MoSS: A MONITORING AND SURVEILLANCE SYSTEM FOR THE EARLY DETECTION AND IDENTIFICATION OF (RE-)EMERGING ANIMAL DISEASES

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INTRODUCTION

The analysis of the process that led to the identification Bluetongue in Belgium (2006) identified a lack of structured communication between field veterinary practitioners, confronted with an emerging disease, and experts scattered over several institutions. There was a need for an accelerated disease identification process to restrict animal discomfort and economic losses linked with decreased production. As a response to that, the Belgian Veterinary Authorities promoted the development of a focal point based on the online reporting of atypical syndromes by veterinary field practitioners and experts in different fields of expertise, in order to identify and characterize the causative agent of possibly emerging syndromes as soon as possible. The project led to the development of the online web application 'MoSS' (Monitoring and Surveillance System). The objectives of the MoSS project are i) to facilitate the early detection and identification of emerging animal diseases and ii) to develop a strong network of (veterinary) experts, assisting in the diagnostic process and identification of the causative agent of possible emerging syndromes. In the past 2 years, a feasibility study, functional analysis and software development resulted in a first implemented version of the MoSS website (www.moss.be) and preliminary results.

MATERIALS & METHODS

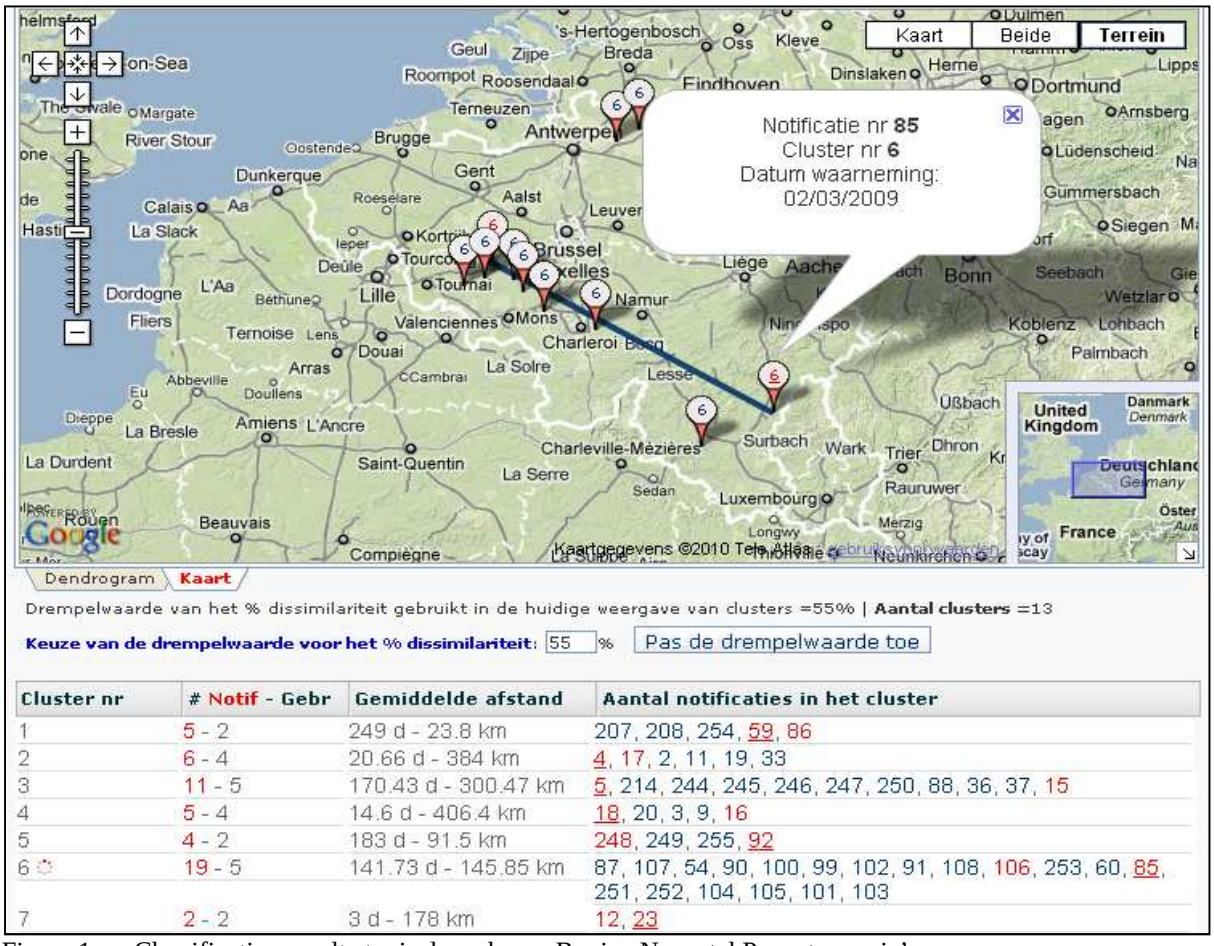
To detect new emergences, the MoSS project is based on active reporting of atypical syndromes by veterinary field practitioners and veterinary experts. Registered web users are encouraged to record three categories of atypical syndromes into the system: i) unknown, emerging syndromes/diseases, ii) known diseases with an unusual clinical expression and/or non-responding to the usual treatment and iii) rare or inadequately documented sporadic disease and re-emerging or endemic diseases. After entering the secured website, the user has access to an enquiry form which collects information about cases with regard to geographical location, clinical signs, epidemiology and possible factors of importance related to the occurrence of the observed case. Subsequently, records are clustered based on the real-time automatic comparison between new records and all previous records. Similar cases with regard to clinical signs, affected animal categories and spatio-temporal distances are automatically grouped using a fully definable hierarchical ascending clustering process. The outcome of this clustering process identifies several groups of notifications aggregated by similarity level. Aiming at fast disease identification and control, an alert signal provided by the onset of a new cluster will be followed by efficient communication between veterinary field practitioners and experts organised on one dedicated Forum page per identified cluster. The forum, for which the first version is expected in November 2010, will connect all levels of expertise and facilitate the diagnostic approach.

The present version of the website also allows for the reporting of any identified endemic or re-emerging animal disease for which the website is activated. A light version of the questionnaire used for the clinical description of atypical syndromes is offered, as well as the same geo-referencing system. Complementary specific questionnaires could be incorporated in the website and support active data collection and monitoring of specifically targeted

diseases. As well, records of identified atypical syndromes can be transferred to the database of endemic diseases.

PRELIMINARY RESULTS

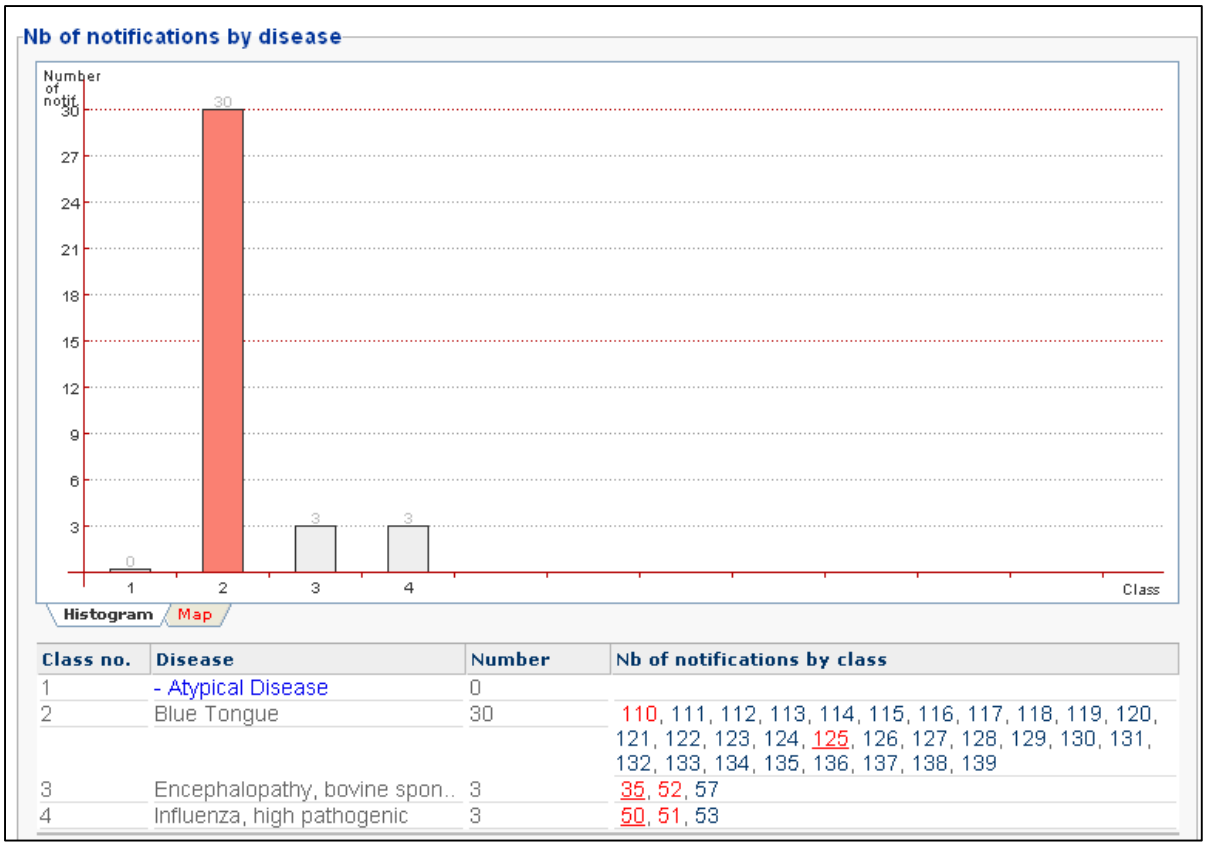
The MoSS-website is still under construction and not officially launched, yet records of atypical syndromes have been made by a number of veterinary experts who are acquainted with the system. The clustering and mapping processes were tested using real life data: records of BNP (Bovine Neonatal Pancytopoenia), a currently emerging syndrome in calves in Europe. Several records were registered in the system by different observers and subsequently compared with "noise". Figure 1 shows the BNP cluster (n°6), a classification result in MoSS. This cluster contains 19 records made by 5 users, with a chosen intra-cluster dissimilarity threshold of 55 %. When the BNP cluster was created, none of the remaining records in the database concerned BNP, which shows the system's potential to discriminate the related BNP cases from the surrounding noise, irrespective of personal variation between the authors of BNP records.



Cluster nr	# Notif - Gebr	Gemiddelde afstand	Aantal notificaties in het cluster
1	5 - 2	249 d - 23.8 km	207, 208, 254, 59, 86
2	6 - 4	20.66 d - 384 km	4, 17, 2, 11, 19, 33
3	11 - 5	170.43 d - 300.47 km	5, 214, 244, 245, 246, 247, 250, 88, 36, 37, 15
4	5 - 4	14.6 d - 406.4 km	18, 20, 3, 9, 16
5	4 - 2	183 d - 91.5 km	248, 249, 255, 92
6	19 - 5	141.73 d - 145.85 km	87, 107, 54, 90, 100, 99, 102, 91, 108, 106, 253, 60, 85, 251, 252, 104, 105, 101, 103
7	2 - 2	3 d - 178 km	12, 23

Figure 1 Classification result atypical syndrome Bovine Neonatal Pancytopoenia'

The website also gives the opportunity to collect information on specifically targeted diseases, such as endemic or potentially re-emerging diseases. Descriptive analytical tools are provided to allow assessment and monitoring of reporting frequencies and to provide summarizing reports to users. Histograms of the number of records per species, disease, time period and region are available (Figure 2, fictive data).



Class no.	Disease	Number	Nb of notifications by class
1	- Atypical Disease	0	
2	Blue Tongue	30	110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139
3	Encephalopathy, bovine spon..	3	35, 52, 57
4	Influenza, high pathogenic	3	50, 51, 53

Figure 2 Distribution of records by surveyed disease

DISCUSSION

The preliminary results show an efficient identification of clusters by the hierarchical ascending clustering method implemented in the website. However, internal and external validations are needed to test and optimize MoSS' analytical characteristics. Internal validation and calibration should lead to the most optimum combination of parameter settings, to detect a potential emerging syndrome with maximum timeliness and sensitivity. External validation will be performed using historic data of model diseases. The expected results would clearly identify the clusters of notifications grouped by model disease.

Although the identification process of potentially emerging diseases will be led by qualified and carefully selected experts, the outcome of the clustering process should be constantly assessed by the Health Authorities to decide when a specific action has to be organized in the field. This decision needs to be taken directly when the disease is identified and officially controlled. As long as no notifiable disease has been suspected no official action may be launched.

As for any system based on clinical observations, MoSS relies to a great extent on the experience, scientific knowledge and alertness of veterinary practitioners, as well as their goodwill to report unusual findings to contribute to sanitary health. In addition, veterinary practitioners might be somewhat reluctant in reporting a case of a potential emerging disease, perhaps afraid for the consequences that it might have. All these aspects create a great importance of providing good training and education to both veterinary practitioners and experts on the MoSS project. Also, the various functions of the website, the added value for the veterinarian of reporting a case in MoSS, the foreseen communication and actions in the case of a potential emerging disease need to be constantly communicated in a comprehensive manner. Providing a fast feedback to reporters is of great importance for both detection of a

potentially emerging disease (identify possible causative agent as soon as possible and restrict negative consequences) as monitoring of endemic diseases (immediate information on disease incidences and history).

The MoSS focuses on production animals but is open to all species on request. The website allows for multilingual management and cross-border reporting. The system is currently being tested in “real life” by a network of sentinel veterinary practitioners before becoming accessible to all Belgian practitioners. The MoSS is based on the centralization and analysis of available information provided by veterinary field practitioners and will be a critical tool aiming at shortening the detection time of any health-related event of importance in domestic animals. It is a first significant step in the preparedness for detection of the ‘unexpected’, as well as monitoring of endemic and epidemic diseases.

ACKNOWLEDGEMENTS

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CONTROL OR ERADICATION OF ENDEMIC DISEASES: WHAT DO WE LEARN FROM EXPERIENCE AND FROM EPIDEMIOLOGICAL AND SIMULATION STUDIES?

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The objective of this paper is to illustrate and discuss insights which can be obtained from experience and from research to support policy-making for the management of endemic diseases.

Definition of an endemic disease varies between users. It generally refers to a disease being present in a population, with no expected spontaneous extinction. The distinction with an epidemic disease is somewhat relative. Indeed, many diseases considered today as epidemic (e.g. foot-and-mouth disease, classical swine fever) are characterised by a peak frequency when the pathogen is introduced into a naïve population, followed by an endemic stage, i.e. equilibrium with a non-zero frequency if no control measure is implemented. In veterinary medicine, what is usually called endemic diseases includes the so-called production diseases or multifactorial diseases (e.g. mastitis), present in most of the herds at varying prevalence, and transmissible diseases which have been present in a population for a while and are perceived as likely to persist. In this paper, we focus on the latter.

For transmissible diseases, eradication means eliminating the pathogen from a population, generally in a defined geographical area. By contrast, control consists of maintaining the frequency and the burden of the disease at an acceptable level. The question of who defines a threshold as acceptable is then raised.

We learn from experience in different countries that it is feasible to eliminate a pathogen from a geographical area. Some recent examples in Europe are the viruses responsible for BVD (bovine viral diarrhoea) in Scandinavian countries, Aujeszky's disease in Western Europe, IBR (infectious bovine rhinotracheitis) in France. In these examples, methods to eradicate the pathogen have relied either on sanitary measures, or on massive vaccination, or a combination of both. It is interesting to analyze why, for the same pathogens, some countries decided to "live with the disease". It is also interesting to realize that for other pathogens, there is kind of implicit consensus that eradication is not the way to go, as e.g. for paratuberculosis.

With observational epidemiological studies, looking at the literature, we find in fact many studies identifying risk factors for occurrence of a disease (defined at large, and including presence of a pathogen), a few of them aiming at understanding risk of reoccurrence after a previous elimination of a pathogen (see for example a review of risks factors for BVD occurrence or reoccurrence in Lindberg *et al*., 2006). Descriptive studies can inform on the frequency of the disease after a control programme has been implemented and document the follow-up of the programme, ideally by measuring not only prevalence, but also incidence of the disease, which is a more precise indicator of effectiveness of preventing new infections. Intervention studies should allow comparing situations with and without an intervention and to assess, ex post, effectiveness of control strategies. In fact, there are very few intervention studies available for control programmes of endemic diseases, probably because they are not deemed feasible (practically and for ethical and/or political reasons), and because of their cost. Overall, available observational studies provide information useful to understand the determinants of disease occurrence, and therefore to construct control programmes aiming at controlling them, but are limited in estimating the effectiveness of control programmes. Moreover, their external validity must be thoroughly discussed before using results in a new population.

In complement to observational studies, in the last decades, epidemiological modelling and simulation studies emerged as a method to evaluate control actions for endemic diseases (see e.g. for BVD, Viet *et al.*, 2006, Ezanno *et al.*, 2008, Courcoul-Lochet and Ezanno, 2010). Among their

advantages, a large number of possible actions can be compared and ex ante evaluation is possible. Simulation models can be developed to account for different contexts, for example, different farming systems, high or low risk of introduction of a pathogen, high or low density of contacts between animals and herds. Uncertainty can also be accounted for and shown in the outcomes, which is of interest for a decision-maker. It is even possible to use models to characterize the required efficacy of a still theoretical control action to achieve to obtain a good effectiveness at the population level. One of the difficulties in such studies is to determine which simplifications of a real system can be done in a model to still predict the expected behaviour of the system. Then information (from observed data) is necessary to parameterize and calibrate the model. Although full model validation is generally not feasible for complex models, they can be used under well-defined assumptions to understand the determinants of persistence versus extinction of a disease in a population, and to rank control strategies according to their relative effectiveness, in a variety of contexts of interest.

Effectiveness of control actions is not sufficient an information for policy-makers to define a control strategy. Besides, socio-economic components are of importance (Saatkamp *et al*, 2006). One the one hand, quantification of diseases losses, on costs of the control programme and on its expected efficiency is necessary (Fourichon *et al*, 2005). On the other hand, even a programme theoretically efficient can fail if there is poor compliance of farmers to apply it. Then, expectations, motivations and obstacles for farmers to implement control actions should be understood (Fourichon *et al*, 2008). Again, modelling can be an interesting approach to link epidemiology and economics. Decisions of farmers can be simulated, and with dynamic models, it is even possible to account for risk aversion and for adaptation of farmers to a changing environment with a variable level of risk for their herd (Rat-Aspert and Fourichon, 2010).

REFERENCES

Courcoul-Lochet A., Ezanno P. 2010. Modelling the spread of Bovine Viral Diarrhoea Virus (BVDV) in a managed metapopulation of cattle herds. Veterinary Microbiology 142, 119-128

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

Fourichon C., Beaudeau F., Bareille N., Seegers H. 2005. Quantification of economic losses consecutive to infection of a dairy herd with bovine viral diarrhoea virus. Preventive Veterinary Medicine 72, 177-181

Fourichon C., L'Hotel L., Frappat B., Pécaud D. 2008. Participation and compliance to a voluntary BVDV control scheme. The Society for Veterinary Epidemiology and Preventive Medicine (SVEPM), Liverpool, United-Kingdom, 26-28 March 2008

Lindberg A., Berriatua A., Fourichon C., Mintiens K., Houe H. 2006. Epidemiology and risks. in EU Thematic network on BVDV control, FP5. Position paper, 24-72, Published on-line at http://www.bvdv-control.org/uploaded_files.asp?meny=22

Rat-Aspert O., Fourichon C. 2010. Modelling collective effectiveness of voluntary vaccination with and without incentives. Preventive Veterinary Medicine 93, 265-275

Saatkamp H.,W., Stott A.W., Humphry R.W., Gunn G.J. 2006. Socio-economic aspects of BVDV control. in EU Thematic network on BVDV control, FP5. Position paper, 99-133, Published on-line at http://www.bvdv-control.org/uploaded_files.asp?meny=22

Viet A.F., Fourichon C., Seegers H. 2006. Simulation study to assess the efficiency of a test-and-cull scheme to control the spread of the bovine viral-diarrhoea virus in a dairy herd. Preventive Veterinary Medicine 76, 151-166

ERADICATION OF ENDEMIC DISEASES: POINT OF VIEW OF THE INDUSTRY

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Endemic diseases may be widespread in the livestock population. Sometimes they cause significant production loss, they may be important for export, and may infect humans.

Individual farmers have the main responsibility for the control of endemic diseases on farm level. Farmers will benefit from maintaining a high level of bio security on their farm. Despite some legal requirements on bio security, there is a wide range of bio security practiced between producers.

Individual producers are responsible for control and management of endemic diseases. But are producers also responsible for the coordination of control measures? When diseases, even endemic diseases, have a significant impact in the markets, the industry or animal welfare, involvement or intervention of authorities is needed. Intervention could be a creation of legislative tools like “annulment of sale”, quarantine, notification) or even financial support in the programs themselves. A general passive surveillance system must be in place to detect and investigate those diseases.

On the other hand, some diseases may be classified as endemic diseases where no regulatory action is required and eradication heavenly depends on the management by the farmer.

In Belgium, voluntary programs to eradicate endemic diseases do not work. Only mandatory programs seem to be successful.

EVALUATION OF THE BELGIAN SALMONELLA SPECIFIC ACTION PLAN

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INTRODUCTION

In EU Regulation No 2160/2003 to control *Salmonella*, the Commission has set deadlines for its Member States to start *Salmonella* surveillance programs in the different livestock species that contribute to the risk of food borne infections in humans (Anonymous, 2003). To control *Salmonella* in pigs at the pre-harvest stage, the implementation of a surveillance and control program should be established before July 2009.The Federal Agency for the Safety of the Food Chain installed a national *Salmonella* surveillance program in pig production farms in January 2005. The *Salmonella* infection status of the herds is established from the serological testing of pigs blood samples collected every 4 months. Since July 2007, farms are assigned as *Salmonella* risk farm based on their serological profile. If the farm's mean S/P ratio is above 0.6 during 3 consecutive sampling rounds, then it is assigned as risk farm. After this assignment, a *Salmonella* Specific Action Plan (SSAP) (checklist, bacteriology and specific measures) is completed in the herd.

The objectives of the present report were: 1. to investigate the effect of the SSAP on the overall serology of the pig farms 2.to focus on risk farms by evaluating the time they remain in the SSAP and exploring the effect of several risk factors on that time.

METHODS & RESULTS

Temporal evolution of overall serology

The temporal evolution of the herd-specific average S/P ratio was analyzed in relation to the SSAP's onset for the total pig production population. At this overall level, the impact of the plan was not notable. A linear mixed model was used to have statistical insight of the impact of the SSAP on the S/P ratio and the effect of the plan was found to be non-significant ($p>0.1$).

Spatial analysis of risk farms

A purely spatial Bernouilli model was used to scan for high rates of farms declared at risk in data aggregated by municipality. Figure 1 shows the relative risk for each municipality that is the estimated risk within that area divided by the estimated risk outside this same area. The most likely cluster is also represented on the figure. Its relative risk was 2.68 ($p=0.001$).

Analysis of the time at risk for a problematic herd

The objective was to analyze the time 'at risk' for a herd, i.e. the time between the beginning and the end of the SSAP for a given herd. A survivor function was estimated using the Kaplan-Meier function. Different survival curves were estimated for different types of farm. The Wilcoxon test was used to see whether the survivor functions in the subcategories were equal. Subsequently, a Cox regression model was applied to the survival data to investigate simultaneously the influence of

several factors (type of farm, season, size of farm, density of pig farms in the municipality) on the survival time. Figure 2 shows the overall survival curve. The cumulative probability of a farm to still be in the plan after 4 months is more or less of 50% and after 2 years almost null. In Figure 3 the survival curve is presented for the types of farm. There was statistical significance of the test's result for the difference between the survival functions according to the type of herd: closed herds stay longer in the SSAP compared to the meat and mixed farms ($p=0.035$). Table 1 presents the results of the Cox regression model. At any point in time after the onset of the SAP, withdrawals were happening at a 27% and 22% higher rate in respectively the mixed and the meat farms compared to the closed farms.

Mapping of the main risk factor

In addition, the proportion of closed farms on the total number of pig farms in each municipality was mapped and compared to figure 1. The Spearman's rank correlation coefficient was estimated to have insight of the correlation between relative risk and proportion of closed herds at the municipality level. A coefficient of 0.4 ($p<0.0001$) was found, indicating a positive correlation between the two variables.

CONCLUSIONS

The overall results for the pig production farms did not show any significant change in serology since the SSAP was implemented two years and a half ago. This finding raises many questions: Do we have sufficient hindsight? Is the plan itself really efficient? Is there enough control of the measures taken in the risk farms? Are we focusing on the right target? etc. In this study, we saw that after four months half of the farms were not at risk anymore. This highlights the impact of the action plan and its efficiency for the situation of the most problematic herds of the country. However, long term situations (in the SSAP for more than a year) have arisen too. The analysis provided some insight on the role the farm's composition may play on the persistence of *Salmonella* in a herd. The higher risk of closed herds seems to be explained by the presence of sows in this particular type of holding structure.

REFERENCES

Anonymous 2003. ZAP Salmonella Update, a zoonosis action plan for the British pig industry (The British Pig executive), p. 12.

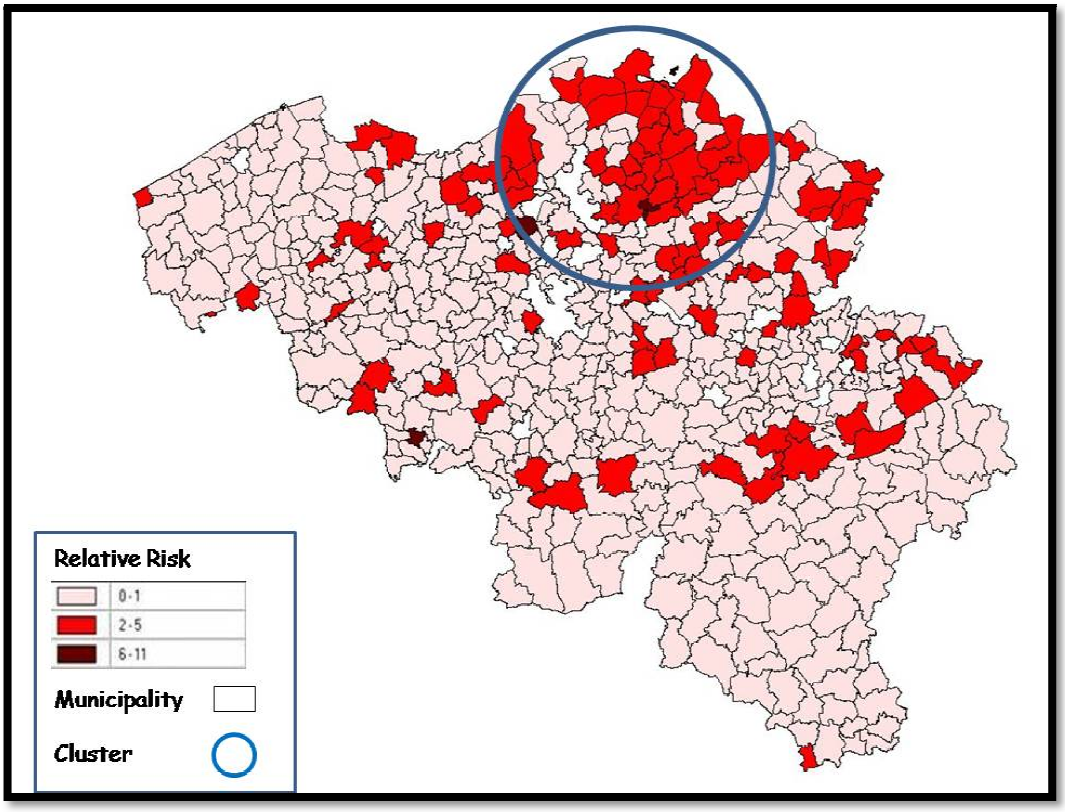

Figure 1 Results of the spatial analysis on the risk of being a farm at risk for Salmonella (Jul 2007 -Jan 2010)

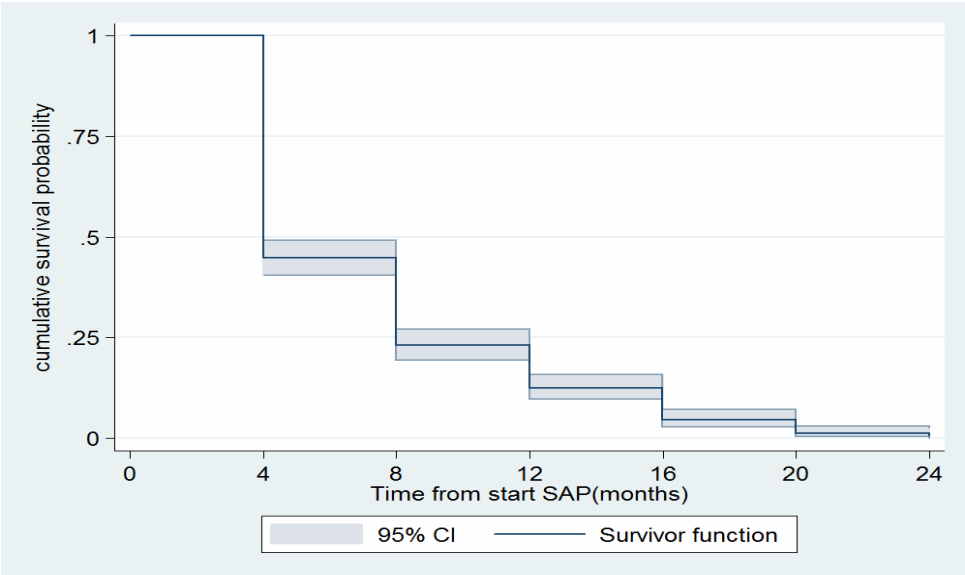

Figure 2 Kaplan-Meier survivor function with 95% confidence interval for the total 491 herds in the SSAP

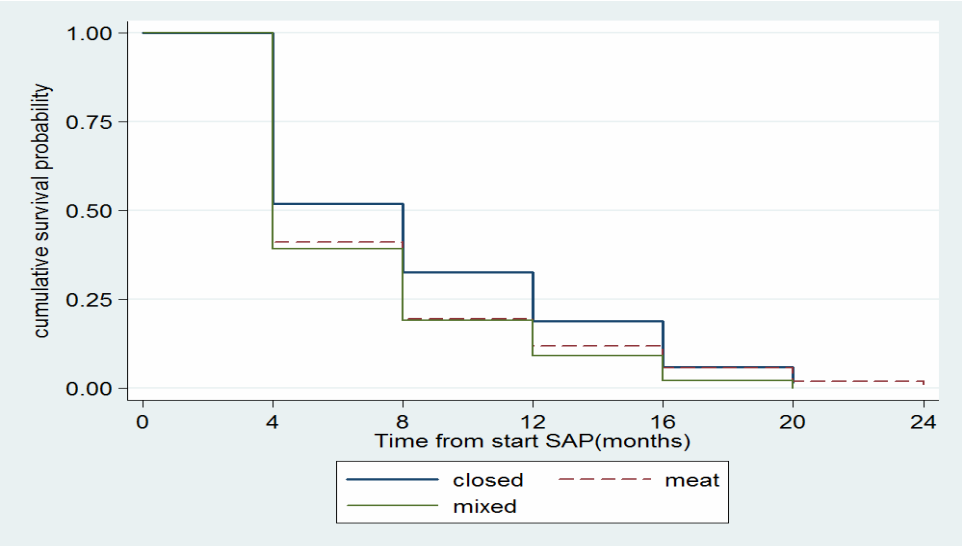

Figure 3 Kaplan-Meier survivor functions for 193 meat farms, 153 mixed farms and 111 closed farms in the SSAP

Table 1 Results of the final Cox regression model analyzing the time for withdraw from the SSAP expressed in terms of hazard ratios (HRs), standard errors (SEs) and associated p-value

Predictor	HR	SE	P-value
Season*[1]=autumn	0.99	0.3	0.99
Season*[1]=winter	1.05	0.3	0.86
Season*[1]=spring	1.22	0.32	0.54
Type of herd*[2]=meat	1.22	0.17	0.19
Type of herd*[2]=mixed	1.27	0.13	0.07
Density of municipality	1.08	0.07	0.24
Size of the herd	1	0	0.84

*[1] reference=summer *[2] reference=closed

LOW PATHOGENIC H5 AND H7 AVIAN INFLUENZA: ASSESSMENT OF TRANSMISSION PARAMETERS AND THE INFLUENCE OF HOUSING CONDITIONS ON TRANSMISSION

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INTRODUCTION

Avian influenza viruses are orthomyxoviridae that can cause a wide variety of symptoms in various bird species. In the phosfolipid bilayer envelope of the virion, two glycoproteins are embedded: Heamagglutinin (HA) and Neuraminidase (NA) [1]. So far, 16 HA and 9 NA antigenic subtypes have been identified in birds [2].

Avian influenza is subdivided in two pathotypes, based on the symptoms observed after infection in poultry. The high pathogenic pathotype (HPAI, formerly: "fowl plague") can cause severe mortality and disastrous outbreaks in domestic poultry with serious economical and ethical consequences. HPAI has only been observed among the H5 and H7 serotypes. The low pathogenic pathotype (LPAI) has been found among all 16 HA serotypes. In poultry, LPAI can cause no, mild or severe respiratory symptoms whether or not associated with secondary infections, depending on factors such as viral strain or bird type [3]. Occasionally an outbreak of LPAI can evoke high mortality rates [4].

The aim of this study was to characterise the excretion and to assess the impact of housing conditions on the transmission of low pathogenic H5 and H7 Avian Influenza viruses in poultry. Hereto, a selection of suitable strains needed to be made in a first series of infection experiments. These allowed selecting low and high infective viruses based on the observed viral excretion and seroconversion. Subsequently, the selected viruses were each used in a transmission experiment comprising two separate groups of SPF poultry, the only difference being soil coverage.

MATERIAL AND METHODS

Animals

All animal experiments were carried out with twelve 4-6 weeks old SPF layer chickens (Lohman-Valo, Germany, Cuxhaven). The animals were housed in a BSL3-isolator with negative air pression. The isolators' inside is approximately 80cm wide, 150cm long and 72cm high. Feed and water were provided at libitum. The drinking bowl contains approximately 300ml of water and is connected to a large reservoir that allows continuous replenishment.

Infection experiments

Each infected bird was oculo-nasally inoculated with a total of 100µl of a 10^7 EID_{50}/ml viral solution diluted in sterile phosphate buffered saline (PBS). Blood samples were collected prior to infection and on day 7, 10, 14 and 21 after inoculation. At 1, 3, 6 and 10 days after inoculation, oropharyngeal and cloacal swabs were collected. The swabs were submerged in 1,5ml Brain Heart Infusion Broth containing penicillin, kanamycin, gentamycin and streptomycin after which they were stored at -80°C until further analysis [5].

Transmission experiments

For the transmission experiments, a 50/50 ratio inoculated/susceptible (I/S) animals was chosen for all viruses [6]. As mentioned above, all transmission experiments were carried out in twofold: one group was housed on a grid, allowing faeces etc. to fall into a dropping pit, another group was housed on approximately 1,5 kg of wood shavings. The 6 susceptibles were introduced 24 hours after inoculation of the 6 inoculated ones [7-9]. To minimize the possibility of direct infection of the susceptibles with inoculum spread in the isolator by the seeders, shortly after inoculation, or inoculum left behind due to gestures by the operators at the time of inoculation, drinking bowls were refreshed and wood shavings (if required) were added 6 hours after inoculation. Blood samples were collected prior to infection and on day 14 and 21 after inoculation. Oropharyngeal and cloacal swabs were collected daily for 10 days and stored the same way as above.

Serology

Sera obtained from blood samples were submitted to hemagglutination inhibition test, according to WHO guidelines [10], or to an indirect antibody ELISA test (ID-Vet, Montpellier, France).

Virology

Viral RNA from swabs was automatically extracted with a commercial viral RNA extraction kit (MagMaxTM AI/ND-96 Viral RNA, Applied biosystems, Lennik, Belgium). RNA was amplified and quantified by means of a fluorescent marker with real time RT-PCR (7500 real time PCR cycler, Applied Biosystems, Lennik, Belgium). The received data from the real time RT-PCR were the number of thermal cycles (Ct) when fluorescence was detected. Ct values higher than 40 were regarded as negative.

Statistical analysis

Using SAS, *beta* for each virus under given conditions was determined with a Generalized Linear Model, using results obtained from real time RT-PCR. The *reproduction ratio (R_0)* was then calculated by multiplying beta with the average infectious period of the contact animals in each group. An animal is regarded "infected" between the first and the last observed moment of excretion of viral RNA. Therefore, the infectious period is the number of days between these two moments.

RESULTS

Infection experiments

Table 1 Average Ct-values from oropharyngeal and cloacal swabs (negative results were excluded). Results from H7N1 A/Ch/Italy/1067/v99 are obtained by Marché et al. [11]. Bars indicate no detection of viral RNA; n.t.=not tested.

		H5N2 A/Ch/Belgium/150VB/99			H5N3 A/Anas Plathyrhynchos/Bel/272/09			H7N1 A/Tadoma Tadorna/Bel/3441/P3/09			H7N1 A/Ch/Italy/1067/v99		
Swab type	Days p.i.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.
Oropharyngeal	1	25,51	2,02	12	31,54	3,20	5	27,21	2,87	12	n.t.	n.t.	n.t.
	3	27,33	2,49	12	33,86	2,17	3	27,97	2,36	11	29,80	4,44	11
	6	33,10	3,42	11	-	-	-	29,91	2,62	11	35,75	1,36	2
	10	36,76		1	-	-	-	-	-	-	-	-	-
Cloacal	1	-	-	-	-	-	-	-	-	-	n.t.	n.t.	n.t.
	3	-	-	-	38,60		1	30,31	5,75	9	29,65	2,45	3
	6	32,36	6,39	2	-	-	-	30,42	5,66	9	31,42	3,44	7
	10	34,87		1	-	-	-	32,38	1,46	2	31,32	2,56	6

Infection experiments showed differences in quantities of excreted viral RNA and excretion route between the tested viruses. Oculonasal inoculation with *H5N2 A/Ch/Belgium/150VB/99*, *H7N1 A/Tadorna Tadorna/Bel/3441/P3/09* and *H7N1 A/Ch/Italy/1067/v99* proved to successfully establish infection in almost all test animals (Table 1).

H5N3 A/Anas Plathyrhynchos/272/09 appears to be a low infective virus, since only 5 out of 12 inoculated birds excreted viral RNA on the observed days. On HI-test only 3 birds had seroconverted, whilst ELISA showed seroconversion in 8 birds (results not shown). Both tested H7 viruses evoked stronger cloacal excretion than both tested H5 viruses.

Based on these results, H5N2 A/Ch/Belgium/150VB/99 (High infective yet weak cloacal excretion), H7N1 A/Ch/Italy/1067/v99 (High infective and strong cloacal excretion) and H5N3 A/Anas Plathyrhynchos/272/09 (Low infective) were selected for use in further experiments.

Transmission Experiments

Results proved transmission of *H7N1 A/Ch/Italy/1067/v99* and *H5N2 A/Ch/Belgium/150VB/99* from the infected animals to the contact animals. Average Real Time RT-PCR and HI-test results obtained from these two transmission experiments are shown in table 2 and 3.

Table 2: Average Ct Values of oropharyngeal and cloacal swabs from transmission experiments with H7N1 A/Ch/Italy/1067/v99 and H5N2 A/Ch/Belgium/150VB/99. n.t.=not tested; St. Dev=standard deviation; Nb. Pos.=number of animals with Ct < 40; green=all animals negative; yellow=at least one animal positive.

		H7N1 A/Ch/Italy/1067/v99 GRID - INOCULATED			H7N1 A/Ch/Italy/1067/v99 WOOD SHAVINGS - INOCULATED			H7N1 A/Ch/Italy/1067/v99 GRID - CONTACT			H7N1 A/Ch/Italy/1067/v99 WOOD SHAVINGS - CONTACT		
Swab type	Days p.i.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.
Oropharyngeal	1	27,84	3,45	6	26,42	3,56	6	Introduction of animals			Introduction of animals		
	2	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	29,95		1	34,16	4,66	3
	3	30,38	4,16	6	28,19	2,17	6	27,81		1	33,73	5,34	3
	4	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	36,69	2,73	4	34,06	5,24	6
	5	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	31,83	7,71	2	34,91	5,37	5
	6	29,72	0,92	6	30,42	3,19	6	34,78	6,61	2	30,28	5,64	5
	7	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	32,88	2,65	3	27,09	1,43	4
	8	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	34,97	1,25	3	29,92	4,23	5
	9	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	27,85	4,08	4
	10	-	-	-	-	-	-	-	-	-	30,16	5,53	4
	11	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	27,35	0,82	2
Cloacal	1	-	-	-	-	-	-	Introduction of animals			Introduction of animals		
	2	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	-	-	-
	3	34,52	7,51	2	23,49		1	-	-	-	-	-	-
	4	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	36,05		1
	5	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	38,86		1
	6	26,40		1	25,25	1,19	2	-	-	-	-	-	-
	7	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	36,82		1
	8	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	-	-	-
	9	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	37,20	0,21	2
	10	-	-	-	39,20		1	n.t.	n.t.	n.t.	-	-	-
	11	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	-	-	-

		H5N2 A/Ch/Belgium/150VB/99 GRID - INOCULATED			H5N2 A/Ch/Belgium/150VB/99 WOOD SHAVINGS - INOCULATED			H5N2 A/Ch/Belgium/150VB/99 GRID - CONTACT			H5N2 A/Ch/Belgium/150VB/99 WOOD SHAVINGS - CONTACT		
Swab type	Days p.i.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.	Ave. Ct	St. Dev.	Nb. Pos.
Oropharyngeal	1	31,32	3,32	5	30,36	2,60	6	Introduction of animals			Introduction of animals		
	2	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	38,05	1,55	4	35,92	4,14	3
	3	30,65	4,69	6	26,12	1,76	6	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	4	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	31,84	2,46	6	29,45	3,18	6
	5	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	6	30,89	3,38	5	34,65	1,70	5	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	7	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	31,05	3,54	5	30,43	2,96	4
	8	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	9	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	10	-	-	-	39,01		1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	11	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	39,99		1	37,41		1
Cloacal	1	-	-	-	-	-	-	Introduction of animals			Introduction of animals		
	2	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	-	-	-
	3	-	-	-	-	-	-	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	4	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	-	-	-
	5	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	6	-	-	-	33,08		1	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	7	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	-	-	-
	8	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	9	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	10	37,52		1	-	-	-	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	11	n.t.	n.t	n.t.	n.t.	n.t.	n.t.	-	-	-	-	-	-

Table 3: Average HI titers of sampled serum from transmission experiment with H7N1 A/Ch/Italy/1067/v99 and H5N2 A/Ch/Belgium/150VB/99. n.t.=not tested; St.Dev=standard deviation; Nb. Pos.=number of animals with HI titer > or = 16.

		H7N1 A/Ch/Italy/1067/v99 GRID - INOCULATED			H7N1 A/Ch/Italy/1067/v99 WOOD SHAVINGS - INOCULATED			H7N1 A/Ch/Italy/1067/v99 GRID - CONTACT			H7N1 A/Ch/Italy/1067/v99 WOOD SHAVINGS - CONTACT		
		Ave. UHA	St. Dev.	Nb. Pos.	Ave. UHA	St. Dev.	Nb. Pos.	Ave. UHA	St. Dev.	Nb. Pos.	Ave. UHA	St. Dev.	Nb. Pos.
HI-Test	14	160,00	105,16	6	320,00	350,54	6	128,00	52,26	1	115,20	28,62	5
	21	352,00	365,98	6	917,33	1562,26	6	256,00	104,51	1	153,60	97,06	5

		H5N2 A/Ch/Belgium/150VB/99 GRID - INOCULATED			H5N2 A/Ch/Belgium/150VB/99 WOOD SHAVINGS - INOCULATED			H5N2 A/Ch/Belgium/150VB/99 GRID - CONTACT			H5N2 A/Ch/Belgium/150VB/99 WOOD SHAVINGS - CONTACT		
		Ave. UHA	St. Dev.	Nb. Pos.	Ave. UHA	St. Dev.	Nb. Pos.	Ave. UHA	St. Dev.	Nb. Pos.	Ave. UHA	St. Dev.	Nb. Pos.
HI-Test	14	149,33	87,44	6	533,33	400,02	6	128,00	52,26	5	125,33	193,70	6
	21	298,67	174,88	6	1024,00	0,00	6	256,00	104,51	5	333,33	382,18	6

Transmission experiment with H7N1 A/Ch/Italy/1067/v99:

In both housing types, all inoculated animals excreted viral RNA starting 1 dpi and kept on doing so for at least 6 days. 4 contact animals in the group housed on grid were infected and showed solely oropharyngeal excretion of viral RNA, while 2 contact animals remained negative for the entire course of the experiment. Only 1 contact animal seroconverted. All contact animals from the group on wood shavings excreted viral RNA, mainly via the oropharyngeal route, but 3 animals showed some cloacal excretion. All of the contact animals seroconverted.

Transmission experiment with H5N2 A/Ch/Belgium/150VB/99:

All inoculated animals excreted viral RNA mainly via oropharyngeal route. Cloacal excretion in these animals was not detected until 6 dpi. In both housing types, all contact animals had excreted viral RNA at least once oropharyngeally at 3 dpi. None of the contact animals showed cloacal excretion of viral RNA on the observed days. 1 contact animal from the group housed on grid did not seroconvert.

Reproduction ratio

Reproduction ratio (R_0) obtained for both transmission experiments are shown in table 4. The results are based on limited data and contain some assumptions. In the *H7N1 A/Ch/Italy/1067/v99* experiment, all three parameters are higher for the group on wood shavings, suggesting better transmission. No significant difference between groups housed on wood shavings or grid was found on student's t-test.

Table 4 Beta, infectious period and R_0 obtained for transmission experiments with H7N1 A/Ch/Italy/1067/v99 and H5N2 A/Ch/Belgium/150VB/99.

		Beta		Infectious Period		R_0
		Beta	Confidence Limits	Average	St. Dev.	
H7N1	Grid	0,24	[0,09 - 0,65]	3,17	2,93	0,77
A/Ch/Italy/1067/v99	Wood shavings	1,28	[0,56 - 2,93]	7,67	1,37	9,84
H5N2	Grid	2,97	[1,21 - 7,30]	8,17	1,72	24,27
A/Ch/Belgium/150VB/99	Wood shavings	2,01	[0,86 - 4,74]	7,33	2,25	14,77

DISCUSSION

H7N1 A/Ch/Italy/1067/v99.

The observed excretion and seroconversion in contact animals was different in the two tested housing types. The contact animals from the group housed on grid excreted small amounts of viral RNA and, more interestingly, only 1 animal seroconverted. Contact animals from the group housed on wood shavings all became infected and seroconverted. They excreted larger amounts of viral RNA as well. All together, these results indicate influence from housing conditions on transmission of this particular virus. This could be due to stronger accumulation of faeces, respiratory excretions, feather dust etc. when housed on wood shavings.

H5N2 A/Ch/Belgium/150VB/99

The observed differences between both housing types in the H7N1 experiment are not present in the H5N2 experiment. The difference in excretion patterns for these two viruses, as observed in the infection experiments, might be an explanation for this. However, repeating the same experiments is necessary to validate these results. Great variability in transmission of viruses when repeating the same experiment has been demonstrated [9].

By selecting viruses with few or late onset of cloacal excretion, the role of oropharyngeal excretion in transmission can be assessed.

REFERENCES

1. Webster RG, et al., *Evolution and ecology of influenza A viruses.* Microbiol. Rev., 1992. **56**: p. 152-179.
2. Fouchier, R.A.M., et al., *Characterization of a Novel Influenza A Virus Hemagglutinin Subtype (H16) Obtained from Black-Headed Gulls.* Journal of Virology, 2005. **79**(5): p. 2814-2822.
3. Alexander, D., *A Review of avian influenza in different bird species.* Veterinary Microbiology, 2000. **74**: p. 3-13.
4. Halvorson, D.A., et al., *Outbreaks of low pathogenicity avian influenza in U.S.A.* Avian Diseases, 2003. **47**(Special Issue, Fourth Internationl Symposium on Avian Influenza, 1997 Proceedings): p. 36-46.
5. Commission, E., *Approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC.* Official Journal of the European Union, 2006. **L237**: p. 1-27.
6. Velthuis, A.G.J., et al., *Design and analysis of small-scale transmission experiments with animals.* Epidemiol. Infect., 2007. **135**: p. 202-217.
7. Kim Min-Chul, et al., *Pathogenicity and transmission of H7N7 avian influenza virus isolates from feces of magpie origin in chickens and magpie.* Veterinary Microbiology, 2010. **141**: p. 268-274.
8. Pillai, S.P., et al., *Pathogenicity and transmission studies of H5N2 parrot avian influenza virus of Mexican lineage in different poultry species.* Vet Microbiol, 2008. **129**(1-2): p. 48-57.
9. Van der Goot, J.A., et al., *Comparison of the transmission characteristics of low and high pathogenicity avian influenza A virus (H5N2).* Epidemiol. Infect., 2003. **131**: p. 1003-1013.
10. WHO (World Health Organisation), *Manual on Animal Influenza Diagnosis and Surveillance.* WHO/CDS/CSR/NCS/2002, 2002: p. 7-67.
11. Marché, S., B. Lambrecht, and T.v.d. Berg, *Evaluation of different Serologic Markers for the early Detection of Avian Influenza Infection in Chickens.* Avian Diseases, 2009. **54**: p. 690-698.

ASSOCIATION OF *CXCR1 (IL8RA)* POLYMORPHISMS WITH UDDER HEALTH AND MILK PRODUCTION OF DAIRY HEIFERS

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INTRODUCTION

Mastitis in dairy cows, an inflammation of the mammary gland, is mostly caused by bacterial intra-mammary infection (IMI). It occurs both in heifers and multiparous cows and is considered as the most important disease in dairy. The prevalence and incidence of heifer mastitis differs among farms. Yet, an even larger variation is observed among heifers within the same farm under the same management, suggesting genetic predisposition of mastitis susceptibility[1].

Innate immune responses in general and migration of large numbers of neutrophils towards the infection site in specific, form the first line of defence against invading mastitis pathogens[2]. Interleukin 8 (IL-8), the most crucial chemo-attractant in this progress[3] exerts its function by binding on the neutrophil receptors CXCR1 and CXCR2. Polymorphisms in the genes coding for these receptors might explain a part of the genetic variation in mastitis susceptibility. One single nucleotide polymorphism (SNP) in the coding region[4] and one SNP in the 5' upstream region[5] of *CXCR1* were found to be associated with mastitis susceptibility. However, these associations have been questioned[6] and so far no papers have been published which confirm these findings in European cattle.

The objective of this research was to screen the entire coding region of the *CXCR1* gene for polymorphisms and to identify potential associations with udder health and milk production in Belgian dairy heifers.

MATERIALS AND METHODS

Phenotypic data

The database for the current research consisted of phenotypic data on udder health and milk production gathered by Piepers et al. (2010)[7] combined with genetic information on DNA released from blood samples. In total, 140 heifers from 20 Flemish farms were enrolled in the study.

Intra-mammary infection (IMI) status at calving for all heifers was determined based on the results of bacteriological culture of quarter milk samples. These samples were aseptically collected twice for each heifer; the first time between 1 and 4 days in milk (DIM), the second time between 5 and 8 DIM. An interval of at least 3 days between both samplings was respected. Heifers were considered non-infected if all quarters were culture-negative at both samplings. Heifers were considered infected with a major pathogen (*Staphylococcus aureus*, esculine-positive streptococci, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*) if at least one quarter was culture-positive for the same major pathogen at both samplings. Heifers were considered infected with coagulase-negative staphylococci (CNS, generally accepted to be minor pathogens) if at least one quarter was culture-positive for CNS during both sampling periods and if no quarters were culture-positive for (a) major pathogen(s). Heifers not complying with these conditions were regarded as culture-positive.

Composite somatic cell count (SCC; number of cells in the udder, an indicator for presence of IMI) and daily milk production at test-day were available for all heifers as a part of the dairy

herd improvement program organized by the Flemish Cattle Breeding Association (CRV, Oosterzele, Belgium). Data on these parameters from 14 until 285 days lactation were used for further analysis.

Genotyping

Blood samples were taken from the tail vein and stored in EDTA vacuum tubes at -20°C. After thawing, DNA was released from each sample. Released DNA was used as a template to amplify simultaneously both copies of the complete *CXCR1* coding region (1236 base pairs) in a single polymerase chain reaction (PCR). Primers which bind specifically at the place of interest (close before the start codon and close after the stop codon of bovine *CXCR1*) were designed for this PCR. Next, the PCR products were sequenced by direct sequencing using the same primers as sequencing primers. Sequences of the coding region of *CXCR1* of all samples were compared with the reference sequence (GenBank Gene ID: 281863) and with each other. Differences indicate the presence of a SNP, named as "SNP x" where the "x" stands for the position relative to the start codon.

Association study

Because prior results from literature[4] suggest the importance of the SNP at position 735, it was decided to focus on the latter. The association between SNP 735 and IMI status at calving was tested using logistic mixed regression models. The association between the heifers' genotype at position 735 and test-day SCC and milk yield was analysed using a linear mixed model. To avoid bias of additive effects of background genes only heifers (n = 86) of different sires were included in the analyses.

RESULTS

In total, 20 SNPs were found. Ten of these SNPs have an impact on the amino acid sequence of the receptor protein (non-synonymous polymorphisms).

A large proportion of the heifers (n = 31) had at least one infected quarter after calving. Statistical analysis showed a borderline non-significant association between SNP 735 and presence of IMI at calving. Heifers with genotype GG were more likely to have IMI caused by all pathogens (P = 0.063) and IMI caused by CNS specifically (P = 0.055) compared to heifers with genotype GC and CC (Fig. 1).

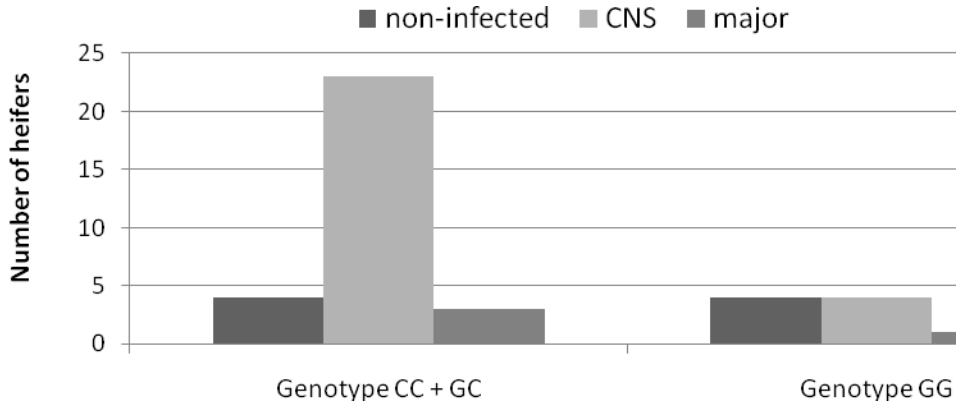

Figure 1. The number of non-infected heifers, heifers infected with coagulase-negative staphylococci (CNS) and heifers infected with a major pathogen (*Staphylococcus aureus*, esculin-positive streptococci, *Streptococcus agalactiae* or *Streptococcus dysgalactiae)*,with genotype CC or GC and genotype GG, respectively. Only heifers with different sires were included in the analysis (n = 86).

Overall, no differences in SCC or milk production over time in first lactation were seen between heifers with genotype CC or GC and heifers with genotype GG.

DISCUSSION AND CONCLUSION

Mastitis is a multi-factorial disease and not only pathogen-specific but host-specific factors as well determine whether or not a heifer becomes infected and whether or not this infection persists. In this study, we investigated if the genotype of the coding region of *CXCR1* can be considered as one of these host-specific factors for Flemish dairy heifers.

To be a host-specific factor, different genotypes should occur in the population. Our research differed from previous research[8,9] as not a segment of the coding region of *CXCR1* but the whole coding region was screened for polymorphisms. This resulted in the discovery of 15 SNPs that have not been described in literature before. The 5 previously identified SNPs were present as well.

In a second step, we performed an association study to analyse whether the different genotypes were associated with different mastitis susceptibility. A borderline non-significant association between SNP 735 and mastitis phenotype was found confirming findings from others[4] in which genotype GG was described as being associated with mastitis resistance. Although heifers and cows with genotype GG seem less susceptible to IMI, neither in this nor in other studies a lower SCC or a higher average daily milk production during first lactation could be demonstrated. Surprisingly, the association between SNP 735 and mastitis susceptibility looked to be pathogen specific, as suggested by our results. Therefore animals with genotype GG might be more resistant to CNS IMI which are less likely to induce high SCC and severe production losses during lactation[10].

However, more research is required to further elucidate the role of polymorphism at position 735 and other SNPs and their relevance for susceptibility of heifers against pathogen-specific IMI, SCC and milk traits in first lactation.

REFERENCES

1. De Vliegher S., Laevens H., Barkema H.W., Dohoo I.R., Stryhn H., Opsomer G., de Kruif A. (2004). Management practices and heifer characteristics associated with early lactation somatic cell count of Belgian dairy heifers. Journal of Dairy Science 87, 937-947.
2. Paape M.J., Shafer-Weaver K., Capuco A.V., Van Oostveldt K., Burvenich C. (2000). Immune surveillance of mammary tissue by phagocytic cells. Advances in Experimental Medicine and Biology 480, 259-277.
3. Barber M.R., Yang T.J. (1998). Chemotactic activities in nonmastitic and mastitic mammary secretions: presence of interleukin-8 in mastitic but not nonmastitic secretions. Clinical Diagnostic Laboratory Immunology 5, 82-86.
4. Youngerman S.M., Saxton A.M., Oliver S.P., Pighetti G.M. (2004) Association of CXCR2 polymorphisms with subclinical and clinical mastitis in dairy cattle. Journal of Dairy Science 87, 2442-2448.
5. Leyva-Baca I., Schenkel F., Martin J., Karrow N.A. (2008). Polymorphisms in the 5' upstream region of the CXCR1 chemokine receptor gene, and their association with somatic cell score in Holstein cattle in Canada. Journal of Dairy Science 91, 407-417.
6. Goertz I., Baes C., Weimann C., Reinsch N., Erhardt G. (2009). Association between single nucleotide polymorphisms in the *CXCR1* gene and somatic cell score in Holstein dairy cattle. Journal of Dairy Science 92, 4018-4022.
7. Piepers S. ,Opsomer G., Barkema H.W., de Kruif A., De Vliegher S. (2010) Heifers infected with coagulase-negative staphylococci in early lactation have less cases of clinical mastitis and a higher milk production in their first lactation than non-infected heifers. Journa l of Dairy Science 93, 2014-2024.
8. Grosse W.M., Kappes S.M., Laegreid W.W., Keele J.W., Chitko-McKown C.G., Heaton M.P. (1999). Single nucleotide polymorphism (SNP) discovery and linkage mapping of bovine cytokine genes. Mammalian Genome 10, 1062-1069.
9. Youngerman S.M., Saxton A.M., Pighetti G.M. (2004). Novel single nucleotide polymorphisms and haplotypes within the bovine CXCR2 gene. Immunogenetics 56, 355-359.
10. Timms L.L., Schultz L.H. (1987). Dynamics and significance of coagulase-negative staphylococcal intramammary infections. Journal of Dairy Science 70, 2648-2657.

GENOTYPIC CHARACTERIZATION OF EHEC O157, O26, O103, O111 AND O145 ISOLATES FROM CATTLE, FOOD AND HUMANS IN BELGIUM

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INTRODUCTION

EHEC

Enterohaemorrhagic *Escherichia coli* (EHEC) is an important food-borne pathogen, that can cause a variety of clinical outcomes, ranging from haemorrhagic colitis (HC) to the life-threatening complication haemolytic uremic syndrome (HUS) (Beutin et al. 2004; Karmali 1989). EHEC comprise a highly pathogenic subset of the zoonotic Shiga-toxin-producing *E. coli* (STEC). Most human infections of HC and HUS in Europe have been attributed to strains belonging to the serotype O157:H7 (EFSA, 2007). However, serotypes O26:H11, O103:H2, O111:H8, O145:H28, have also frequently been isolated from clinical cases (Bettelheim 2007).

Niche

The main reservoir of EHEC is cattle (Blanco et al. 2004; Verstraete et al., 2010). Cattle play an important role in the epidemiology of human infections (Griffin et al., 1991). Transmission generally occurs by faecal contamination of food, water or direct animal contact. A small percentage of cattle in a herd shed higher levels of EHEC (> 10^4 CFU g^{-1} faeces). They are called super-shedders and aid at maintaining EHEC on the farm and increase the potential risk of contaminating the food chain (Low et al., 2005; Omisakin et al., 2003).

Virulence

Principal virulence factors of EHEC are Shiga-toxin 1 and 2 (*stx1* and *stx2* genes), responsible for kidney damage. The adhesin intimin (*eae* gene) mediates intimate attachment of EHEC to the intestinal epithelial cells and causes attaching and effacing lesions in the intestinal mucosa. EHEC cells colonize the host gut by modifying the host enterocytes (gut epithelial cells) and so creating their niche. From this position, EHEC produce toxins that attack vital organs in the host body. Different variants of the virulence factors were described, mediating different biological activities (*stx2* variants) or host tissue tropism (*eae*-type) (Persson et al. 2007; Ramachandran et al. 2003). Stx1 and Stx2 are phage-encoded toxins and ehx, katP and espP plasmid-encoded toxins. The presence of these genes in the genome is not stable and transmission between strains is obvious. This makes that EHEC virulence can evolve fast.

In the current study, EHEC isolates from a wide variety of sources were genetically characterized, in order to determine pathogenicity and visualize relatedness and transmission.

MATERIALS AND METHODS

Strains

The study included 169 O157:H7 EHEC strains from bovine, food and human sources and 58 non-O157 EHEC belonging to serotypes O26:H11 (25), O26:H7 (1), O26:H28 (1), O103:H2 (13), O111:H8 (7) and O145:H28 (11) from human clinical sources, isolated in Belgium between 2000 and 2007.

PCR

Virulence gene detection and serotyping was performed by multiplex PCR (mPCR) and singleplex PCR. The Applied Molecular Genomics (AMG) group of the VIB Department of Molecular Genetics (UA-VIB) designed a proprietary 33 amplicon mPCR assay, containing 5 serogroups (O26, O103, O111, O145, O157), 5 FliC types (H2, H7, H8, H11, H28), virulence genes stx*1* with three variants (ab, c, d), *stx2* with six variants (c, c-O118, d_{act}, e, f, g) and *stx2* consensus, *eae* with five variants (β1, γ1, γ2, ε, ζ), *ehx*, *tir*, *katP*, *espP* and *saa* (Nielsen et al., 2003). In addition, results were completed (*stx2*, *cdtV*) and confirmed by singleplex PCRs described in literature.

PFGE

Fingerprinting was performed by PFGE (pulsed field gel electrophoresis) separation of *Xba1*-digested genomic DNA in accordance with the PulseNet Europe protocol. BioNumerics® (Applied Maths, Austin, Texas, USA) software was used to group isolates based on band pattern similarities where types and subtypes were defined to have resp. >95% and 100% dice similarity.

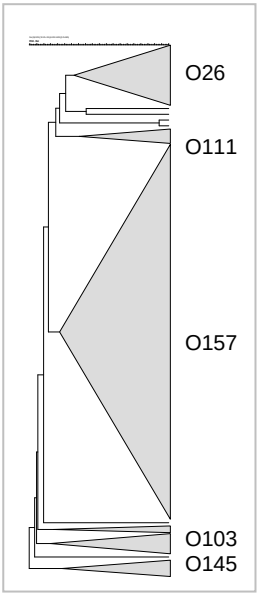
RESULTS

PCR typing resulted in diverge virulence patterns, listed in Table 1.

Table 1. PCR typing results of 169 O157:H7 and 58 non-O157 EHEC strains

O157:H7 EHEC	Non-O157 EHEC
100 % *stx* • 35 % *stx2c* • 32 % *stx2* • 17 % *stx2* + *stx2c* • 7 % *stx1* + *stx2c* • 5 % *stx1* + *stx2* • <5 % *stx1d*, *stx2d*$_{act}$ 100 % *eae*γ_1 100 % *ehx*, 99 % *espP*, 98 % *katP*	100 % *stx* • O26 89 % *stx1* • O103 92 % *stx1* • O111 57 % *stx1;* 43 % *stx2* • O145 55 % *stx2;* 26 % *stx1* 100 % *eae* (β, γ_1, γ_2, ε) 93 % *ehx*, 84 % *espP*, 76 % *katP*

A dendrogram based on similarities of *Xba1*-PFGE patterns was created (Fig.1). Results showed that strains clustered according to serogroup. An MDS view of human clinical EHEC isolates only, coloured according to their clinical manifestation, showed that related isolates did not have the same clinical outcome (Fig. 2). Results in Fig. 3 showed that related strains grouped together according to their *stx*-profile.



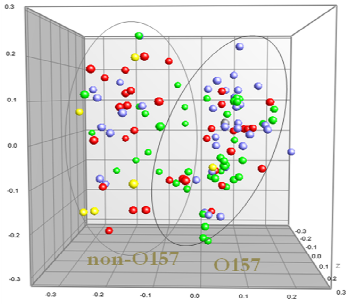


Figure 1 (left) PFGE-based clustering of EHEC isolates investigated in this study.

Figure 2 (above) MSD of PFGE-based clustering of human clinical EHEC isolates coloured according to their clinical manifestation, yellow: asymptomatic, red: diarrhoea, purple: bloody diarrhoea, green: HUS.

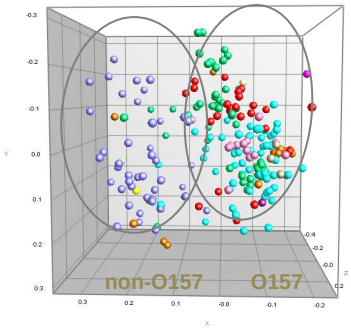
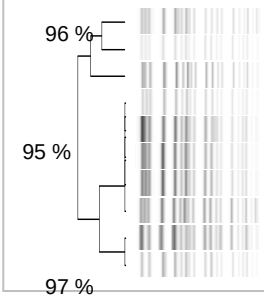


Figure 3 MSD of PFGE-based clustering of EHEC isolates coloured according to *stx*-profile.

PFGE-types (> 95 % similarity) had very similar virulence profiles, as demonstrated in Fig. 4. PFGE-types were associated to a variety of clinical outcomes. They infected as well animal as humans and were found in food products also. The PFGE-type persisted during several years.



O157:H7	**Cattle**	-	04/2005	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	Bloody Diarrhoea	05/2005	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	Diarrhoea	03/2003	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	not known	03/2004	*stx2 + stx2c*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	not known	05/2003	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	**Meat**	-	05/2003	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	**Meat**	-	05/2003	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	not known	07/2006	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	HUS	04/2003	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*
O157:H7	Human	Diarrhoea	09/2004	*stx2*	*eaeγ1*	*ehx*	*espP*	*katP*

Figure 4 PFGE-based clustering of a persisting PFGE-type, infecting animal, food and human.

DISCUSSION

A broad variety of virulence profiles among EHEC strains was observed, that correlated with the PFGE-based clustering of the strains. Possible routes of transmission were visualised, as identical PFGE-types were found in animals, food and humans. This indicates an intensive exchange exists between the EHEC-reservoir (cattle) and the environment.

REFERENCES

Bettelheim, 2007. The non-O157 Shiga-toxigenic (Verocytotoxigenic) *Escherichia coli*; Under-rated pathogens. Crit. Rev. Microbiol, 33, 67-87.

Beutin, L., Krause, G., Zimmermann, S., Kaulfuss, S., Gleier, K., 2004. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. 42, 1099–1108.

Blanco, M., Padola, N., Kruger, A., Sanz, M., Blanco, J., Gonzalez, E., Dahbi, G., Mora, A., Bernardez, M., Etcheverria, A., Arroyo, G., Lucchesi, P., Parma, A., Blanco, J., 2004. Virulence genes and intimin types of Shiga-toxin-producing *Escherichia coli* isolated from cattle and beef products in Argentina. Internat. Microbiol. 7, 269-276.

EFSA, 2007. Monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. The EFSA Journal 579, 1-61.

Griffin, P., Tauxe, R., 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other Enterohemorrhagic *Escherichia coli*, and the associated Hemolytic Uremic Syndrome. Epidemiol. Rev. 13, 60-98.

Karmali, M.A., 1989. Infection by verocytotoxin-producing *Escherichia coli*. Clin. Microbiol. Rev. 2, 5-38.

Low, J., McKendrick, L., McKechnie, C., Fenlon, D., Naylor, S., Currie, C., Smith, D., Allison, L., Galy, D., 2005. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. Appl. Envir. Microbiol. 71, 93-97.

Nielsen, E., Andersen, M., 2003. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5 ' nuclease PCR assay. J. Clin. Microbiol. 41, 2884-2893.

Omisakin, F., MacRae, M., Ogden, I., Strachan,N., 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. Appl. Envir. Microbiol. 69, 2444-2447.

Persson, S., Olsen, K., Ethelberg, S., Scheutz, F., 2007. Subtyping method for *Escherichia coli* Shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. J. Clin. Microbiol. 45, 2020-2024.

Ramachandran, V., Brett, K., Hornitzky, M., Dowton, M., Bettelheim, K., Walker, M., Djordjevic, S., 2003. Distribution of intimin subtypes among *Escherichia coli* isolates from ruminant and human sources. J. Clin. Microbiol. 41, 5022-5032.

Verstraete, K., De Zutter, L., Messens, W., Herman, L., Heyndrickx, M., De Reu, K., 2010. Effect of the enrichment time and immunomagnetic separation on the detection of Shiga-toxin-producing *Escherichia coli* O26, O103, O111, O145 and sorbitol positive O157 from artificially inoculated cattle faeces. Vet. Microbiol. 145, 106-112.

HORIZONTAL STUDY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ON TWO INDEPENDENT BELGIAN PIG FARMS

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) in animals has been reported since 2005 with increasing frequencies. Livestock-associated MRSA (LA-MRSA), with clonal complex ST398 has emerged in productive livestock, especially in pigs. This type has also been isolated from cattle, horses, poultry and even humans. Various studies report the transmission of this type of MRSA to humans, especially people in close contact with farm animals. There is an urgent need to evaluate the prevalence of LA-MRSA in pigs and other animals. This, to determine risk factors and transmission mechanism with as final endpoint controlling and reducing the occurrence of MRSA in farms with livestock.

The objectives of this horizontal study were (i) to evaluate if age is correlated with MRSA prevalence (ii) to study the MRSA contamination in the direct environment (iii) to determine possible sources for acquisition.

MATERIALS & METHODS

This study is part of a larger project in which a total of six farms were sampled: two closed pig farms, two poultry-pig farms and two cattle-pig farms.

Two independent closed Belgian pig farms were thoroughly sampled. Within each farm, a statistical number of nasal samples were collected from pigs of different ages: pre-weaning (1, 2, 3 and 4 weeks), post-weaning (age 4-10 weeks), fattening pigs (2.5-6 months) and sows (>7-8 months). In addition, the direct environment of the pigs was also sampled (floor, wall and air).

All swab specimens were enriched overnight in Mueller Hinton broth (Oxoid, Germany) supplemented with 6.5% NaCl. After 18-24 hours a loopful was plated onto MRSA-ID (BioMérieux, France). Characteristic colonies were interpreted following the manufacturers instructions and further analyzed by multiplex-PCR for 16S *r*RNA, *mec*A and *nuc* gene (1).

RESULTS

The pre-weaning prevalence in farm A and B differed significantly: 4.8% (4/84) and 18.8% (15/80). Post-weaning prevalences were 81.5% (66/81) and 95.9% (70/73), respectively on

farm A and B. In fattening pigs, the prevalence was still high on both farms, respectively 85.7% (72/84) and 86.8% (79/91). However, in sows MRSA was detected in very low numbers and this on both farms: 1.4% (1/73) and 3.0% (2/66). In the direct environment MRSA was most often isolated in barns with a high number of MRSA colonized pigs. Suggesting contamination of the barn environment with MRSA from the pigs, and vice-versa. Further the farmer and his family were sampled on several occasions. Family members working on the farm were at least MRSA positive on one occasion. Molecular typing of two human isolates originating from farm A, confirmed that the MRSA isolates belonged to the clonal complex ST398. This two human isolates shared the same *spa* type t567, which was also found as one of the *spa* types present in the pigs on that farm. Insinuating the transmission of this type of MRSA from pigs to humans, as was previously established by others (2).

DISCUSSION

This data demonstrate an increase in MRSA colonization after weaning, which remains high until slaughter age. Weaning is known to be a very stressful period, which can cause a decrease of immunity. This could lead, in combination with a high infection pressure in the barn, to an increase in MRSA colonization. On the contrary, MRSA colonization in the piglets and sows remains low on both farms. The prevalence in sows and in piglets may be related. Further, a low MRSA contamination of the environment both in the farrowing house as in the gestation stable, was found on both farms. This strengthens the idea of an existing correlation between the number of MRSA colonized pigs and the contamination rate of the barn environment.

Although this is a single point-in-time sampling, it gives an idea of the age-related MRSA colonization in pigs and also about the contamination of the direct barn environment.

REFERENCES

1. Maes et al., 2002
2. Huijsdens et al., 2006

PRELIMINARY RESULTS ON THE LINK BETWEEN BIOSECURITY STATUS AND HERD CHARACTERISTICS, DAILY WEIGHT GAIN, MORTALITY AND THE USE OF ANTIMICROBIAL DRUGS

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INTRODUCTION

Biosecurity gains importance for the health management of pig farms. It is believed that biosecurity influences production results, nevertheless, few studies succeed in demonstrating this link. In order to quantify the biosecurity status on pig herds a biosecurity scoring system was developed. This scoring system is used to study the relation between the biosecurity on a farm and herd characteristics, daily weight gain and mortality.

Furthermore, the high use of antimicrobial drugs on pig herds is becoming a problem. In this study the link between the biosecurity status of a pig herd and the amount of antimicrobial drugs used has also been investigated.

MATERIALS AND METHODS

Sixty randomly selected Belgian pig herd were visited in the last year. The herds were all closed (sows, piglets and fattening pigs at the same location) and had at least 80 sows and 400 fattening pigs. During a herd visit, the biosecurity status of the herd was quantified by means of a biosecurity scoring system (Laanen et al., 2010). This scoring system is available online in Dutch and in English on www.biocheck.ugent.be. After filling in the accompanying questionnaire, the scoring system provides a score between 0 (worst possible situation) and 100 (best possible situation) for both external and internal biosecurity.

During the herd visit, additional data concerning the herd and production characteristics such as: number of sows, number of piglets, number of fattening pigs, age of the buildings, years of experience of the farmer, daily weight gain and mortality of fattening pigs and the use of antimicrobial drugs were collected.

The data about the use of antimicrobial drugs were used to calculate the Treatment Incidence based on the Animal Daily Dose Pig (TI_{ADDpig}) according to the method of Timmerman et al, 2005.

RESULTS

Herd characteristics:

The correlations between the different herd characteristics and the external and internal biosecurity are shown in table 1.

Performance of fattening pigs:

The daily weight gain of the fattening pigs was positively correlated with both external ($r = 0.30$, $p = 0.02$) and internal ($r = 0.22$, $p = 0.09$) biosecurity. The correlation between mortality of fattening pigs and the external biosecurity was slightly negative ($r = -0.12$, $p = 0.38$).

Table 1. The correlation between herd characteristics and the biosecurity on pig herds.

Herd characteristic	Biosecurity	Correlation coefficient	P-Value
Number of sows on the herd			
	External	0.39	<0.01
	Internal	0.30	0.02
Number of piglets (<25kg) on the herd			
	External	0.39	<0.01
	Internal	0.29	0.03
Number of fattening pigs on the herd			
	External	0.26	0.05
	Internal	0.17	0.19
Age of the buildings			
	External	-0.21	0.10
	Internal	-0.39	<0.01
Years of experience of the farmer			
	External	-0.03	0.85
	Internal	-0.17	0.21

Use of antimicrobial drugs:

The average TI_{ADDpig} was 131.5, which means that for a total of 1000 pigs, 131.5 pigs were treated daily with a standard dose of an antimicrobial drug. The correlation between the TI_{ADDpig} and the external ($r = -.010$, $p = 0.43$) and internal ($r = -0.13$, $p = 0.33$) biosecurity was slightly negative.

DISCUSSION AND CONCLUSIONS

These results clearly indicate the existence of a positive correlation between the number of sows, piglets or fattening pigs on a herd and the level of biosecurity, indicating that farmers with larger herds pay more attention to biosecurity than those with smaller herds. The larger a herd becomes, the more professional and well managed it becomes. Yet it should be acknowledged that these correlations remain moderate to low indicating that many other factors also play a role in the level of biosecurity.

It is also noticeable that the biosecurity increases with a decreasing age of the buildings. This illustrates that in more modern infrastructure more attention is paid to biosecurity. The same holds for the years of experience of the farmer suggesting that younger farmers are more interested in, and willing to apply biosecurity measures.

When evaluating the link with daily weight gain of fattening pigs it appears that this increases with an increasing biosecurity, which is very interesting for farmers. Also the negative correlation between the mortality and the external biosecurity is of interest since this suggests that mortality decreases with an increasing external biosecurity.

The negative correlation between the biosecurity and the TI_{ADDpig} shows that on herds with a higher biosecurity less antimicrobial drugs are used when compared to herds with a lower biosecurity. This means that the use of antimicrobial drugs on a herd could be decreased by increasing the biosecurity level. The low correlation indicates that a lot of other factors, like the vaccination status of the herd, play a part in the amount of antimicrobial drugs used.

Several of the found correlations are not statistically significant and therefore need to be interpreted with care. Yet, in this study only the results of the first 60 herds out of an ongoing study on 100 herds are presented. Therefore it is believed that with increasing sample size several of the observed trends will become statistically significant. Moreover, the data will also be further explored to identify the parts of biosecurity that are most influential on the use of antimicrobial drugs, health and production parameters.

REFERENCES

Laanen M., Beek J., Ribbens S., Vangroenweghe F., Maes D., Dewulf J., 2010. Bioveiligheid op varkensbedrijven: ontwikkeling van een online scoresysteem en de resultaten van de eerste 99 deelnemende bedrijven. *Vlaams Diergeneeskundig Tijdschrift* 79 (4), 302-306

Timmerman T., Dewulf J., Catry B., Feyen B., Opsomer G., de Kruif A., Maes D., 2006. Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. *Preventive Veterinary Medicine* 74, 251-263

GLOBAL ERADICATION OF RINDERPEST: THE FINAL ACT

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Rinderpest is a contagious viral disease affecting all cloven-hoofed animals, mainly cattle and Asian domestic buffalo. Classical symptoms in cattle include ocular and nasal discharge, accompanied by fever, erosive lesions in the mouth, profuse diarrhoea and dehydration. In wildlife species, the clinical picture is more complex with African buffalo *(Syncerus caffer)* showing similar signs to cattle but in others e.g. lesser kudu (*Tragelaphus imberbis*), the only signs might be corneal opacity and blindness (11, 14). Mortality can attain 100% in immuno-naive cattle herds and 60% in free-ranging immuno-naïve buffalo populations (11). The presentation however will focus on strains of the virus, hypo-virulent in indigenous cattle, causing so called 'mild rinderpest', with very discrete clinical signs in livestock (but often as virulent in a range of wildlife species). Veterinary science distinguishes three lineages of the rinderpest virus :

1. Lineage 1 of the virus which caused classical rinderpest in Africa.
2. Lineage 2 of the virus which caused both hypo-virulent and virulent rinderpest in Africa.
3. Lineage 3 of the virus which caused rinderpest in Asia.

While cattle and buffalo are the iconic victims of rinderpest, several other species, both domesticated and wild, are also sensitive to the virus, including sheep and goats, wild suids, giraffe and a broad range of antelopes mainly of the genus *Tragelaphus*, though with different symptomatology. Based on these sensitivity differences, the clinical and serological surveillance of wildlife has played a major role in the eradication efforts of rinderpest on the one hand because of the high sensitivity of certain species to the virus and hence, ease of (clinical) detection, but on the other hand certain species also as serological sentinel animals due to the high morbidity and post-epidemic seroprevalence at the population level. Some species show low sensitivity to the virus (and high survival rate) but often in these seroprevalence is lower, in others mortality levels might be so high that little antibody can be detected in the population as there are few survivors. This approach has been especially important in cases when and where vaccination against rinderpest was applied in livestock (2, 6, 12, 13, 19).

Rinderpest diagnosis is usually based on clinical signs (the 3 D's : diarrhoea, discharge and death), but as these signs are common to other diseases and as there exists the occurrence of mild rinderpest, serology and virus isolation are regarded as essential. Several tests are recommended by the OIE, such as cell culture, histopathology, AGID and RT-PCR, while for serology two ELISA tests and one VNT have been recommended. Rinderpest control and eventually eradication has relied on vaccination mostly, using a live attenuated vaccine, initially produced through serial passages in goats (caprinised vaccine), but since 1970 produced on tissue cultures (RBOK-BK-VERO). Vaccine – induced immunity is reliable, protective, including lactogenic transmission, and long-lasting (2, 17).

More than 1,500 years ago rinderpest emerged to take its toll on humankind's domesticated animals. It is the only animal disease credited with changing the course of history. We tend to think of rinderpest as a tropical disease, but the virus was present in most of Europe and the Far-east at some point. Rinderpest was first recognized as a distinct plague (*the cattle plague*)

in 376-386 (1). Rinderpest outbreaks were later documented in paintings as far back as the early 18th century in the Netherlands and the 13th century in China and Mongolia.

The last outbreaks in Western Europe date back to the 19th century : the Netherlands (1869), France and Germany (1870), the United Kingdom (1900). In 1924, recurrent rinderpest outbreaks in some parts of Europe, including Belgium (1920), led to the creation of the *Office International des Epizooties* (OIE), now renamed *World Organisation for Animal Health.* The initiative was spearheaded by the Belgian Government, but it was the French Government which eventually provided the headquarters of the Organisation.

After the last of the European nations got rid of the disease well before the second World War, Africa and Asia became the new focus of eradication efforts. These efforts were conducted in Asia without too many technical set-backs, but this was not the case in Africa. Since 1962, the African States, with the assistance of the European Union (and its predecessors), had been involved in these eradication efforts, initially through the *Joint Programme 15.* The last continent-wide vaccination effort was conducted under PARC (*Pan-African Rinderpest Campaign*) from 1986 to 1999 and led to the near-eradication of the disease in most parts of Africa (7, 18) as verified through the activities of the *Pan-African programme for the Control of Epizootics* (PACE) from 2000 to 2007. While e.g. Botswana reported its last outbreak in 1899 and the Republic of South Africa in 1902, outbreaks still occurred nevertheless as late as 1987 in Nigeria and 1997 in Tanzania (4, 20).

The aforementioned silent circulation of 'mild rinderpest' in the area encompassing northern Kenya, eastern Ethiopia and southern Somalia, the so-called *Somali Eco-System*, became a major eradication challenge as soon as it became formally recognised in 1994 (15), not just because of the behaviour of the virus, but also because of the behaviour of the animals and their herdsmen (nomadic) and of the ongoing civil war in Somalia. Despite these harsh conditions, sustained serology and participative epidemiology (10) eventually led to the demonstrated eradication of the disease and the infection from both wildlife (13) and livestock in this area (in 2010).

The last occurrence of rinderpest in Asia was reported in livestock in Pakistan, in the year 2000 (4, 16), while the last confirmed outbreak of rinderpest in Africa occurred in wild African buffalo in Meru National Park (Kenya) in 2001 (5, 8, 11, 12, 13).

In 1994, encouraged by the results obtained in various parts of the world, OIE and FAO established the *Global Rinderpest Eradication Programme* (GREP). This Programme, a key element within the FAO's *Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases* (EMPRES), was conceived as an international coordination mechanism to promote the global eradication of rinderpest and verification of rinderpest freedom, while providing harmonised technical guidance to achieve these goals. From the outset, GREP was a time-bound programme, due to declare rinderpest freedom by 2011 (1, 3).

Over the years, a total of 266 country-applications from OIE Members and non-Members have been submitted and scrutinised by the OIE Scientific Commission for Animal Diseases, with various degrees of acceptance as a result (numerous applications were referred back to the countries at least once). In most of these cases, countries submitted first a case for recognition of disease freedom, i.e. shortly after the end of vaccination campaigns, and two or more years later a submission for recognition of infection freedom, i.e. the serological absence of any rinderpest related traces.

From a worldwide perspective, almost all countries known to keep rinderpest susceptible livestock have been recognized by the OIE as being officially free from rinderpest infection. A handful of remaining countries are in the process of submitting documented evidence to

OIE for evaluation and the epidemiological situation in their corresponding region has not indicated any circulation of the disease or its virus in the natural host for many years.

Out of the 177 OIE Member countries and territories, only 2 have not submitted a request for official recognition yet : Kazakhstan and Sri Lanka. Another 3 are currently being processed for evaluation or awaiting clarification of pending issues, namely the Federated States of Micronesia, Kyrgyzstan and Turkmenistan. Out of the remaining 21 non-OIE Members, only one requires special efforts to enable the country to be declared free before the deadline, i.e. Liberia.

In addition, the global eradication of rinderpest demands that the international community establishes an inventory on existing rinderpest virus stocks in order to prevent the re-emergence of the disease through release of rinderpest virus from laboratory sources. To this end FAO and OIE, through a newly established Joint Committee, have committed themselves to establish the principles of international oversight and regulations for facilities holding rinderpest virus containing material. Specific guidelines are being developed to ensure secure handling and sequestration of rinderpest virus in the post-eradication era. Additionally, countries are encouraged to safely reduce the number of rinderpest virus repositories under official supervision in order to minimise the risk of accidental release.

The launching of GREP was founded on the scientific understanding that the eradication of rinderpest was feasible. Not only has eradication proved feasible, it has probably already been achieved. If everything goes according to plan, worldwide rinderpest eradication could be announced jointly by the two Directors General of OIE and FAO, on both occasions, during the May 2011 OIE General Session and the June 2011 FAO Conference. Thirty one years after smallpox was eradicated by the *World Health Organisation* (WHO), rinderpest will be the second disease ever to be eradicated from the planet. The same principles, though not immediately in view of world-wide eradication, are now being developed for the progressive control of foot-and-mouth disease (9).

REFERENCES

1. Anon. (1999) FAO EMPRES Transboundary Animal Diseases Bulletin: Issue No. 11 (September 1999), Rome. Online : http://www.fao.org/docrep/X3444e/x3444e00.htm
2. Anon. (2009) OIE Terrestrial Animal Health Code, Paris. (volume 2) 486 – 492.
3. Anon. (2010) FAO GREP, Rome : http://www.fao.org/ag/againfo/programmes/en/grep/home.html
4. Anon. (2010) OIE World Animal Health Information Database, Paris. Online : www.oie.int/wahid
5. Bastiaensen P., Denormandie N., Squarzoni C., Bidjeh K., Diop B.A., Bessin R. (2007) *"Eradication planétaire de la peste bovine : la dernière ligne droite"* [Planetary eradication of rinderpest : the last straight line]. Tropicultura. 25 (2) 113-118.
6. Couacy-Hymann E, Bodjo C, Danho T, Libeau G, Diallo A. (2005) Surveillance of wildlife as a tool for monitoring rinderpest and peste des petits ruminants in West Africa. Rev Sci Tech. Dec ; 24(3): 869-77.
7. Couacy-Hymann E, Bodjo SC, Koffi MY, Danho T. (2007) Observations on rinderpest and rinderpest-like diseases throughout West and Central African countries during rinderpest eradication projects. Res Vet Sci. Oct ; 83 (2): 282-5.
8. Diop BA, Bastiaensen P. (2005) Achieving full eradication of rinderpest in Africa. Vet Rec. Aug 20 ; 157 (8): 239-40.
9. Domenech J, Lubroth J, Sumption K. (2010) Immune protection in animals: the examples of rinderpest and foot-and-mouth disease. J Comp Pathol. Jan ; 142 Suppl 1: S120-4. Review.
10. Jost CC, Mariner JC, Roeder PL, Sawitri E, Macgregor-Skinner GJ. (2007) Participatory epidemiology in disease surveillance and research. Rev Sci Tech. Dec; 26 (3): 537-49. Review.
11. Kock R.A., Wambua J.M., Mwanzia J., Wamwayi, H.. Ndungu, E.K Barrett, T. Kock, N.D.. Rossiter P.B (1999) Rinderpest epidemic in wild ruminants in Kenya 1993-7 Vet Rec. 145, 275 – 283
12. Kock R.A. (2006) Rinderpest and wildlife. In: Rinderpest and Peste des Petits Ruminants Virus. Plagues of large and small ruminants. Edited by Thomas Barrett, Paul-Pierre Pastoret, and William Taylor Biology of Animal Infections Elsevier publications Academic Press, London. Chap. 7 pp. 144-162

13. Kock R.A., Wamwayi, H. M., Rossiter P. B., Libeau G., Wambwa, E., Okori J.,.Shiferaw, F. S, Mlengeya, T. D. (2006). Rinderpest in East Africa: continuing re-infection of wildlife populations on the periphery of the Somali ecosystem. Prev. Vet. Med Vol 75/1-2 pp. 63-80.
14. Kock N.D., Kock R.A., Wambua J., Mwanzia. J. (1999) Pathological changes in free ranging African Ungulates during a rinderpest epiizootic in Kenya 1993 - 1997 Vet. Rec. 145, 527 – 528
15. Mariner JC, Roeder PL. (2003) Use of participatory epidemiology in studies of the persistence of lineage 2 rinderpest virus in East Africa. Vet Rec. 2003 May 24;152(21):641-7.
16. Matin MA, Rafi MA. (2006) Present status of rinderpest diseases in Pakistan. J Vet Med B Infect Dis Vet Public Health. Dec ; 53 Suppl 1: 26-8.
17. Roeder PL, Lubroth J, Taylor WP. (2004) Experience with eradicating rinderpest by vaccination. Dev Biol (Basel); 119: 73-91. Review.
18. Roeder PL, Taylor WP. (2007) Mass vaccination and herd immunity: cattle and buffalo. Rev Sci Tech. Apr ; 26 (1): 253-63. Review.
19. Rossiter P, Wamwayi H, Ndungu E. (2006) Rinderpest seroprevalence in wildlife in Kenya and Tanzania, 1982-1993. Prev Vet Med. Jul 17; 75 (1-2): 1-7.
20. Taylor WP, Roeder PL, Rweyemamu MM, Melewas JN, Majuva P, Kimaro RT, Mollel JN, Mtei BJ, Wambura P, Anderson J, Rossiter PB, Kock R, Melengeya T, Van den Ende R. (2002) The control of rinderpest in Tanzania between 1997 and 1998. Trop Anim Health Prod. Nov ; 34 (6): 471-87.

THE RELEVANCE OF MANAGEMENT, METEOROLOGICAL AND ENVIRONMENTAL FACTORS IN THE SPATIAL DISTRIBUTION OF *FASCIOLA HEPATICA* IN DAIRY CATTLE IN FLANDERS.

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INTRODUCTION

Fasciola hepatica, a trematode parasite with a worldwide distribution, is the cause of important production losses in the dairy sector. The diagnosis in adult dairy cows is hampered by the fact that the infection is subclinical. To increase awareness and develop regionally adapted control methods, knowledge on the spatial distribution of economically important infection levels is needed. Previous studies modelling the spatial distribution of *F. hepatica* are mostly based on single cross-sectional samplings and have focussed on climate and environment factors, often neglecting management factors. Also, they have typically been used to describe the presence of infection, omitting to take into account the level of infection and economic impact. Therefore the objectives of this study were to (1) assess the prevalence and spatial distribution of economically important *F. hepatica* infections over three consecutive years in dairy herds in a temperate climate area (Flanders, Belgium), (2) link the interannual changes in prevalence and spatial distribution with meteorological factors and (3) assess the importance of management, climatic and environmental factors in the spatial distribution of economic *F. hepatica* infections.

MATERIALS AND METHODS

A bulk-tank milk antibody ELISA was used to measure *F. hepatica* infection levels in a random sample of 1762 dairy herds in the autumn of 2006, 2007 and 2008. Since the test results have been shown to be associated with production, a cut off was used identifying herds likely to suffer production losses due to fascioliosis (ODR>0.8). The infection levels were included in a Geographic Information System next to meteorological and environmental parameters. Management parameters were determined for a subset of 464 herds using a questionnaire, and were included in the database.

Logistic regression models were used to determine associations between possible risk factors and infection levels in 2006, comparing three different models: 1) including only climate and environment factors, 2) including only management factors and 3) including factors from both management and climate and environment. The models were validated by a K-fold cross-validation procedure combined with Receiver Operating Characteristic (ROC) analysis. For that purpose, the dataset was partitioned in 10 groups of around 160 cases for model 1 and in 5 groups of around 100 cases for models 2 and 3, each run using one of the groups as validation set and the other as training set. For each run (10 for model 1 and 5 for models 2 and 3) the Area under the Curve (AUC) of the ROC curve was calculated in the validation set, and from these values the average AUC was calculated.

RESULTS

The prevalence of *F. hepatica* was stable, with small interannual differences in prevalence, being 37.3% (95%CI: 35-40) in 2006, 40.4% (38-43) in 2007 and 40.2% (38-43) in 2008. The spatial distribution was also relatively stable with small differences in size and location of clusters (Figure 1). Considerable variation in meteorological factors was observed (Figure 2).

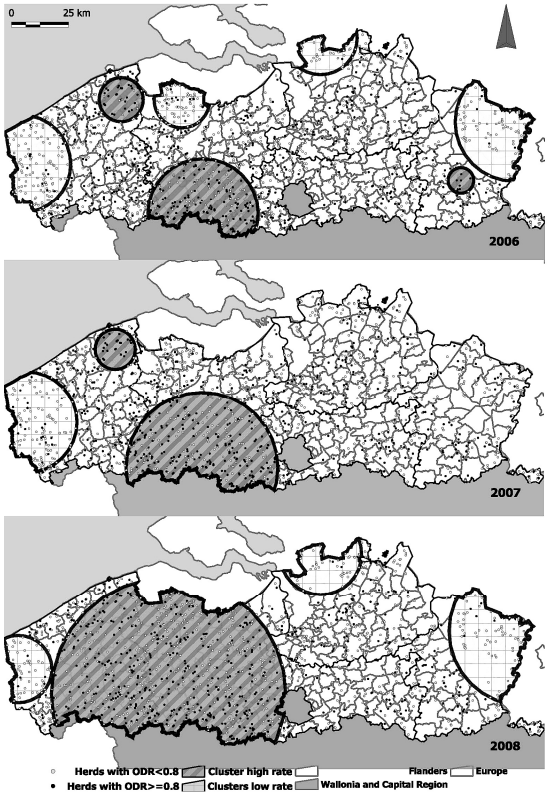

Figure 1 Spatial distribution of *F. hepatica* economically positive herds (ODR>0.8) in 1680, 1545 and 1481 georeferenced bulk-tank milk samples collected from dairy herds in Flanders (Belgium) in the autumn of 2006, 2007 and 2008, respectively.

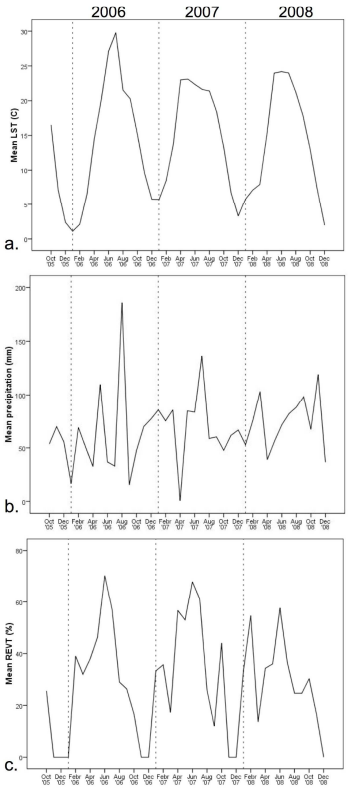

Figure 2 Graph of the monthly means of LST (a), precipitation (b) and REVT (c) from October 2005 till December 2008.

Model 1, based on climatic/environmental factors, included annual rainfall, elevation and slope, soil type. The logistic regression model based on management (model 2) included the factors mowing of pastures, proportion of grazed grass in the diet and length of grazing season as significant predictors. Model 3, including both management and climate/environment, consisted of the same risk factors as model 2, adding annual rainfall. The models are shown in Table 1. The AUC was 0.62, 0.68 and 0.68 for model 1, 2 and 3, respectively. A risk map was built based on model 3 combining management and climatic/environmental factors, using Ordinary Kriging to interpolate the probability outcome of the model. To provide insight into the uncertainty of the produced risk map, a map displaying the standard deviation (SD) of the probabilities over the 5 runs was also kriged. Both maps are shown in Figure 3. The riskmap was shown to be useful: when the 1216 georeferenced herds not included in the management sample were used as a validation set, an AUC of 0.66 was found.

Table 1. Multivariate logistic regression model of climate and environment factors associated (model 1), management (model 2) and a combination of the two (model 3) with economically important *F. hepatica* infection levels as dependent variable.

Model	Variables	B	S.E.	P
Model 1 (n=1680)	Elevation	-0.006	0.002	0.006
	Slope	0.131	0.035	<0.001
	Rainfall nov2005-oct2006	-0.064	0.011	<0.001
	Humid loam	0.664	0.219	0.002
	Schor polders	1.704	0.675	0.012
	Sand	-0.233	0.121	0.053
	Constant	3.338	0.707	<0.001
Model 2 (n=464)	Herd size			0.029
	>60	Baseline		
	30-60	0.566	0.283	0.046
	<30	-0.094	0.378	0.804
	Mowing (cows)			<0.001
	No mowing	Baseline		
	Mowing	-1.337	0.31	<0.001
	Mowing partly	-0.729	0.294	0.013
	Length grazing season (cows)	0.41	0.136	0.003
	Grass % in diet (cows)			0.003
	100% grazing	Baseline		
	>50%	-1.779	0.479	<0.001
	<50%	-1.527	0.501	0.002
	No grazing	-1.155	1.362	0.396
	Constant	-1.483	1.078	0.169
Model 3 (n=464)	Rainfall nov2005-oct2006	-0.061	0.025	0.016
	Herd size			0.031
	>60	Baseline		
	30-60	0.566	0.284	0.047
	<30	-0.088	0.38	0.817
	Mowing (cows)			<0.001
	No mowing	Baseline		
	Mowing	-1.334	0.313	<0.001
	Mowing partly	-0.733	0.297	0.014
	Length grazing season (cows)	0.376	0.137	0.006
	Grass % in diet (cows)			0.004
	100% grazing	Baseline		
	>50%	-1.777	0.485	<0.001
	<50%	-1.583	0.508	0.002
	No grazing	-1.295	1.37	0.345
	Constant	1.163	2.241	0.604

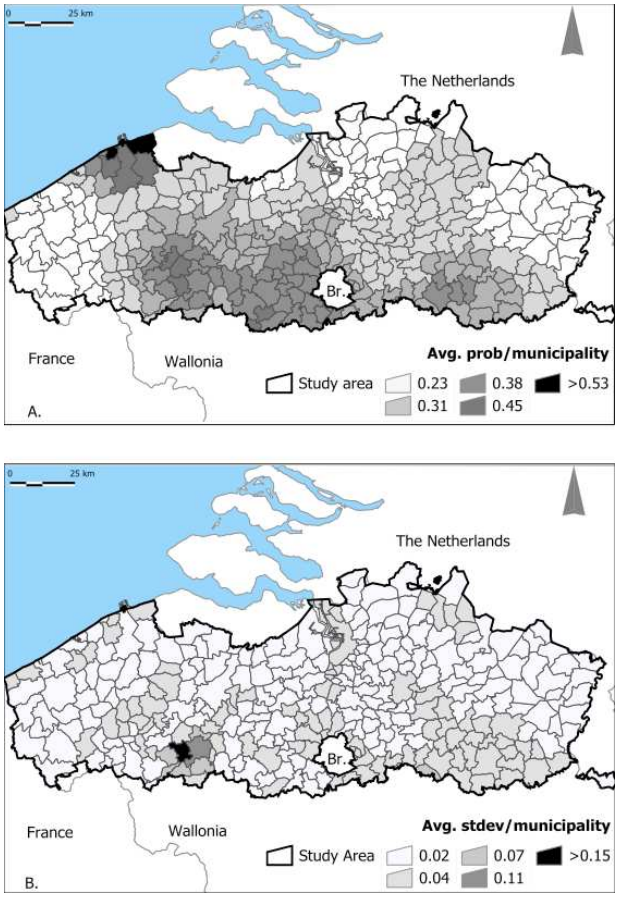

Fig. 3. (A) Risk map based on model 3, displaying the probability averaged on municipality level. The best probability cut off for economically important infections was 0.38. (B) Map displaying the average standard deviation of the probability over the 5 runs. Br.= Brussels.

CONCLUSION

The results of the modelling indicate that in temperate climate zones without large climatic and environmental variation, management factors are essential to the spatial distribution of *F. hepatica*, and should be included in future spatial distribution models. The higher the exposure to pasture, the higher the infection risk. Mowing had a protective effect, which could be explained by the removal of last year's metacercariae or eggs and miracidia. The effect of herd size is probably related to unmeasured management factors. The negative effect of yearly rainfall could indicate that in Flanders, rainfall is generally high and above a certain threshold can cause a wash away effect of free-living stages and snails.

The inclusion of management factors in risk models provides the opportunity to deliver not only risk maps, but also regional management advice resulting from these maps.

Since the prevalence of *F. hepatica* was relatively stable in the study period a longer monitoring period is needed to study the link between the temporal distribution of *F. hepatica* and interannual meteorological differences.

EFFECT OF ORAL SUPPLEMENTATION OF MEDIUM CHAIN FATTY ACIDS (AROMABIOTIC®) ON BLOOD AND MILK NEUTROPHIL VIABILITY OF DAIRY HEIFERS AND COWS IN EARLY LACTATION

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INTRODUCTION

Polymorphonuclear neutrophilic leukocytes (PMN) play an important role in the first line immune defence of the mammary gland[1]. Both heifers and multiparous cows suffer from immune suppression around parturition, characterized by a higher proportion of apoptotic (= less viable) blood and milk PMN[2]. This phenomenon is most probably associated with the higher prevalence and increased severity of mastitis in that particular period. Because of public concerns about emergence of antibiotic resistance and drug residues in milk, attempts have been made to evaluate the efficacy of alternatives to antibiotic therapy in treating and controlling mastitis. Medium chain fatty acids (MCFA) have been hypothesized to modulate immunity in humans[3]. Based on experiences from the field, oral supplementation of MCFA (Aromabiotic®) seems to improve udder health in bovine as well. However, results from clinical trials including both treated and control animals are still lacking although they are warranted to ascertain the potential efficacy of these lipid molecules as well as to reveal their potential effect on bovine blood and milk PMN survival.

The objective of the present study was to explore the effect of orally supplemented MCFA to heifers and multiparous cows starting 6 to 8 weeks prior to calving on blood and milk PMN apoptosis between 1 and 3 days after calving in a double-blinded clinical trial including treated as well as control animals kept under the same management.

MATERIALS AND METHODS

Study design

A randomized double-blinded clinical trial was conducted from June 2009 to June 2010 on the research dairy farm of Ghent University (Biocentrum Agri-Vet, Melle, Belgium). Twelve animals from all lactating cows in the herd as well as 10 animals from all pregnant heifers in the herd were selected according their expected calving date. These 22 animals were randomly assigned to either the control group (n = 11) or the MCFA (test) group (n = 11). From the start of the trial (on average 53 days before calving) until 4 months post partum, pregnant heifers and dry cows assigned to the test group received 25 gram of an MCFA-containing powder daily (Aromabiotic®) top-dressed on the feed while locked in the feeding barrier.

Data and sample collection

At the onset of the trial period, blood samples were collected from the tail vein of all multiparous cows and heifers included in the study to determine blood PMN viability. Additionally, composite milk samples for determination of the milk PMN viability (100 mL) and somatic cell count (30 mL), and duplicate quarter milk samples for bacteriological culturing were collected from the multiparous cows only just before they were dried off. Between 1 and 3 days after calving, duplicate quarter milk samples for bacteriological culturing were again collected from all multiparous cows as well as heifers enrolled in the study. The viability of PMN in both blood and milk was estimated by determining the proportion of apoptotic PMN using flow cytometry as described by Piepers et al. (2009)[4].

Bacteriological culturing and isolate identification was done as previously described[5].

Also, composite milk somatic cell count (cells x 1000 per ml) and milk yield (kg of milk per day) at test-day for the first 4 recordings after calving were used per animal on a four-weekly basis as part of the Dairy Herd Improvement program (VRV, Oosterzele, Belgium).

Statistical analyses

To evaluate the effect of MCFA supplementation before calving on the blood PMN viability shortly after calving, a linear mixed model with animal as random effect was fit. The model with blood PMN viability as outcome variable included supplementation before calving (main predictor of interest), parity, period and the different interaction terms as categorical predictor variables. An identical linear mixed regression model with animal as random effect was fit to determine the effect of MCFA supplementation before calving on the evolution of the milk PMN viability across the dry period of the multiparous cows. The association between MCFA supplementation before calving and milk PMN apoptosis of both heifers and cows shortly after calving was evaluated fitting a linear regression model including supplementation (main predictor of interest), parity and the interaction term between both variables as categorical predictor variables. A backward stepwise modeling procedure was used to eliminate non-significant terms from the initial models. Statistical significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

At the onset of the study, significant differences in neither blood nor milk PMN viability were found between treated and control animals. In non-supplemented animals, blood PMN apoptosis significantly increased between start of supplementation and the first days after calving ($P < 0.001$) whereas no substantial change in blood PMN apoptosis could be observed in the MCFA supplemented animals ($P = 0.69$) (Figure 1). As was expected based on literature, blood PMN apoptosis shortly after calving was higher in multiparous cows than in heifers ($P < 0.05$). Still, an identical effect of oral MCFA supplementation on blood PMN apoptosis was observed for both heifers and multiparous cows. Similar results were obtained for milk PMN apoptosis in multiparous cows. Overall, the proportion of apoptotic milk PMN in early lactation was lower in the MCFA supplemented group compared to the non-supplemented group ($P < 0.001$) (Figure 2). As compared to blood PMN apoptosis, milk PMN apoptosis shortly after calving was higher in multiparous cows than in heifers as well ($P < 0.01$).

CONCLUSIONS

Oral supplementation of MCFA to heifers and multiparous cows from 6 to 8 weeks before calving appears to curb the natural “dip” in the systemic as well as the local innate immunity shortly after calving independently from the cows’ parity. To what extent the observed differences in blood and milk PMN viability will eventually result in a lower prevalence of intramammary infections and a better udder health and higher milk production throughout lactation merits further research.

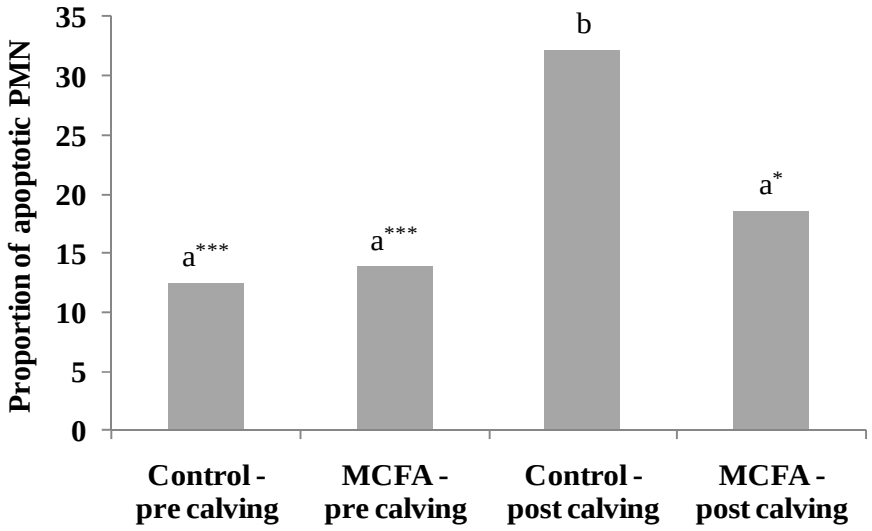

Figure 1 The proportion of apoptotic blood PMN before and after calving of both control and MCFA supplemented multiparous cows and heifers. Different superscript letters indicate significant differences: * ($P < 0.05$); ** ($P < 0.01$) and *** ($P < 0.001$).

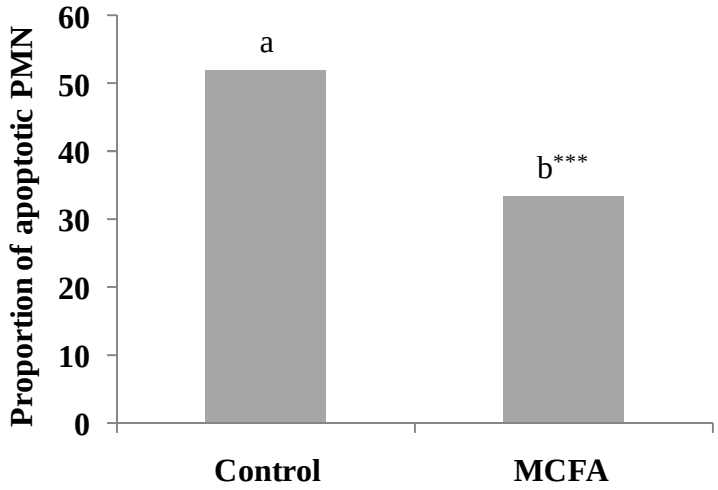

Figure 2 The proportion of apoptotic milk PMN after calving of both control and MCFA supplemented multiparous cows and heifers. Different superscript letters indicate significant differences: * ($P < 0.05$); ** ($P < 0.01$) and *** ($P < 0.001$).

REFERENCES

1. Rainard, P. and Riollet, C. (2009). Innate immunity of the bovine mammary gland. Vet. Res. 37:369-400.
2. Van Oostveldt, K. et al. (2001). Apoptosis and necrosis of blood and milk polymorphonuclear leukocytes in early and midlactating healthy cows. Vet. Res. 32:617-622.
3. Gagnon, L. et al. (2007). Medium-chain length fatty acids, glycerids and analogues as neutrophil survival and activation factors. World Intellectual Property Organization WO 02/083120 PCT/CA02/00535.
4. Piepers, S. et al. (2007). Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J. Dairy Res. 74:478-483.
5. Piepers, S. et al. (2009). Heifer and quarter characteristics associated with periparturient blood and milk neutrophil apoptosis in healthy heifers and in heifers with subclinical mastitis. J. Dairy Sci. 92:4330-4339.

ETHICAL ASPECTS OF ENDEMIC DISEASE ERADICATION

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INTRODUCTION

It is important to correctly define what we mean with "ethics" when dealing with any specific topic. Ethics, as many scholarly disciplines, is not a unified, monolithical field of work. Many subdisciplines possibly draw on the same basic principles, but are concerned with entirely different subjects. One can in this case refer to ethical subdisciplines such as medical ethics, veterinary ethics, environmental ethics, food ethics and many more.

Next to this "horizontal" differentiation between ethical subdisciplines, there is an analogue diversification along "vertical" lines. There is indeed a difference between the fundamental work of authors such as Kant (1785) and the writings of e.g. Singer (1975) on animals. This difference is not just in scope, but there is also a major difference in the way it relates to the physical world, with respect to its subjects, its methodology and, in part, with respect to the way its conclusions are to be valuable for daily life.

All the above mentioned disciplines are essentially concerned with the same fundamental question: "What is good?". Some approach this question at a very fundamental level (e.g. Kant), others at a more apllied level (e.g. Singer), but all try to differentiate between right and wrong. The methodology used is not always the same, some use virtues (e.g. Aristotle), others rights (e.g. Kant), consequences (e.g. Bentham), etc., but with all it is clear that ethics is not "an option". The ethical assessment of an action is not something that can be done parallel to – or even worse: after – the action, but needs to be an integral part of it in order for the action to be genuinely ethical.

ANIMALS' SOCIETAL AND MORAL STATUS

The individual animals' status depends on the specific human-animal relation (bond) it is a part of. As discussed at large elsewhere (De Tavernier et al., 2005), there are a multitude of different possibilities (utility animals, pets, hobby animals, …) that determine how the animals' "value" is understood. Many of the contemporary societal discussion on animal use seems to be rooted in a shift of the "reference animal": historically the utility animal was the animal that was ubiquitous, now it is the pet.

In ethics we have moved from an animal protection ethic to an animal welfare and even animal rights ethic:

"Our duties towards animals are merely indirect duties towards humanity. […] [a man] must practise kindness towards animals, for he who is cruel to animals becomes hard also in his dealing with men." (Kant, 1785)

No matter what the nature of the being, the principle of equality requires that its suffering be counted equally with the like suffering - insofar as rough comparisons can be made - of any other being. (Singer, 1975)

It is clear that current agricultural practices are at odds with the ideas of anybody adhering to the strong version of any welfare or rights ethics. A moderately anthropocentric approach on the

other hand (see De Tavernier et al., 2005) yields the possibility to introduce some of the concerns raised by welfare and rights ethicists, without necessarily condemning all animal husbandry.

If progress is to be made within societal debates on animal use, it seems important to distinguish to important aspects within those debates: (1) what matters to the animal (= animal welfare), and (2) what matters to humans (= human ethics).

ETHICAL ANALYSIS TOOLS

The Ethical Matrix – A principlist approach

A first tool that is often used within agricultural and food ethics contexts, is the Ethical Matrix, first developed by Mepham (1996). In an EM, the complexity of ethical deliberation is reduced by dividing the problem along two different lines. The first step is to construct a list of relevant *prima facie* ethical principles (the columns of the matrix). These are ethical principles that are conditional, meaning that a stronger claim can overrule a weaker one. The four *prima facie* principles used are those introduced by Beauchamp and Childress (2001) in the context of biomedical ethics: non-maleficence, beneficence, autonomy and justice (commonly dubbed the "Georgetown Mantra"). Mepham has combined non-maleficence and beneficence in the term "well-being", which reduced the size of his EM by a quarter without loosing information.

Another element is necessary: a list of (relevant) agents that have interests (the rows of the matrix). This list will depend on the matter under investigation, but there will normally be human interest groups (producers, consumers, …) and often animals (laboratory animals, farm animals, …). It may also include plants or "the environment" as such (see e.g. Mepham and Tomkins, 2003). Not everyone will accept non-sentient entities as an interest group; one can therefore opt to assign the concerns they represent to other groups (such as citizens or consumers).

The ethical issues connected to the issue being analysed will then be represented by the cells of the matrix (i.e. the combination of the conditional principles and the interest groups, see table 1). The analysis itself consists in deciding – cell by cell – whether the three principles have been respected.

Table 1: Empty version of the Ethical Matrix

	Well-being	**Autonomy**	**Justice**
Farmers			
Consumers			
Farm animals			
Environment			
…			

Using this matrix, one could systematically analyse different eradication options for a certain endemic disease. One would to take all available scientific information and draw conclusions from that, this could be called an "open" application of the EM. This approach seems only feasible when the EM is used for creating scientific or policy reports and maybe in public participation exercises, when time is available to investigate all positions thoroughly. This exercise will result in a clearer "topography" of the disease eradication case, but it will not automatically result a clearcut answer to which option is best.

Autonomy – Rights and duties

Respect for autonomy implies respecting the right to make autonomous decisions and this has to be considered within each stakeholder group. In other words, it plays on the autonomy-paternalism dilemma. In this context, this means balancing the autonomy of the farmer against the (probably inherent) paternalism of the disease management authorities. Three concepts or "rights" can be invoked in this context: the "right to know", the "right not to know", and the "duty to know".

If we value the autonomy of an individual animal owner (farmer), it seems that his/her right to know (whether an animal is infected) is important, but also that he/she can choose not to have an animal tested (right not to know). On an individual level, we already encounter a dilemma: which right takes priority when an animal is sold? The new owners right to know whether an animal is infected, or the old owners right not to know?

On the broader level (sector, region, country, …), this dilemma is even more complex. Can the individuals right not to know take priority over the interests of the sector in general? Is it not everyone's duty (to know) to protect the common good? This has been discussed at length elsewhere (Evers et al., 2008)

A discussion about the balance between these three principles, and thus about our position on the autonomy-paternalism continuum, will definitely inform competent authorities about which options are within the range of possibilities. These may then be fed into the Ethical Matrix analysis.

Learning from experiences with epidemics

There is a striking difference between public reaction to stamping-out and the lack of reaction against the killing of the same animals for food, which is an economic goal in its own right. Although the difference in media coverage probably contributes to this (day-to-day slaughtering procedures are not often shown in the media), it is a sign that non-economic considerations such as animal welfare and environmental issues are gaining importance in the animal disease control debate. The underlying question, therefore, is "how to balance different (legitimate) concerns?"

Even for people professionally involved with animal disease control it is clear that the classical approach to the problem is no longer sufficient. Unfortunately, veterinarians and animal production specialists are often untrained in such sociological and ethical discussions. Moreover, during an outbreak, there is often no time for anything but "crisis management", leaving little time for a thorough analysis of the alternatives to the classical approach (which has been proven to be successful in a variety of situations). The fundamental question to be resolved in this chapter is therefore how to identify the ethically best animal disease intervention (i.e. control, eradication, …) strategy *in tempore non suspecto*.

The Animal Disease Intervention Matrix (ADIM; in Dutch "Ethische Matrix Dierziektebestrijding", EMD) has been developed by Aerts and Lips (2006) to be able to analyse and discuss the different eradication options. The general problem is segmented into smaller parts: the different objectives that a good disease control scenario should achieve. Through intensive discussions with all stakeholders and disease control experts, 15 objectives have been identified:

1. Protecting the health of control personnel and farmers

2. Protecting public health
3. Protecting animal health
4. Ensuring animal welfare
5. Respecting the human-animal bond
6. Limiting environmental damage
7. Limiting the psychological impact on the farmer
8. Limiting the psychological impact on the control personnel
9. Respecting food
10. Limiting disturbance of social life
11. Limiting economic losses in agriculture
12. Limiting economic losses in non-agricultural sectors
13. Ensuring practicality
14. Ensuring food security
15. Protecting valuable animals

Not all of these are equally relevant when endemic diseases are considered and not epidemic diseases. A more systematic review of the matter is certainly necessary, but in a preliminary assessment, it seems that the following sets of "technical" and "societal" objectives are to be retained.

- Technical: protecting animal health, ensuring animal welfare, limiting economic losses in agriculture, ensuring practicality, and protecting valuable animals;
- Societal: respecting the human-animal bond, respecting food, ensuring food security.

While considering the elements connected to these eight remaining objectives, it is important to not limit oneself to agricultural or veterinarian situations and issues. Also in endemic disease cases, pets, hobby animals, exotic animals, and other groups of animals can be affected. The high number of owners, and the radically different cost-benefit analyses may prove as challenging here as in the case of an epidemic.

ANALYSIS OF ENDEMIC DISEASE ERADICATION

Within the criteria listed lies the answer to the question posed: "Endemic disease eradication: how far must we go?" Unfortunately, this question is not entirely unambiguous. Does one mean "How far do we have to go?"; which essentially asks to define the minimum level of action to be taken. Or can the question be paraphrased as "How far are we allowed to go?"; i.e. laying down the maximum action level. The only thing that is clear, so it seems, is that one is not trying to aim for the absolute maximum; something that could be translated as "How far are we able to (can we) go?".

A preliminary account of the issue suggests that different criteria are limiting for each of these different questions. The minimum acceptable effort seem to be defined by "animal health" and "economy", while the maximum effort seem to be limited by "economy" and societal objectives.

CONCLUSION

Relating to the account given above, is a daunting question: "how far *do* we go?" How are we *now* doing with regard to the criteria and principles mentioned? Are we promoting well-being, preventing harm, securing autonomy and doing justice to all? Do we succeed in protecting animal health, applying practical solutions, etc? How far are we off the reasonable maximum effort?

It is my suggestion that we should aim for the maximum ethically acceptable effort. This means that we, as a society, as a sector, as veterinarians, farmers, stakeholders need to do more than what we "must" do. In my – be it preliminary – analysis this can only be the case if we let the collective take priority over the individual, replacing the "right not to know" by the "duty to know", even if this means that little autonomy is left to the animal's owner.

REFERENCES

Aerts, S. & Lips, D. (2006). Development of an Animal Disease Intervention Matrix (ADIM). In: M. Kaiser & M. Lien (eds.). *Ethics and politics of Food.* Wageningen Academic Publishers, Wageningen. p. 233-238.

Beauchamp, T.L. and Childress, J.F. (2001). *Principles of biomedical ethics (5th ed.).* New York, Oxford University Press.

De Tavernier, J., Lips, D. and Aerts, S. (2005). *Dier en welzijn.* Leuven, LannooCampus.

Evers, J., Aerts, S. & De Tavernier, J. (2008). An ethical argument in favor of nano-enabled diagnostics in livestock. *Nanoethic* , 2(2): 163-178.

Kant, I. (1785). *Fundamental principles of the metaphysic of morals. (10th ed.).* (Translated by Abbott, T.K.). London, Longmans. 102 p. (1962 edition).

Mepham, B. (1996). A framework for the ethical analysis of novel foods: the ethical matrix. *Journal of Agricultural and Environmental Ethics*, 12(2): 165-176.

Mepham, B. and Tomkins, S. (2003). *The Ethical Matrix* [online]. www.ethicalmatrix.net

Singer, P.A. (1975). *Animal liberation. A new ethics for our treatment of animals*. New York, New York Review/Random House.