herpesvirus-1 In The Belgian Cattle Population." *Prev.Vet.Med.* 45(3-4): 285-295.
- Boelaert, F., H. Deluyker, D. Maes, J. Godfroid, A. Raskin, H. Varewijck, M. Pensaert, H. Nauwynck, F. Castryck, C. Miry, J. M. Robijns, B. Hoet, E. Segers, V. Van, I, A. Robert, And F. Koenen (1999). "Prevalence Of Herds With Young Sows Seropositive To Pseudorabies (Aujeszky's Disease) In Northern Belgium." *Prev.Vet.Med.* 41(4): 239-255.
- Boelaert, F., K. Walravens, P. Biront, J. P. Vermeersch, D. Berkvens, And J. Godfroid (2000b). "Prevalence Of Paratuberculosis (Johne's Disease) In The Belgian Cattle Population." *Vet.Microbiol.* 77(3-4): 269-281.
- Bollaerts, K.E., Messens, W., Delhalle, L., Aerts, M., Van Der Stede, Y., Dewulf, J., Quoilin, S., Maes, D., Mintiens, K., Grijspeerdt, K. 2009. Development Of A Quantitative Microbial Risk Assessment For Human Salmonellosis Through Household Consumption Of Fresh Minced Pork Meat In Belgium. *Risk Anal.* 29(6):820-40.
- Dewulf, J., F. Koenen, S. Ribbens, A. Haegeman, H. Laevens, and A. De Kruif (2005). "Evaluation of the epidemiological importance of classical swine fever infected, E2 sub-unit marker vaccinated animals with RT-nPCR positive blood samples." *J Vet Med B Infect Dis Vet Public Health* 52(9): 367-71.
- Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2000a). "Airborne transmission of classical swine fever virus under experimental conditions." *The Veterinary Record* 147: 735-738.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2001a). "An E2 sub-unit marker vaccine does not prevent horizontal or vertical transmission of classical swine fever virus." *Vaccine* 20(1-2): 86-91.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2001b). "Evaluation of the potential of dogs, cats and rats to spread classical swine fever virus." *Vet.Rec.* 149(7): 212-213.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2001c). "An experimental infection with classical swine fever virus in pregnant sows: transmission of the virus, course of the disease, antibody response and effect on gestation." *Journal of Veterinary Medicine Series B* 48(8): 583-591.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2002). "An experimental infection to investigate the indirect transmission of classical swine fever virus by excretions of infected pigs." *Journal of Veterinary Medicine Series B* 49(9): 452-456.

Dewulf, J., H. Laevens, F. Koenen, K. Mintiens, and A. de Kruif (2004). "Efficacy of E2-sub-unit marker and C-strain vaccines in reducing horizontal transmission of classical swine fever virus in weaner pigs." *Prev Vet Med* 65(3-4): 121-33.

Dewulf, J., H. Laevens, F. Koenen, H. Vanderhallen, K. Mintiens, H. Deluyker, and A. de Kruif (2000b). "An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs [In Process Citation]." *Vaccine* 19(4-5): 475-482.

Hendrickx G, Gilbert M, Staubach C, Elbers A, Mintiens K, Gerbier G, Ducheyne E. 2008. A Wind Density Model To Quantify The Airborne Spread Of Culicoides Species During North-Western Europe Bluetongue Epidemic, 2006. *Prev Vet Med.* 87(1-2):162-81. Epub 2008 Jul 17.

Laevens, H., H. Deluyker, F. Koenen, G. Van Caenegem, J. P. Vermeersch, and A. de Kruif (1998a). "An experimental infection with a classical swine fever virus in weaner pigs. II. The use of serological data to estimate the day of virus introduction in natural outbreaks." *Vet.Quart.* 20(2): 46-49.

Laevens, H., F. Koenen, H. Deluyker, and D. Berkvens (1998b). "An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response." *Vet.Quart.* 20(2): 41-45.

Laevens, H., F. Koenen, H. Deluyker, and A. de Kruif (1999). "Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response." *The Veterinary Record* 145(9): 243-248.

Laevens, H., Mintiens, K. 2002. Een Bewakingsprogramma Ter Reductie Van De Salmonella Prevalentie In Belgische Varkensbedrijven. Rapport Ccdd Voor Favv:2002:1-9.

Mintiens, K., H. Deluyker, H. Laevens, F. Koenen, J. Dewulf, And A. De Kruif (2001). "Descriptive Epidemiology Of A Classical Swine Fever Outbreak In The Limburg Province Of Belgium In 1997." *J.Vet.Med.B* 48(2): 143-149.

Mintiens K, Verloo D, Venot E, Laevens H, Dufey J, Dewulf J, Boelaert F, Kerkhofs P, Koenen F. 2005. Estimating The Probability Of Freedom Of Classical Swine Fever Virus Of The East-Belgium Wild-Boar Population. *Prev Vet Med.* 70(3-4):211-22.

Catry, B., Dewulf, J., De Kruif, A., Vanrobaeys, M., Haesebrouck, F., Decostere, A. 2007. Accuracy Of Susceptibility Testing Of *Pasteurella Multocida* And *Mannheimia Haemolytica*. *Microb Drug Resist.*13(3):204-11.

Persoons, D., Van Hoorebeke, S., Hermans, K., Butaye, P., De Kruif, A., Haesebrouck, F., Dewulf, J. 2009. Methicillin-Resistant *Staphylococcus Aureus* In Poultry. *Emerg Infect Dis.*15(3):452-3.

Ribbens, S., Dewulf, J., Koenen, F., Maes, D., De Kruif, A. 2007. Evidence Of Indirect Transmission Of Classical Swine Fever Virus Through Contacts With People. *Vet Rec.* 160(20):687-90.

Zepeda, C., Salman, M., Ruppner, R., 2001. International Trade, Animal Health And Veterinary Epidemiology: Challenges And Opportunities. *Prev. Vet. Med.* 48:261-271.

RISK APPROACH MODELS TO ESTIMATE THE SENSITIVITY FOR DIFFERENT SURVEILLANCE COMPONENTS: BLUETONGUE IN BELGIUM A CASE STUDY

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Introduction

Bluetongue (BT) is an arthropod-borne viral disease of both wild and domestic ruminants. The distribution of the virus is dependant on environmental and climatic conditions which allows the vector to accomplish its transmission cycle. In August 2006, from the original focus in the area where Belgium, the Netherlands and Germany share borders, an epidemic of Bluetongue Virus (BTV) serotype 8 gradually disseminated throughout the North-Western European countries, causing the most severe outbreak of this disease ever recorded (Méroc et al., 2008). The EU Legislation 1266/2007 modified by 1108/2008 prescribes the implementation of passive clinical surveillance and sentinel surveillance and a combination of serological and/or virological surveillance, as well as a targeted risk based monitoring. Each country is recommended to adapt its surveillance system in order to meet the objectives and prove the efficacy of its system.

The study has been done in this context for Belgium and three major components characterize the Belgian BTV surveillance, monthly sentinel (sero) surveillance, yearly cross sectional serological survey ('winter screening'), clinical passive surveillance. The aim of this study was to evaluate these three major components of BTV surveillance in Belgium.

Material and Methods

The scenario trees as illustrated by Martin et al. (2007) were used to conduct this study. A scenario tree for each surveillance component was designed in different Excel spread sheets. All factors interfering with the probability of infection or detection were taken in account. In this study it was assumed the components were all independent.

The first node identified was an infection node "Country status" to which the design prevalence (DP) was attributed.

The following nodes were the category nodes "Zone", "Vector activity", and "Specie", the major factors retained in the tree influencing the risk of infection. Relative risks (RR) and respective population proportions (PPr) as well as sampled population proportion (SPr) were attributed to each of these category nodes.

The infection node "Herd status" for each combination of category nodes "Zone" and "Vector activity" above was obtained. The parameters RR and PPr entered above enabled the calculation of the adjusted risk of infection (AR) for each herd type combination, which in turn would provide the herd effective risk of infection (HEPI). The same was done within each herd taking in account the "Specie" category node, which provided an "Animal status" node with its respective animal effective risk of infection (AEPI).

At the end of each limb of the tree, effective probability of detection (EPD) for each limb of the tree were calculated, with the multiplication of the SPr in each risk group and the effective probabilities of infection obtained above as well as the herd and animal sensitivities (HSe and ASe respectively). The animal sensitivity and the herd sensitivity took in account the sampling probability as well as the expected prevalence and the diagnostic test properties. These were computed in EpiTools (AusVet©). A range of different expected prevalence, and sample sizes were simulated to identify the minimum, most likely and maximum HSe and ASe. The diagnostic test characteristics were based on the competitive Elisa test from Vandenbussche, et al. (2008).

The computation of each probability of detection for each risk group provided a unit sensitivity (USE), which is the probability of detecting the disease given the country is infected by randomly sampling one unit in the whole population, and a component sensitivity (GSe) which is the probability of detecting the disease given the country is infected by sampling all units sampled in that surveillance component.

The DP's were obtained from the regulation E.C.1266/2007. The SPr's and PPr's were obtained from national databases. The RR's were obtained following empirical statistical methods (Faes, 2009), through literature review, as well as expert opinion. In order to account for the uncertainty and variability of the different parameters, appropriate distributions were fitted.

The monthly surveillance data/component enabled as well the computation of the posterior probability of freedom (PFree) for that given month as well as the probability of infection (PInf) for that given month, taking in account the probability of introduction (PIntro). The latter was set to 0 from January till March, the vector free period, and to 0.5 from April to December onwards the vector activity period (in accordance to the Belgian definition of vector activity period).

Results

Table I: Results obtained for the unit sensitivity, per component after a full year of surveillance. The mean (Mean USE), the minimum (Min USE), maximum (Max USE) and standard deviation (Std Dev) are shown.

Component	Mean USE	Max USE	Min USE	Std Dev
Winterscreening	0,000273	0,000407	0,000195	0,0000361
Sentinel	0,000387	0,000557	0,000206	0,0000506
Outbreak	0,000336	0,000338	0,000129	0,0000431

Discussion

The scenario trees as illustrated by Martin et al. (2007), used to conduct this study, have proven to be very useful tools in evaluating disease surveillance programs, as all ready seen in the passed (Hadorn et al., 2009; Martin et al., 2007; Welby et al., submitted).

Out of this study, it appears that the passive clinical surveillance provides good estimation of the current disease status in the country. Nevertheless its minimum value can be very low in comparison to the other components. Thus this might underline that the efficiency of this surveillance component is strongly dependant on the level of disease awareness.

The simulations done per month could enable policy makers to have a clear insight on the uncertainty around this probability freedom for each month. Interestingly we observe that the sentinel surveillance though seemingly provides less evidence towards freedom probability, it is the first component to detect the disease. Here again, this illustrates the importance of the level of disease awareness, as proven in other countries too (Elbers et al., 2009; Hadorn et al., 2009).

It is evident that the output of this study is strongly dependant on the assumptions, fitting distributions around most of the parameters taking in account the uncertainty and variability around them, allowed to have a good insight on the different surveillance systems running. The simulations done in Epitools (AusVet©) taking in account the possible ranges of herd and

animal sensitivity depending on the disease awareness, expected prevalence, and sampling probability enabled a more appropriate representation of the uncertainty, and variability. But having empirical data on those parameters would of course bring added value, as all these current simulations were based on assumptions. The input parameters could be improved as well regarding the different relative risks.

Similar simulations would have to be run regarding an early detection system. Also it would be interesting to run a cost benefit analysis as this is a key element guiding decision makers in their choices for the design of a surveillance system in many countries.

As a main conclusion, this study has enabled to underline important elements to quantify the sensitivity of whole surveillance system taking in account all the components as well as risk factors, sampling probability, and expected prevalence, which is a useful tool to meet the international standards when implementing disease surveillance in a country.

References

- Elbers A.R., van der Spek A.N., van Rijn P.A., 2009, Epidemiologic characteristics of bluetongue virus serotype 8 laboratory-confirmed outbreaks in The Netherlands in 2007 and a comparison with the situation in 2006. *Prev Vet Med* 92, 1-8.
- Faes C., 2009, Spatial risk analysis for bluetongue in Northern Europe. Appendix 2 Attachment 2 of Work package 6.6 EPIZONE Epidemiology and Surveillance of BTV report.
- Hadorn D.C., Racloz V., Schwermer H., Stark K.D., 2009, Establishing a cost-effective national surveillance system for Bluetongue using scenario tree modelling. *Vet Res* 40, 57.
- Martin P.A., Cameron A.R., Greiner M., 2007, Demonstrating freedom from disease using multiple complex data sources 1: a new methodology based on scenario trees. *Prev. Vet. Med.* 79, 71-97.
- Méroc E., Faes C., Herr C., Staubach C., Verheyden B., Vanbinst T., Vandebussche F., Hooyberghs J., Aerts M., De Clercq K., Mintiens K., 2008, Establishing the spread of bluetongue virus at the end of the 2006 epidemic in Belgium. *Vet Microbiol* 131, 133-144.
- Vandebussche F., Vanbinst T., Verheyden B., Van Dessel W., Demeestere L., Houdart P., Bertels G., Praet N., Berkvens D., Mintiens K., Goris N., De Clercq K., 2008, Evaluation of antibody-ELISA and real-time RT-PCR for the diagnosis and profiling of bluetongue virus serotype 8 during the epidemic in Belgium in 2006. *Vet Microbiol* 129, 15-27.
- Welby S, van den Berg T., Marché S., Houdart P., Hooyberghs J., Mintiens K., Redesigning the serological surveillance programme for notifiable Avian Influenza in Belgian professional poultry holdings (submitted).

