THE ETIOLOGY OF CHRONIC PLEURITIS IN PIGS: A PATHOLOGICAL AND SEROLOGICAL STUDY

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1. INTRODUCTION

Prevalence of chronic pleuritis (CP) in slaughter pigs has increased in the Netherlands from 12% in 1990 to 22.5% in 2004. Although studies on infectious agents causing CP in pigs often show a relation with *Actinobacillus pleuropneumoniae* and *M.hyopneumoniae* (Enoe *et al.*, 2002), the etiology of pleuritis is not fully understood (Andreasen *et al.*, 2001). Aim of the present study was to identify pathogens that are primarily involved in the origin and occurrence of pleuritis in swine herds with a high prevalence of pleuritis in slaughter pigs.

2. MATERIALS AND METHODS, RESULTS, DISCUSSION AND CONCLUSION

Five finishing farms with a history of high pleuritis prevalence in slaughter pigs were selected from abattoir records. At each farm one compartment was monitored from start of the fattening period (age 10-12 weeks) until slaughtering (age 26-30 weeks).
Pathological study: Each compartment was clinically inspected every two weeks and the percentage lethargic as well as the percentage coughing pigs was recorde and pigs were examined. Individual pigs showing fever (body temperature ≥40ºC) and/or abnormal respiratory type were suspected to be acute phase representatives (APRs) of acute pleuritis. A maximum of 3 APRs per inspection were submitted the next day for pathological and bacteriological examinations. At slaughter, lungs of the remaining pigs were sampled and gross pathology performed.
Serological study: Blood samples of all pigs were collected three times during the fattening period: at the start of the fattening period, six weeks later and at slaughter. After slaughter, only serum samples of pigs showing CP at slaughter were included in the serological assays. Antibodies against the following infectious agents were measured: PRRSV; Influenza H1N1, H1N2, H3N2; PCV2; *M.hyopneumoniae;* ApxIV toxin; *A..pleuropneumoniae; H. parasuis*.
Overall results are summarized in table 1. Only 3 herds showed high pleuritis scores at slaughter (herd 1 32%, herd 2 27%, herd 5 27%) and were included in the serological studies. Pigs with pleuritis did not show cause specific findings when examined pathologically.
Symptoms of acute pleuritis in fattening pigs are not recognized and thus, farmers cannot intervene in the acute stage of pleuritis. The causal agent(s) of pleuritis cannot be pinpointed and farms with CP show positive antibody titres for most of the major respiratory agens. Therefore, reducing high pleuritis scores will require general management improvements rather than pathogen specified strategies.

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4. ACKNOWLEDGEMENT

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Table 1 **Results of serological tests on antibodies** of pigs with chronic pleuritis at slaughtering that were sampled three times during fattening (percentage of tested animals positive in assay). **Clinical scores** of lethargy and coughing (percentage positive animals of total herd) recorded during clinical inspections. **Pathology results of APRs**, selected during clinical inspections (number of animals).

	Herd 1									Herd 2										Herd 5									
Test	**N**	**Moment in time (weeks)**								**N**	**Moment in time (weeks)**									**N**	**Moment in time (weeks)**								
Serological assay	**45**	**0[a]**	**2**	**4**	**6**	**8**	**10**	**12**	**14[b]**	**15**	**0[a]**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16[b]**	**28**	**0[a]**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16[b]**
M.hyopneumoniae		0			0				59		0			7					60		7			3					68
PRRSV		93			82				53		93			73					67		21			71					89
App ApxIV toxin		18			0				100		60			13					100		82			97					100
App CFT		2			7				89		27			27					87		71			100					96
H.parasuis		24			58				96		13			40					80		25			69					86
*Influenza H1N1**		56			16				27		7			0					0		0			0					71
*Influenza H1N2**		9			0				47		0			0					0		0			0					0
*Influenza H3N2**		11			0				71		0			0					0		7			4					0
Clinical scores	**138**									**80**										**132**									
Coughing		5	0	0	20	15	3	2			1	10	0	1	0	1	2	16	20		0	0	0	2	5	2	2	2	20
Lethargy		5	1	3	50	25	10	1			5	10	0	1	1	1	2	0	0		5	0	2	5	5	1	2	5	5
Results of APRs	**13**									**7**										**15**									
Acute pleuritis																										1	1	1	
Chronic pleuritis					3	1	1	1			1	1		1			1		1				1	1		2			2
Pneumoniae		2	1		3	3	3	1			1	3							1					3	2	1	2	2	3
Negative																													

[a] Start of fattening period [b] Week of slaughtering

*For Influenza, column "0" shows the percentage of positive pigs; column "6", column "14[b]" and "16[b]" show the percentage seroconverted pigs

VACCINATION AGAINST CLASSICAL SWINE FEVER: EPIDEMIOLOGICAL CONSEQUENCES

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1. INTRODUCTION

Emergency vaccination will possibly be used in controlling a future outbreak of Classical Swine Fever (CSF) in the Netherlands (see 'Concept Beleidsdraaiboek Klassieke Varkenspest' (Anonymous, 2005)). A marker vaccine is available that enables the distinction between infected vaccinated animals and noninfected vaccinated animals. However, concerns exist that animals are only slowly protected by this type of vaccination and they may be infected subclinically. Using mathematical modelling, this research project (Bergevoet et al., 2007) will address two questions:

- Which emergency vaccination strategies can effectively be applied to control CSF epidemics?
- How can we declare areas free of infection and do emergency vaccination strategies increase the risks encountered in declaring freedom of infection?

2. MATERIALS AND METHODS

We developed a mathematical model that describes the effects of marker vaccination and transmission of CSF virus between individual animals, between pens and between farms. The results of transmission experiments and the outbreak data of the CSF epidemic that occurred in the Netherlands in 1997 and 1998, serve to calibrate the multi-level model. We applied this model on the situation of 2006, with in total 9000 pig farms. Distinctions were made between finisher farms (consisting only of finishing pigs), and multiplier farms (consisting of separate sow and piglet sections). Different control strategies were compared: three emergency vaccination strategies (in 1 km, 2 km and 5 km rings) and preemptive ring culling in 1 km radius around a detected herd. Thousand simulations were carried out for each control strategy. The resulting simulated epidemics were subjected to six end screening scenarios that differ in the number of animals sampled per farm type.

3. RESULTS

In Table 1 results are summarized for outbreaks that occurred mainly in pig farm dense areas in the Netherlands and that were controlled using different control strategies. As a measure for the effectivity of a control strategy, the outbreak size, the duration and the effective reproduction number between herds R_h of the simulated epidemics are evaluated.

Table 1 Results for outbreaks which have started with 11-20 infectious herds at the moment of the first detection of an infected herd (between brackets the two-sided 95% interval).

control strategy	number of detected herds	number of not detected herds	duration (days)	R_h*
1 km ring culling	18 (9-57)	0 (0-1)	92 (36-278)	0.49 (0.08-1.22)
1 km ring vaccination	22 (9-84)	1 (1-9)	111 (36-313)	0.53 (0.09-1.30)
2 km ring vaccination	19 (9-49)	2 (2-8)	95 (36-233)	0.46 (0.08-1.08)
5 km ring vaccination	15 (8-29)	2 (2-8)	71 (34-171)	0.35 (0.05-0.84)

* The effective reproduction number between herds R_h is here defined for 'second generation herds': this is the number of infections that is caused by a herd that was infected by a herd that was infectious at the moment of the first detection of an infected herd.

The results show that 1 km ring vaccination is less effective than 1 km ring culling. This is not surprising as it takes some time for vaccination to build protection (typically two weeks), whereas culling works instantaneoulsy. The effectiveness of vaccination in 2 km radius around an infected herd is comparable to 1 km ring culling. The most effective strategy is 5 km ring vaccination, which yields an effective reproduction number significantly below unity.

Vaccination increases the chance that a within-farm outbreak remains undetected during the epidemic, because more small outbreaks occur on vaccinated farms that were infected before the vaccine gave full protection. The number of these undetected outbreaks increases with increasing vaccination radius, compared to the total epidemic size. After the epidemic they need to be detected during the end screening to prevent them entering the food chain. The chance that they also escape detection during the end screening depends on the sample sizes taken on the different type of farms (finishers, sows or piglets and vaccinated or unvaccinated).

The recommended end screening scenario is to sample 1 animal per pen on all vaccinated farms, 1 animal per pen on unvaccinated finisher farms and a random sample as required by the EU for unvaccinated multiplier farms (i.e. 32 piglets and 61 sows). Using this scenario, the absolute number of seropositive animals which are missed by the end screening is on average 3-5 animals in the entire country, with an upper boundary of 10-18 animals (95% quantile). Applying more stringent end screening scenarios (e.g. sampling 2 animals per pen instead of 1) can't lower these numbers much. The most important result however, is that the risk of missing infected animals during the end screening is not different for preemptive culling or emergency vaccination strategies.

4. DISCUSSION

In conclusion, emergency vaccination can be as effective a control strategy as pre-emptive culling to control CSF epidemics, provided that a larger vaccination radius is used. However, it is to be expected that the end screening will detect a number of small outbreaks on vaccinated farms, which would set back the infection free status. Therefore it is recommendable to start with (intermediate) screenings as soon as seems acceptable. When a sufficiently stringent end screening scenario is used, vaccination does not increase the risk of missing seropositive animals.

The simulation results have also been used by LEI for the economical analysis. They concluded that the largest part of the losses is caused by the decreased revenues of animals slaughtered due to welfare problems, and not the decreased value of meat of vaccinated animals. The extent of these problems depends on duration of the outbreak and the size of the area with movement restrictions.

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ESTIMATING *SALMONELLA* PREVALENCE IN PIGS: A NEW METHOD TO ESTIMATE THE TRUE PREVALENCE DIRECTLY FROM ANTIBODY LEVELS

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1. INTRODUCTION

Examing animals for infectious diseases is often done by assessing the level of disease-specific antibodies in serum samples. Typically, a threshold value is used to dichotmize the antibody level data to serological data, based on which epidemiological parameters are estimated. However, the use of a threshold value in order to diagnose each individual animal as being non-diseased or diseased for a specific disease is always prone to false positives, false negatives or inconclusive classifications. Bollaerts *et al*. (2007) proposed an alternative method to estimate epidimiological parameters, in particular the true prevalence and the force of infection, directly from antibody level data without the use of a threshold value. The method is based on an underlying mixture model and can be extended to the joint analysis of two or more diseases providing additional insights in disease co-occurrence. The direct estimation method is applied to estimate the true *Salmonella* prevalence in pigs as a function of sampling time to investigate seasonal effects.

2. MATERIALS AND METHODS

2. 1. Data

A serological *Salmonella* surveillance programme, organized by the Belgian Federal Agency for the Safety of the Food Chain (FASFC), has been in place since January 2005 (Van der Stede *et al.*, 2007). *Salmonella*-specific antibodies are determined by an indirect enzyme-linked immunosorbent assay in blood samples, collected from weaners, growing pigs and finishing pigs from all production holdings in Belgium within the Aujeszky disease monitoring programme. Within this programme, 10 to 12 blood samples (depending on the size of the herd) of pigs of different weight categories (<40 kg, 40-59kg, 60-79 kg and ≥ 80kg) have to be collected in each pig production holding every 3 to 4 months. The blood samples are examined for *Salmonella*-specific antibodies with a commercial indirect-ELISA (Idexx Laboratories, HerdCheck* Swine *Salmonella* Antibody Test Kit) according to the manufacturer's recommendations. The results are reported as sample to positive ratios (SP ratio = OD_{sample} - $OD_{neg\ kit\ control}$ / $OD_{pos\ kit\ control}$ - $OD_{neg\ kit\ ontrol}$).

2.2. Methodology

Commonly, the true prevalence π is estimated using seroprevalence data being binary data obtained by assessing the presence or absence of disease-specific antibodies in serum samples where antibodies are assumed to be present if the (log of the) antibody level exceeds a certain user-defined threshold value ζ and are assumed to be absent otherwise or

$$\mathrm{y}_i = \begin{cases} 1, & z_i > \zeta; \\ 0, & z_i \leq \zeta \end{cases}$$

with z_i being the (log of the) antibody level of subject *i*. The mean of the serological data $\bar{y}_\zeta$ is then an unbiased estimate of the seroprevalence *p*. For a perfect test, for which test misclassification does not exist, the true prevalence and the seroprevalence coincide. However, virtually all tests are subject to test misclassification yielding biased estimates of the true prevalence when naively estimated as the mean of the serological data. Correcting for the bias induced by test misclassification yields the Rogan-Gladen estimator (Rogan & Gladen, 1978) or

$$\pi = (\bar{y}_\zeta + SP_\zeta - 1)(SE_\zeta + SP_\zeta - 1)$$

with SE_ζ and SP_ζ being the threshold-specific test sensitivity and specificity, assumed to be known fixed values. However, it is more realistic to relax the latter assumption and assume uncertainty distributions for SE_ζ and SP_ζ instead and obtain an estimate of (the distribution of) π within a bayesian framework using

$$y \sim \text{Bernoulli}(p)$$
$$p = SE_\zeta \pi + SP_\zeta(1 - \pi)$$

and incorporating informative prior information about SE_ζ, SP_ζ and π, which can be obtained from previous studies or expert opinion. However, this information is not always available or easy to obtain. Therefor, Bollaerts *et al* (2007) proposed a direct method to estimate the true prevalence without the need to specify a threshold value nor SE_ζ and SP_ζ. The direct method is based on an underlying mixture model of antibody levels. In particular, the density of the antibody levels is assumed to be a two-component mixture of the non-diseased and diseased population or

$$G(z) = (1 - \pi) f(z|\theta_1) + \pi f(z|\theta_2)$$

,

where $f(z|\theta_i), i = 1, 2$, are the mixing components with parameters $\theta_i$and where π is the mixing probability. In the current setting, $f(z|\theta_1)$ is the density of the non-diseased population, $f(z|\theta_2)$ is the density of the diseased population and π is the true prevalence. Based on the mixture mean

$$E(z) := \mu = (1 - \pi)\mu_1 + \pi\mu_2\,,$$

it is readily obtained that

$$\pi = (\mu - \mu_1)/(\mu_2 - \mu_1).$$

This expression shows that π is equal to the excess of the mixture mean μ to the mean of the non-diseased population μ_1 relative to the difference in the means of the diseased μ_2 and non-diseased populationμ_1. Hence, it is straightforward to estimate π using

$$\widehat{\pi} = (\widehat{\mu} - \widehat{\mu}_1)/(\widehat{\mu}_2 - \widehat{\mu}_1).$$

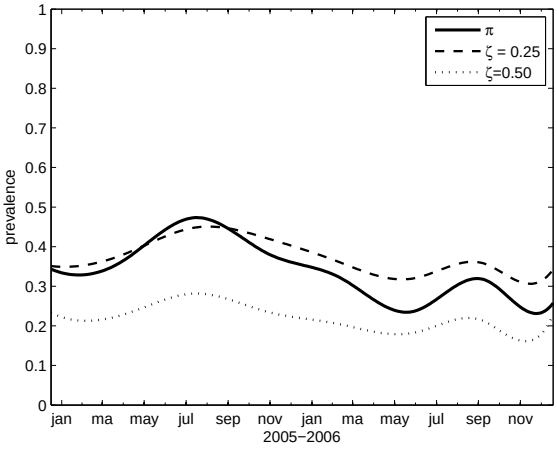
The mixture mean μ can be easily estimated using the average of the antibody level data whereas μ_1 and μ_2 (if not known) can be estimated from the data using EM-type of algorithms [1]. Often interest is in factors x that influence π. As before, we can derive a direct estimate of π based on the underlying mixture model of antibody levels assuming that the mixing probability depends on the covariate information x, denoted as $\pi(x)$. In this case, the true prevalence can be written as

$$\pi(x) = (\mu(x) - \mu_1)/(\mu_2 - \mu_1)$$

and an estimate of π can be obtained as before.

3. RESULTS

The direct estimation method is applied to estimate the true *Salmonella* prevalence in pigs in Belgium from January 2005 to December 2006 as a smooth B-splines function of time to investigate the evolution of *Salmonella* prevalence over time as well as the (known) seasonal effects. The population means μ_1 and μ_2 are estimated using the EM-algorithm assuming a two-component normal mixture (McCulloch, 1997) whereas he mixture mean $\mu_{(time)}$ is estimated using Generalized Estimating Equations (GEE) (Molenberghs & Verbeke, 2005). This approach is used to account for the hierarchical structure of the data since all herds are measured repeatedly over time and at each sampling time, blood samples of several animals are taken. The results are given in Figure 1. The GEE-based estimates of the seroprevalence based on two different threshold values ($\zeta = 0.25$ and $\zeta = 0.50$) are given as well for reasons of comparison. The analysis indicate that the mean seroprevalence based on $\zeta = 0.25$ is slightly overestimating the true prevalence whereas the mean seroprevalence based on $\zeta = 0.50$ is an underestimation of the true prevalence. Furthermore, seasonal effects can be observed with higher *Salmonella* prevalences during the (late) summer months. Finally, the plot suggests an overall decreasing trend over time.



4. DISCUSSION

We applied the direct estimation method developed by Bollaerts et al. (2007) to obtain an estimate of the true *Salmonella prevalence* in pigs in Belgium as a function of time. Seasonal effects are observed as well as a general decreasing trend over time. Major advantage of the direct estimation method is that a choice of threshold, being always prone to testmisclassification, is not needed. Drawback of the direct estimation method is the assumption of covariate-invariant means of the antibody levels for each subpopulation. However, this assumption is comparable to the covariate-invariant threshold commonly used to transform antibody level data to seroprevalence data. The estimation method also needs an estimate of μ_1 and μ_2 in case they are unknown. It is not always straightforward to estimate these parameters especially not if the mixing distributions are not well separated. However, this is comparable to the problems associated with the estimation of test sensitivity and specificity in case of threshold based methods with correction for test misclassification. Taken all together, we believe that direct estimation offers an interesting alternative approach to estimate the true prevalence. In further research, the performance of the direct estimation method will be compared with (bayesian) threshold based methods with correction for test misclassification.

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RE-EMERGENCE OF BLUETONGUE SEROTYPE 8 IN BELGIUM AND THE NETHERLANDS IN 2007

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1. INTRODUCTION

Bluetongue (BT) is an arthropod-borne viral non-contagious disease of domestic and wild ruminants, particularly affecting sheep with severe clinical disease including mortality. At present 24 different BT-serotypes have been identified and the disease is transmitted by biting midges (*Culicoides*). BT has a worldwide distribution between approximate latitudes 35°S and 40°N, although in parts of western North America, China and in Kazakhstan BTV may extend up to almost 50°N. This part of the world contains the habitat of *C. imicola*, the most important BT-vector of the *Culicoides* spp. BT is endemic in southern member-states of the European Union (EU), and several new incursions have been seen in Italy, Greece, Turkey, the island of Corsica, the islands of Menorca and Mallorca and Portugal. BT-serotypes 1, 2, 4, 9 and 16 were involved in epidemics in the EU member-states. Starting August 2006 a major epidemic of BTV serotype 8 was diagnosed in the North-Western part of Europe, affecting The Netherlands, Belgium, Germany and the North of France. The precise route of introduction remains unknown (Mintiens, 2007), but is clear that the incursion of the virus was a very exceptional event.
Many hoped that during the winter season we would get rid of BTV-8, assuming that the chain of transmission would be broken by dying off of infected vectors and cessation of viraemia in infected ruminants.
However, in the course of 2007 it became evident that BTV-8 somehow survived the winter in North-West Europe and a re-emerging epidemic spread exponentially within the original affected countries (Belgium, Germany, France, Luxembourg and The Netherlands), affecting ten thousands of holdings with ruminants (e.g. in Belgium and the Netherlands more than respectively 5,000 and 6,000 holdings with ruminants were affected at the end of November 2007). Besides that, BTV-8 was introduced into the United Kingdom, Denmark and Switzerland (promed, 2007a). The scale of the epidemic in 2007 is so huge that vaccination as a control option is (now taken very) seriously considered within the European Union (promed, 2007b).

2. FIRST NEW BTV-8 OUTBREAKS IN WESTERN EUROPE IN 2007

The very first outbreak in the originally affected area in Western Europe was reported from Germany in May 2007 when a sentinel cow tested positive in the state of North-Rhein Westphalia, followed by reporting of clinical disease (BTV-8 confirmed) in sheep in Belgium on 17 July. A cow without clinical sings tested positive (PCR) while tested for export purposes in France on 19 July 2007. So, it seemed that at several different locations within the originally BT-affected area, new infections popped up.

3. SITUATION IN THE NETHERLANDS IN 2007

In the spring of 2007, a cross-sectional study was executed in the Netherlands to get an impression of the seroprevalence in cattle in different compartments within the Netherlands. The bluetongue prevalence in 2006 was highest in the southern part of the Netherlands, with a gradient decreasing towards the Northern part; more than 50% of the compartments within the Netherlands were at zero or negligible prevalence (Vellema et al., 2007). The BTV-8 outbreaks in the Netherlands in 2006 caused 2.5% morbidity (defined as sum of total number of cattle affected relative to the sum of the total number of cattle present in outbreak herds) and 0.22% mortality (defined as sum of total number of cattle that died relative to the sum of the total number of cattle present in outbreak flocks) in cattle, and 7.7% morbidity and 4.4% mortality in sheep, while 50% of the sick sheep died

(Elbers et al., 2007). Therefore, the overall conclusion at the end of the 2006-epidemic season was that the clinical consequences were considered as relatively mild in cattle and fairly moderate in sheep.
The first new BTV-8 outbreak in the Netherlands was reported on 26 July 2007 in the province of North Brabant in the southern part of the Netherlands: a cow without clinical signs tested positive (PCR) while tested for export purposes. That was the start of an exponentially increasing epidemic, spreading from the South towards the North, East and West of the Netherlands. The overall trend of the epidemic curve indicates a continuing increase in the total number of outbreaks, with a peak in mid-September (week 38).

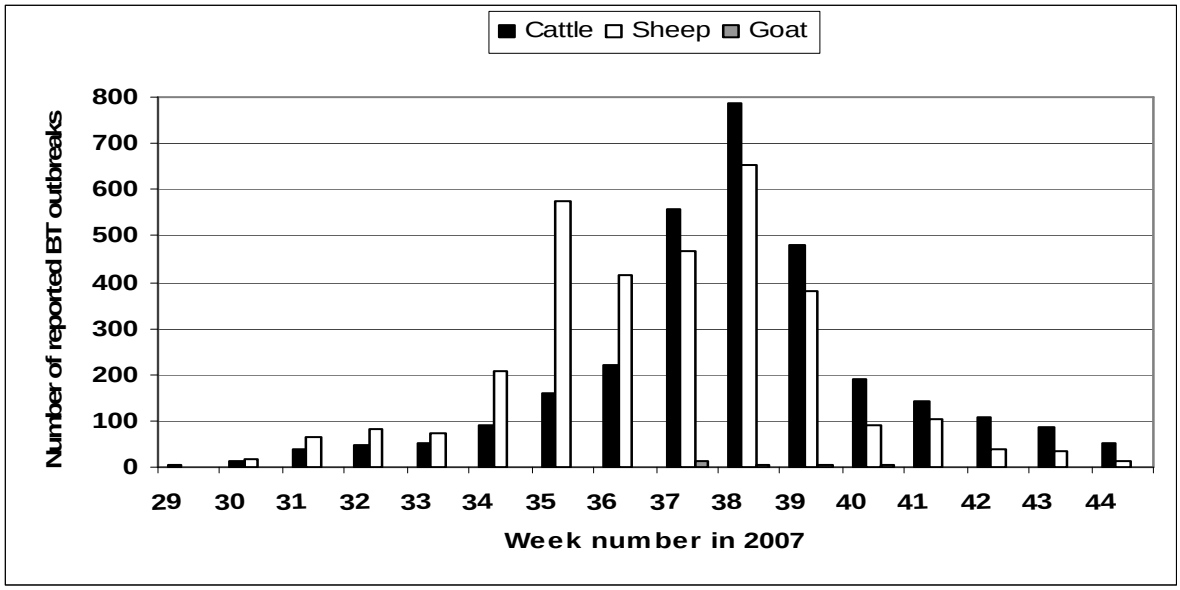

Figure 1. Distribution of the number of reported BTV-8 outbreaks by week in cattle, sheep and goat herds in the Netherlands.

From week 39 on, the number of new reported outbreaks has continually decreased. About an equal number of sheep and cattle holdings reported clinical BT problems in their animals.
During the 2006-epidemic in North-West Europe, no clinically affected goats were reported. However, in week 35 of 2007 the first clinical disease in goats caused by BTV-8 was reported from the Netherlands (Dercksen et al., 2007): in a holding containing 600 Dutch milking goats, 10 goats demonstrated clinical signs of bluetongue, starting with acute drop in milk yield and pyrexia, followed by edema of lips and face, crusts on lips and muzzle, nasal discharge, conjunctivitis and erythema of the udder. Up to week 44 a total of 25 holdings reported clinical disease (BT indicative) in goats.
In 2007, farmers suggested a more severe clinical situation on their farms compared to 2006. This may be explained by a higher number of animals within herds clinically affected compared to 2006. The rendering organisation Rendac in the Netherlands indicated that in August, September and October 2007 respectively 50%, 100% and 10% more sheep cadavers were collected from sheep farms than usual (2,000-2,500 sheep cadavers per week). In October, Rendac indicated that 5% more cattle cadavers were supplied by cattle farms than usually done (6000-6,500 cattle cadavers per week). This is an indication that the 2007-epidemic has a much more severe impact – more affected animals per holding but not necessarily more severe disease within individual animals - compared to the situation in 2006, in which no measurable increase in supply of sheep and cattle cadavers from farms was noticed.
Preliminary results from a longitudinal study in the southern part of the Netherlands (a cooperation between CIDC-Lelystad, Faculty of Veterinary Medicine of the University of Utrecht and Veterinary Practice Heerlen) on 5 cattle herds and 5 sheep flocks show indications that a large number of animals within these farms became infected (PCR and serology positive) in the second half of 2007, and these animals were serological and PCR negative in the Spring of 2007. Animals that were infected in the 2006 epidemic season - and as a result were serological positive in the Spring of 2007 - did not became infected (no PCR positives) again in the Autumn of 2007.

4. SITUATION IN BELGIUM IN 2007

In Belgium, a cross-sectional study to establish the spread of the virus after the 2006 epidemic was performed in winter 2007 (Méroc et al., 2007). As in the Netherlands, a gradient in the herd and within herd prevalence was

found decreasing from the 'area of first infection' around Maastricht-Liège, towards the other parts of Belgium (figure 2).

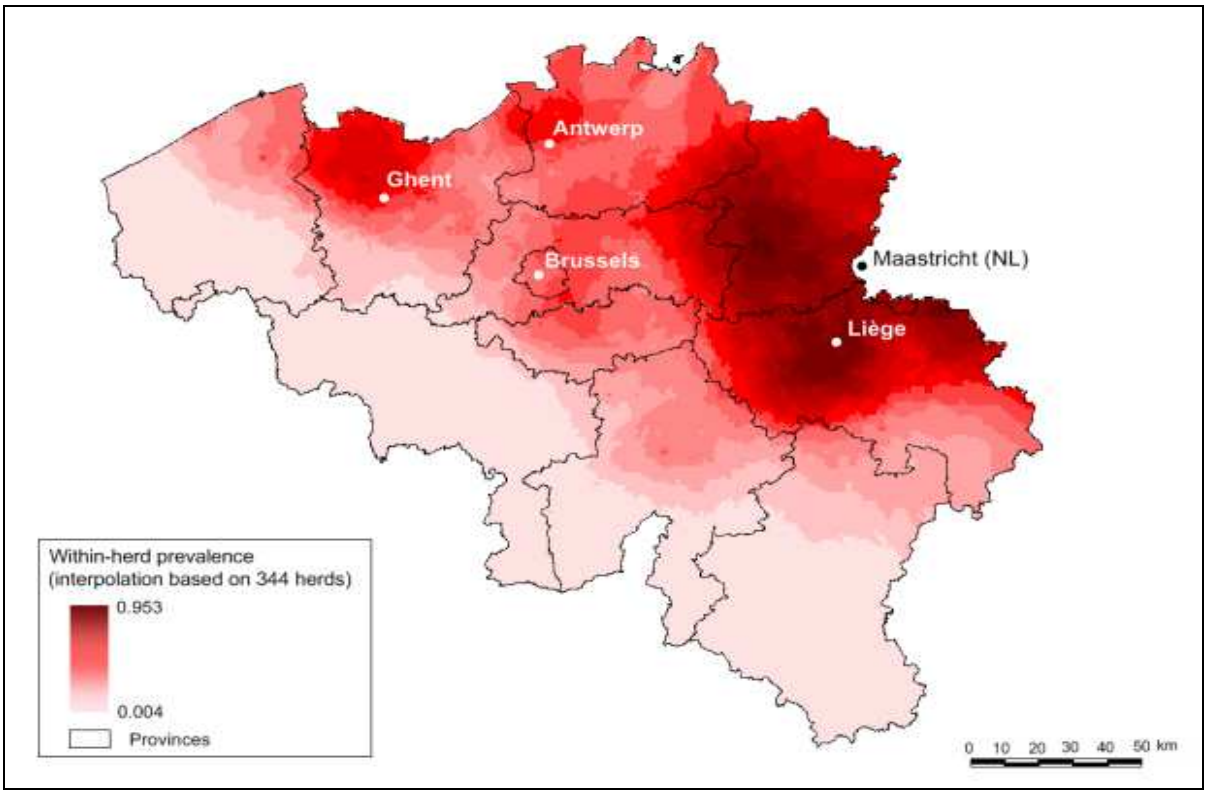

Figure 2. Distribution of within-herd-seroprevalence in Belgium according to the results of the winter screening, Belgium , January 2007 (Méroc et al., 2007).

The first case of the Belgian 2007 BTV-epidemic was reported in a herd located in East Flanders (Olsene), This case was the starting point of a new wave of BT outbreaks in Belgium. The epidemic expanded is such way that within the two first months four times more cases were identified than during the entire 2006 episode. Although the first cases in 2007 mainly occurred in areas with low within-herd prevalence, it soon became clear that the incidence was most probably related to the population density of the susceptible hosts.

At the end of the epizootic in 2006, the case-herd species distribution was nearly identical with 57.3% of sheep herds and 42.7% of cattle herds. Investigations were made in 2006 in the Dutch infected cattle herds and showed that 64.7% were of dairy type (Elbers et al., 2007). The same distribution were again for in 2007 in Belgium..

For 1014 case-herds, the outbreak was further investigated: total numbers of animals that died or were slaughtered because of BT as well as animals that developed BT clinical symptoms were recorded. Clinical prevalence (number of clinically affected animals in a herd divided by the total number of animals at risk) ranged between 0 and 100% in both species case-herds. The overall clinical prevalence in ovine and bovine species was respectively estimated at 27.3 and 6.8%. The mortality rate was estimated at respectively 11.2 % and 0.6% in ovine and bovine species. No mortality was observed in 44% of the ovine flocks and 84% of the cattle herds. In sheep flocks, overall case fatality was 43.2 (range 0 – 100%). In cattle herds, overall case fatality was 6.29 (range 0- 100%). In order to get more reliable information on mortality in Belgium in 2007, rendering plant data since 2005 were required and plotted in figure 3. When comparing with previous mortality patterns, the unusual increase of mortality in small ruminant population since the beginning of August (week 31) reflects clearly. This makes that a serious economical impact of the epidemic can be expected.

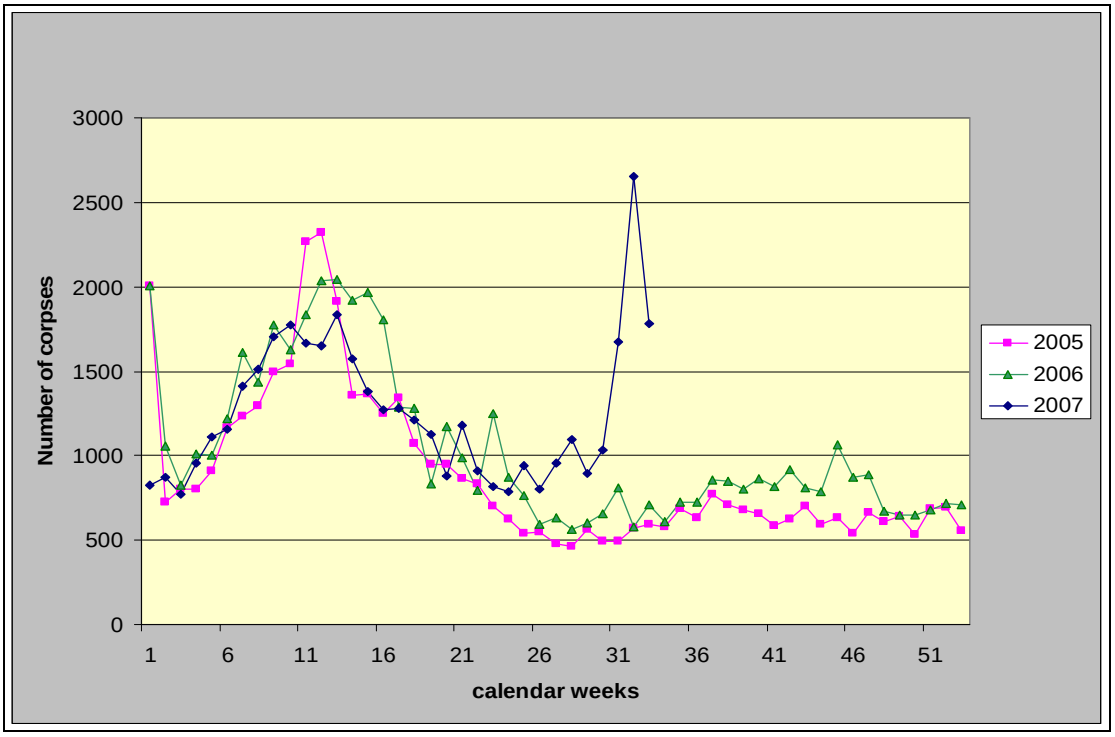

Figure 3. Weekly numbers of small ruminants (ovine/caprine) corpses collected by the rendering plant (Rendac), Belgium, 2005-2007

5. CULICOIDES VECTORS

In 2006, BTV-8 spread across 5 Member States (MS's) and by December 2006 had affected an area of approximately 170.000 km^2. At least 2 species of _*Culicoides*_ i.e. _*C. obsoletus*_ and _*C. dewulfi*_ were shown to be involved in its transmission. All affected MSs initiated national entomological surveillance programmes with the result that _*Culicoides*_ are now monitored widely using mainly Onderstepoort-type blacklight traps. The most significant findings made over the past year are summarised and discussed with emphasis on The Netherlands, where 20 farms are sampled weekly.

▪ _*Culicoides*_ activity during the winter months of 2006-7: In the Netherlands and in Belgium, low numbers of _*Culicoides*_ (almost exclusively of the Obsoletus Complex and excluding _*C. dewulfi*_) were captured almost each week between January and March 2007; 99 percent were freshly emerged nullipars, indicating that low-level breeding had continued throughout the winter.

▪ How did BTV-8 survived the winter between 2006 and 2007? Between January an March 2007 (plus or minus 90 days) the absence of older parous, potentially BTV-infected, previous-season adult midges in light trap collections led to the (false!) hope that BTV would not survive the winter. However, its ferocious recrudescence in 2007 invites many questions, which are discussed

▪ More _*Culicoides*_ in a cooler and wetter 2007: The average number of vectors captured in Holland in 2007 is approximately 10-fold greater than the number collected in 2006, despite it being cooler and wetter and quite unlike last year [2006] (the hottest on record since measurements began in 1706). This would indicate that warmer winters and moderate, normal summers favour vector proliferation and perhaps also allow viruses previously exotic to Europe to become endemic there.

▪ Marked changes in some vector _*Culicoides*_ abundances : the Obsoletus Complex is the most prevalent vector in the Netherlands and dominant on half the farms surveyed. However, in parts of the southern Netherlands, _*C. dewulfi*_ has this year [2007] superseded _*C. obsoletus*_. If a similar reversal has occurred also elsewhere in Europe, it may in part explain the intensity of the current outbreak.

▪ Diurnal biting activity in _*Culicoides*_: _*C. dewulfi*_ and _*C. obsoletus*_ attack livestock in broad daylight while they are at pasture, especially on overcast days. Aggravating the situation, they also enter animal houses after dark. Therefore, the attack of livestock by day and at night, both indoors and outdoors, complicates our fight against BT. At this stage, vector control seems to hold little promise for halting the spread of the disease.

6. CONCLUSIONS

In 2007, BTV-8 continued to spread, including a jump across the English channel. The BTV-8 restriction zone now covers an area of almost one million km^2. There are no obvious geographical or topographic boundaries that might halt the advance of BTV-8, making it likely that it will continue in 2008 (and beyond) until it reaches the (as yet unknown) limits of its range. This is daunting when it is considered that vector _*Culicoides*_ (and susceptible ruminant hosts) occur across the entire Holarctic Region, which includes the Mediterranean Basin where _*C. imicola*_ lies in waiting, and North America, where outbreaks of BTV and Epizootic Haemorrhagic Disease of Deer virus (EHDV, another _*Culicoides*_-borne pathogen) are occurring also. In this respect, it would seem that warmer winters will only add to the conundrum in the future, promoting rather than suppressing virus survival and vector longevity. Vaccination still seems to be the best defence available to us, but have we waited too long?

The underlying factors for the fast expanding nature of the 2007 BT episode merit further investigations. Still, following hypotheses can be suggested:

- A considerable virus reservoir was present in the host populations at the onset of the epidemic
- More *Culicoides* have been captured in a cooler and wetter 2007
- a relative change in some vector _*Culicoides*_ abundances

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CONTACT STRUCTURES OF DUTCH BROILER AND LAYER FARMS: AN AVIAN INFLUENZA PERSPECTIVE

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1. INTRODUCTION

The Netherlands is a leading exporting country of poultry (mainly chicken) meat and eggs (Windhorst, 2006). Although broiler and layer farms are situated throughout the country, they are predominantly concentrated in Gelderland, Limburg, North Brabant and the eastern part of Overijssel with two distinct densely populated poultry areas in the Gelderse Vallei and East Brabant/North Limburg. In 2003, The Netherlands experienced a large Avian Influenza (AI) H7N7 epidemic, which started in the Gelderse Vallei and spread to Brabant and Limburg (Stegeman et al., 2004). The outbreaks appeared in clusters suggesting neighbourhood spread, but also virus spread over larger distances took place (Report DG Sanco, 2003). Although airborne spread may have played a role in neighbourhood spread, in general it is assumed that for AI transmission contacts between farms by e.g. poultry, people or vehicles play a crucial role particularly before control measures are at place (Thomas et al., 2005). These contacts may spread the virus over longer distances. However, data on frequencies and distances of direct and indirect contacts of broiler and layer farms is scarce.

To gain more insight into the contact structure of broiler and layer farms in The Netherlands a questionnaire and logbook study were conducted among 16 broiler and 21 layer farms. The contact structure was investigated in a non-epidemic situation, which should be comparable to the contacts in the period between introduction and detection, the so-called high risk period (HRP). Data on contact structures between farms (frequencies, distances) as collected in this study can serve as a basis for stochastic spatial simulation models. With these models, outbreaks can be simulated and efficacy of control strategies can be evaluated, for example (see: Jalvingh et al. (1999) and Mangen et al. (2001) for CSF, Yoon et al. (2006) for FMD and Sharkey et al. (2007) for AI). Currently a stochastic spatial model to simulate HPAI outbreaks in The Netherlands is being developed.

For the purposes of presentation, we assume that an AI virus is introduced in a broiler or layer farm and that the HRP lasts two weeks. The type, frequency and distances travelled of contacts are presented based on this assumption. In addition, possible links between different poultry sectors and with hobby poultry were investigated as well as biosecurity measures taken.

2. MATERIAL AND METHODS

2.1 Selection of farms

Sixty-five broiler and layer farms were selected from the 85 farms participating in the Agricultural Accountancy Data Network (BIN) of the Agricultural Economics Research Institute (http://www.lei.wur.nl/UK/statistics/Binternet/default.htm). This network collects financial and technical data of a representative sample of farms and holdings of the Dutch Agricultural Census. The administrators of the network made a pre selection of 75 farms; 10 farms were not selected as they had the same owner as other farms in the selection, were very small or because owners were not motivated or were difficult to reach. Of the other 75 farms we approached 65 farms to obtain a reasonable number of farms of each type (broiler and layer) and each housing type (cage, barn, mixed) in the study. Each selected farmer was sent a letter inviting his/her participation in the study, followed by a telephone call a few days later.

The response rate was 60% (39/65 farms). Reasons for not willing to participate were 1) too busy, 2) not interested and 3) participating in too many studies already. With farmers that indicated their willingness to take part, a visit was planned. All farm visits were done by the same person, who interviewed the farmers and filled in a questionnaire. After the interview, the farmers were asked to register all farm visitors (including private visits) in a standardized logbook for 4 weeks starting on the day of the visit. Farmers who participated in this study were paid remuneration.

2.2 Questionnaire

The questionnaire and logbook study is part of a larger study in which also Germany, Austria and Italy are participating. The questionnaire and logbook were originally designed in Germany but were translated and adapted to the Dutch situation. Before the start of the study, a pre-test was conducted with one layer and one broiler farmer. The questionnaire and logbook were adjusted taking into account the comments of the two farmers. After this adjustment, the questionnaire consisted of 63 questions, divided into the following categories: general farm information, management, feed, litter, manure, employees, biosecurity, pest control, rendering, sharing machinery, professional contacts and hobby poultry.

In total 16 broiler farms and 23 layer farms were visited to fill in the questionnaire. One layer farm turned out to be exceptionally small and another 'layer farm' had recently changed into a rearing farm and was therefore excluded from analysis. The final questionnaire dataset thus consisted of 16 broiler farms and 21 layer farms, which is approximately 2% of the Dutch broiler and layer farms. The farms in the survey were properly distributed over the country according to the total broiler and layer farm distribution in The Netherlands, except for the Gelderse Vallei area due to non response.

2.3 Logbook

For a period of four weeks, the farmers recorded the following information on contacts: date and time of visit, reason for visit, access to the poultry houses (yes/no), address and type of business where they were coming from (origin) and where they were going to after the visit (destination), whether poultry was present at the origin or destination address and if yes, specification of the poultry species and whether they had access to these poultry houses. The farmers were asked to record all farm contacts, including private visits, but excluding the movements of the farmer and employees themselves as this was considered infeasible.

The return rate of the logbook was 66% (26 logbooks, 13 of layer farms and 13 of broiler farms). Reasons for not filling in the logbook were 1) filling in was too time consuming and 2) visitors did not want to fill in the addresses out of privacy reasons. The logbook of the rearing farm and one logbook of a layer farm that arrived after analysis was already finished were excluded from analysis. The quality of one of the logbooks of a broiler farm was doubtful as it had only 3 entries and no feed deliveries were registered; it was therefore also excluded from further analysis. The final logbook dataset consisted accordingly of data on 1051 contacts of 12 broiler farms and 11 layer farms.

2.4 Analysis

The data was analysed with SPSS 15.0 software. Distances (km) travelled by visitors were defined as the straight-line distance between addresses and was estimated using Google Earth © 2007 based on street names and postal codes. When only the name of the town was filled in, the centre of the town was taken for estimating distances. Distances could be calculated for approximately 90% of the contacts.
We used the following risk classification: high risk was assigned to movements of live poultry from farm to farm (e.g. reared laying hens) and movements of poultry manure to a poultry farm, medium risk was assigned to persons entering the sheds while poultry was present, other live poultry transports (e.g. day-old-chickens and broilers to slaughterhouse) and transports of poultry manure to destinations other than poultry farms, and low risk was assigned to persons not entering the sheds or only when sheds are empty, deliveries of feed, litter and other materials, egg transports and rendering trucks.

3. RESULTS

3.1 General farm information

Data on general farm information as obtained from the questionnaire is summarized in Table 1.

Table 1: General farm information (questionnaire) for broiler and layer farms

	Broilers (n=16)	Layers (n=21)
Mean # chickens (min-max)	85,425 (24,000-190,800)	70,518 (20,000-176,120)
Mean # poultry houses (min-max)	2.8 (1-6)	2.9 (1-6)
% Farms with cage/barn/mixed housing	100% barn	19% cage, 38% barn, 43% mixed[1]
% Farms with outdoor run	0%	24% (4 covered, 1 uncovered)
% Farms all in/all out or one-age system	94% all in/all out	48% one-age system
Length production round	53 days	14 months
% Farms flock thinning once/twice	87% once (wk 5) 13% twice (wk 5, 6)	-
% Farms with mixed farming poultry	none	none
% Farms with mixed farming other	19% cattle	19% cattle, 5% sheep
% Farms with farm sale	13%	62%
% Farms with sale live poultry	19%; > 4 times/year	10%; ± 2 times/month

[1] Mixed = combinations of cage, barn and/or free range

The 'all in/all out' system in broiler production is comparable to the 'single-age system' in layer production and means that all poultry houses are empty simultaneously between production rounds. The average duration of a broiler production round (from delivery of day-old-chickens to last batch for slaughter) was 43.6 ± 2.8 days; the average duration of the empty period was 9.2 ± 2.6 days. The average duration between deliveries of young hens to a poultry house was approximately 14 months. Three broiler farms and four layer farms kept a substantial number of cattle (100-225 cows) and one layer farm kept a considerable number of sheep (175). Other farm animals (horses, goats, donkeys and pigs) were present in small numbers; none of the farms kept other species of commercial poultry. One broiler farm sold potatoes, and another sold eggs from a non-commercial holder; 13 layer farms sold eggs. Three broiler and two layer farms sold live poultry to non-commercial holders. We did not gather information on crop farming.

3.2 Employees

More than half (52%) of the layer farms in our study had employees; all farms had one or two employees except for a 'zorgboerderij' where more than 20 employees and clients were at work. Almost all employees working on the layer farms entered the poultry houses weekly (96%). The average distance travelled from home to work was 4.3 km (range 0.1-14.0 km). Three employees (14%) also kept poultry at home. Only one broiler farm (6%) had one employee, who had access to the poultry houses, travelled 11 km to work and kept no poultry at home. Forty-eight percent of the layer farmers and 25% of the broiler farmers stated that employees and family members were not informed about avian influenza precautionary measures.

3.3 Biosecurity

On all layer and broiler farms wearing coveralls and changing footwear was common practice. Hand washing before entering the poultry houses was done on 62% of the layer and 75% of the broiler farms. However, hand washing when leaving the poultry houses was only common practice on one layer (5%) and one broiler farm (6%).

Four layer and five broiler farms shared one or two machines with other farms. All but one layer farm had agreed with the other farm to clean the machines after use, the other farm had made no arrangements on this. On 86% of the layer farms egg trays are shared with other farms. The majority of these farms use solely plastic egg trays (89%) which are easier to clean than cardboard egg trays; one farm shared solely cardboard egg trays and another shared both plastic and cardboard egg trays with other farms.

A pest control programme for rodents was carried out regularly (>4 times per year) on all farms. A pest control programme for insects was applied on 63% of the broiler farms (in the empty period) and on 81% of the layer farms (12% regularly, 88% when required). On 71% of the layer and 19% of the broiler farms service companies were contracted for the pest control programmes.

Half of the farms had cats; on average 3 house cats and 8 free roaming cats. Only one layer farm kept 4 captive cage birds indoors.

Fourteen layer farms (67%) and nine broiler farms (56%) had stored poultry manure on the farm premises in the past 12 months. More than half of these farms (56%) had stored this manure uncovered or partly uncovered. The majority of layer farms (76%) and all broiler farms sold their manure to traders in agricultural manure or for industrial purposes. Two of the layer farms (10%) used the manure on own land and three layer (14%) and six broiler farms used it partly on own land and partly for trade.
Cleaning water which contains manure and litter particles from the poultry sheds was present at all broiler farms and on 52% of the layer farms. Most of the cleaning water was stored in the manure basement or expelled to the sewerage. On 56% of the broiler and 14% of the layer farms it was also used on own land and two layer farms (10%) expelled it in a ditch.
The premises of 20% the broiler and 43% of the layer farms were fenced in; information signs were present on 37% of the layer farms and only on one broiler farm.
The average distance to the nearest neighbouring poultry farm was 2.0 km with range [0.7 – 2.0] for broiler and 1.4 km [0.2 – 8.0] for layer farms.

3.4 Contact structure

From the results of the questionnaire we computed that layer farms had on average 14.4 ± 6.2 contacts per 2 weeks of which on average 1.7 contacts entered the sheds (excluding employees). The majority of these contacts are egg transports and deliveries of feed, litter and other materials which are considered low risk contacts. Broiler farms had fewer contacts, 9.4 ± 3.7 contacts per 2 weeks of which on average 2.9 contacts entered the sheds. Table 2 specifies for different type of professional contacts the percentage of farms with that particular contact and the mean frequencies per two weeks as obtained from the questionnaire, as well as access to the sheds and the off-farm distances travelled as recorded in the logbook.

The average off-farm distance travelled (including private contacts) was 18.8 km with range [0.2-360.9] for layer farms and 16.6 km [0.1-181.8] for broiler farms. Particularly transport of broiler chickens to the slaughterhouse, some manure and egg transports and contract workers travelled over large distances. Only 1 high risk contact was found: a manure transport from a layer to a layer rearing farm. For broiler farms the medium risk contacts travelled on average larger distances (32.9 km) than the low risk contacts (11.6 km), ($P < 0.01$, Student T-test). For layer farms low and medium risk contacts travelled on average 17.6 and 21.7 km, respectively.
Based on the logbook data, 94 (23.5%) of the contacts of layer farms travelled directly to other poultry farms (including private contacts); 95% of these off-farm contacts were with layer or layer rearing farms. Egg transports and business advisors accounted for the majority of these contacts. For broiler farms 24 (12.5%) of the off-farm contacts travelled directly to other poultry farms of which 75% were contacts with other broiler farms. Similar to layer farm contacts, business advisors accounted for a large proportion of these contacts. Only two contacts with other poultry species were found: one veterinarian travelled from a broiler to a turkey farm and a pest controller travelled from a layer to a duck farm.
Approximately 16% of the 154 private contacts recorded for layer farms entered the sheds; for broiler farms only 1% of the 184 recorded private contacts entered the sheds. Twelve private contacts travelled directly to another poultry farm, six of them entered the sheds at the off-farm contact only. The average distance travelled for private contacts was 4.6 km with range [0.1-72.8]. Forty contacts of house-sale of eggs were recorded; the average distance travelled was 3.1 km with range [0.1-9.6], none of these entered the sheds.

3.5 Contacts between commercial and backyard poultry

Based on the questionnaire a few contacts between backyard and commercial farms were found: a farm with hobby chickens in the backyard, a broiler farmer that sold eggs from a backyard farm, five farms that sold live poultry to non-commercial holders and three employees of layer farms that kept poultry at home. One broiler farmer visited bird shows twice a year; no poultry were purchased at these shows.

Table 2: Specification for each business contact: the percentage of farms with that particular contact (questionnaire), the mean frequencies per two weeks (questionnaire), access to the sheds (logbook) and the off-farm distances travelled (logbook).

Type of contact	% Farms with contact		Mean freq./2 wks (questionnaire)		In shed (freq. logbook)	Mean off-farm distance (km) to next contact [min-max]	
	Broilers	Layers	Broilers	Layers		Broilers	Layers
Delivery poultry	all	all	0.25	0.05	sometimes (4/8)	46.4 [12.1-71.7]	84.5 (n=1)
Unloading team	19%	71%	0.25	0.07	yes (10/10)	5.5 [1.0-11.9]	82.4 (n=1)
Transport to slaughterhouse	all	all	0.52	0.05	sometimes (2/18)	78.3 [39.6-181.8]	42.3 [4.5-82.4]
Loading team	all	all	0.53	0.07	yes (4/4)	29.1 [13.4-51.6]	no records
Other service teams	63%	all	0.48	0.37	yes (10/16)	1.7 [0.5-4.3]	4.0 [0.2-7.4]
Veterinarian	all	all	0.94	0.15	sometimes (19/24)	23.2 [0.8-92.1]	30.9 [30.4-31.5]
Manure transport	all	86%	0.63	1.49	sometimes (2/27)	4.1 [3.1-7.1]	132.8 [6.1-360.9]
Egg transport	-	all	-	5.48	sometimes (12/100)	-	38.6 [2.0-133.6]
Rendering trucks	all	all	0.97	1.23	no (0/17)	0.8 (n=1)	1.3 [0.2-3.5]
Deliveries	all	all	3.93	4.17	sometimes (1/125)	30.4 [0.8-121.5]	24.5 [0.2-55.8]
Inspectors	all	all	0.15	0.14	sometimes (1/4)	no records	17.4 [5.9-29.5]
Advisors	all	all	0.66	0.88	sometimes (24/70)	25.8 [0.6-93.6]	27.3 [1.6-106.1]
Other	88%	all	0.57	0.51	sometimes (23/83)	14.4 [0.1-66.1]	46.6 [0.1-267.1]

4. DISCUSSION

The results of this study provide first insights into the contact structure of layer and broiler farms in The Netherlands. Since this study examined the contact structure during a non-epidemic situation, it gives an indication about the possibilities for contact-based spread of AI during the HRP and provides information for stochastic spatial simulation models.

The frequency of many professional contacts is strongly dependent on the production cycle in broiler and layer farms and therefore shows little variation between farms. Direct contact with poultry (entering poultry sheds) is generally thought to be a more likely way of AI transmission than contact with the poultry farm premises only. Contacts entering the sheds appear to be mainly those necessary for normal business operations; this may reflect increased biosecurity efforts in the Dutch poultry production sector.

Based on the contact information in the logbook, it is more likely that contact-based transmission occurs within a sector than between sectors and transmission to other species is unlikely.

Distances travelled by the contacts were highly variable and many records in the logbook were missing or unreadable. Distances travelled are regionally dependent due to location of slaughterhouses, packing stations, hatcheries, etc. Since only a low number of farms joined the logbook study and no data on the Gelderse Vallei was available, the data on distances might not be representative for The Netherlands as a whole. However, the ranges indicate that contact(s) could result in virus spread between the two distinct densely populated poultry areas in The Netherlands and that this would most likely be due to professional contacts. Private contacts, sale of farm products and employees are more likely to account for neighbourhood spread. It would be interesting to extend this study to the other levels of the poultry production chain.

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ESTIMATION OF THE ECONOMIC LOSS DUE TO NEW SUBCLINICAL MASTITIS IN DUTCH DAIRIES USING A TEST-DAY MODEL

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1. INTRODUCTION

Most studies regarding the economic losses resulting from mastitis focus on clinical mastitis (Halasa et al., 2007). However, several studies have shown that milk yield losses due to subclinical mastitis can be considerable (Seegers et al., 2003). Results of these studies varied largely and production losses were not estimated precisely enough for economic calculations. Moreover fat and protein loss estimations were scarce and might have underestimated the actual loss (Hortet & Seegers, 1998).
Methodologies to estimate production loss did not succeed to include the natural variations between cows, which might have misestimated the production loss. Random regression test-day modelling (RRTM) was developed to analyze test-day records of cows for genetic evaluation. Because these models are based on herd and cow specific lactation curves, they are more accurate. Our objective was to estimate the economic losses due to new cases of subclinical mastitis, using the RRTM.

2. MATERIALS & METHODS

The Dutch udder health center (UGCN, Deventer, The Netherlands) in cooperation with dairy herd improvement organizations (CR Delta and NRS, Arnhem, The Netherlands) collected cow production and clinical mastitis records in 400 randomly selected dairy farms. Data collection lasted from 1st July 2004 until 30th June 2005. During this period 251,647 TD records from 43,462 lactations of 39,512 cows were collected. Clinical records were based on farmers' diagnosis of abnormal milk color or presence of clots.
The first definition of new subclinical mastitis was based on literature (Doubling50), where a cow was considered to have new subclinical mastitis at test-day i (TD_i) when SCC was < 50,000 cells/ml at TD_{i-1} and > 100,000 cells/ml at TD_i. The second definition (Theshold150/250) was based on Dutch monitoring limits of milk SCC to indicate subclinical mastitis (Schepers et al., 1997). Accordingly a primiparous cow was considered a new case of subclinical mastitis at TD_i if the SCC was < 150,000 cells/ml at TD_{i-1} and > 150,000 cells/ml at TD_i. A multiparous cow was considered a new case of subclinical mastitis at TD_i if the SCC was < 250,000 cells/ml at TD_{i-1} and > 250,000 cells/ml at TD_i. Only the first subclinical mastitis episode was included in the analysis not to bias the results from previous subclinical episodes.
RRTM was used to predict milk, fat, and protein production for each test-day based on previous test-days. Predictions of milk, fat, or protein production were provided for TD_i based on the production at all preceding TDs, corrected for fixed genetic and environmental effects, parity, days in milk (**DIM**) and other important risk factors (De Roos & De Jong, 2006). The predicted production at the subclinical mastitis test-day represents the production of the cow assuming the SCC remained within the healthy limit and the cow follows its expected lactation curve.
The difference (ΔProd) between the actual and the predicted production (milk, fat, or protein) of a cow at TD_i (subclinical TD) reflects the effect of new subclinical mastitis on production for that cow

$$\Delta\text{Prod} = \text{Actual Production at } TD_i - \text{Predicted Production at } TD_i \quad (1)$$

To estimate the loss due to new subclinical mastitis a mixed effect model PROC MIXED in SAS (SAS institute Inc., 2004), was used according to the following equation

$$Y_{hjk} = \beta_o + \beta_1 \times \text{LnSCC} + \beta_2 \times \text{Parity}_h + \beta_3 \times \text{TDInt} + \beta_4 \times \text{DIM}_j + \text{Cow}_k + e_{hjk} \quad (2)$$

where Y_{hjk} is the change in milk, fat, or protein production (ΔProd) at the subclinical mastitis TD (TD_i) for cow k in parity h and DIM class j, β_o is the overall mean ΔProd, β_1 is the linear regression coefficient of the natural logarithm of SCC × 10^3 cells/mL (**LnSCC**), LnSCC is the fixed effect of LnSCC at TD_i, β_2 is the linear regression coefficient of the hth class of parity, Parity_h is the fixed effect of class h of parity (5 classes, parity = 1, 2, 3, 4, and ≥ 5), β_3 is the linear coefficient of TD interval, TDInt is the fixed effect of the time interval (in days) between TD_{i-1} and TD_i, β_4 is the linear coefficient of the jth class of DIM, DIM_j is the fixed effect of class j of DIM (30 classes) at TD_i, Cow_k is the random effect of cow k, and e_{hjk} is the residual error. The model was run separately for each definition of new subclinical mastitis.

The fit of the RRTM was assessed by the residuals normal distribution, fitted values, leverage, and Cook's distance plots. The influence of observations that had Cook's distance > the accepted upper limit according to Dohoo et al. (2004) was assessed based on the following equation

$$\text{Influence} = 3 \times K / N \quad (3)$$

Where Influence is the accepted upper limit for Cook's distance, K is the number of parameters in the model (excluding the intercept) and N is the total number of observations included in the model.

3. RESULTS

Primiparous cows comprised 31.6% of the whole study population. Mean milk production for primiparous cows was 23.2 kg/d with a geometric mean SCC of 65,000 cells/mL. For multiparous cows mean milk production was 28.33 kg/d with a geometric mean SCC of 105,000 cells/mL. the model fitted the data properly, the residuals were approximately normally distributed and the fitted values were homogenously distributed showing no trend. The leverage showed few outliers and their influence was tested using the Cook's distance (Figure 1). The upper limit of the influence was 0.001, and 139 observations exceeded this limit.

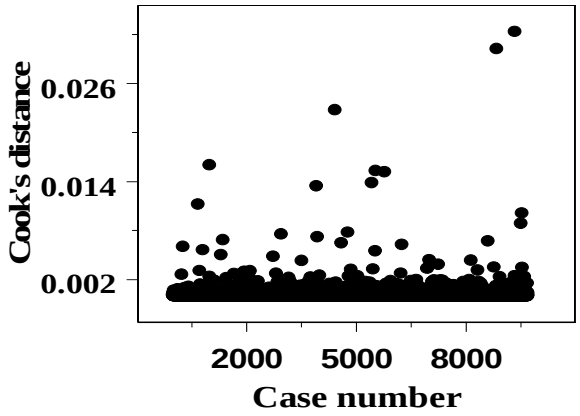

Figure 1. Cook's distance values for the change in milk production model using definition Doubling50.

There was no significant difference in production loss among multiparous cows and therefore results are presented for primiparous and multiparous cows. There was no significant effect of TDInt on the change in milk, fat, or protein production (lowest P-value = 0.15). Similarly, none of the 30 classes of DIM were found to affect the change in milk, fat, or protein production significantly (lowest P-value = 0.65) and therefore TDInt and DIM were not included in the final model. The number of new subclinical mastitis cases was 3030 and 6658 for primiparous and multiparous cows, respectively.

Milk, fat and protein loss due to new subclinical mastitis are shown in Table 1 per definition. Using Doubling50, a primiparous cow is estimated to lose 0.41 kg milk, 4.51 g fat, and 10.23 g protein per day during the period of new subclinical mastitis at the level of SCC 200,000 cells/ml. A multiparous cow is estimated to lose 0.6 kg milk, 10.16 g fat and 12.49 g protein per day during the period of new subclinical mastitis at SCC 200,000 cells/ml. Using Threshold150/250, a primiparous cow is estimated to lose 0.41 kg milk, 4.51 g fat, and 10.23 g protein per day during the period of new subclinical mastitis at SCC 200,000 cells/ml. A multiparous cow is estimated to lose 0.72 kg milk, 15.53 g fat and 9.65 g protein per day during the period of new subclinical mastitis at SCC 300,000 cells/ml. Cows are estimated to lose more milk according to doubling50 for low SCC

compared to Threshold150/250. However, this estimation is opposite for high SCC, which could be explained by the different limits of healthy SCC level.

Table 1. Effects of an increased SCC during a new case of subclinical mastitis on milk, fat and protein production for primiparous and multiparous cows and for definitions Doubling50 and Threshold150/250.

	Doubling50						Threshold150/250					
SCC at TD_i	Milk		Fat		Protein		Milk		Fat		Protein	
	Parity 1	Parity ≥ 2	Parity 1	Parity ≥ 2	Parity 1	Parity ≥ 2	Parity 1	Parity ≥ 2	Parity 1	Parity ≥ 2	Parity 1	Parity ≥ 2
200	0.41	0.60	4.51	10.16	10.23	12.49	0.36	-	0.7	-	9.1	-
300	0.50	0.79	8.91	18.10	13.75	17.64	0.61	0.72	3.14	15.53	15.6	9.65
400	0.56	0.93	12.03	23.67	16.24	21.29	0.78	0.90	4.87	22.00	20.21	17.59
500	0.61	1.03	14.45	28.02	18.17	24.13	0.92	1.05	6.21	27.00	23.8	23.76
600	0.65	1.12	16.43	31.58	19.75	26.44	1.04	1.17	7.31	31.07	26.72	28.79

4. DISCUSSION

Economic damage due to subclinical mastitis has been mainly attributed to the fact that a subclinical cow is a constant source of infection to other cows and to milk production loss (Swinkels et al., 2005). However, fat and protein losses were not included in previous calculations, which could be more important than milk loss in countries such as the Netherlands because farmers are paid according to fat and protein content of the milk.

Using Doubling50, milk production loss was found to be 0.41 and 0.6 kg/d for primiparous and multiparous cows, respectively, at SCC 200,000 cells/mL. Literature estimates of milk production loss for two-fold increase in crude SCC are 0.40 and 0.60 kg/d for primiparous and multiparous cows, respectively (Seegers et al., 2003), which is close to the estimates in this study (would be 0.38 and 0.46 kg/d for the same relationship). In this study, the clinical records were excluded from the analysis, consistent with Hortet et al. (1999) and Koldeweij et al. (1999). Fat and protein production were also affected negatively with increased SCC. Using Doubling50 (Table 1) primiparous and multiparous cows are estimated to lose 4.51 and 10.16 g/d of fat, respectively, during a SCC increase to 200,000 cells/mL. For the same relationship, primiparous and multiparous cows are estimated to lose 10.23 and 12.49 g/d of protein, respectively. Previous research found fat and protein losses of 5 and 4 g/d, respectively (assuming a cow produces 25 kg milk per day) per two-fold increase in SCC, regardless of the parity of the cow and ignoring other risk factors (Hortet and Seegers, 1998). Koldeweij et al. (1999) found close estimates to this study.

There is a general debate in literature regarding the definition of a new subclinical case in relation to SCC. Djabri et al. (2002) found that the average SCC for culture-negative quarters was 68,000 cells/mL. In a review by Seegers et al. (2003) a healthy udder was defined if SCC < 50,000 cells/ml. Threshold150\250 assumes an udder healthy if SCC < 150,000 and < 250,000 cells/mL, for primiparous and multiparous cows, respectively. Doubling50 seems more consistent with recent literature on the definition of a healthy udder. Production loss was found to be different using Doubling50 than Threshold150\250 (Table 1) for the same increased SCC level. This could indicate that the manner of change of the SCC might be as important as the level of increase in SCC.

Ideally, intramammary infections would be monitored using bacterial culturing of milk samples. However, the availability of such datasets is limited to small-scale datasets for research purposes (Schukken et al., 2003). Evaluation of economic damage due to subclinical mastitis demands a large dataset, which is not available due to high costs. The availability of large datasets of SCC makes it an attractive proxy to bacterial culture samples.

In conclusion, Random regression test-day modeling is a good technique to estimate the effect of new subclinical mastitis on production. There is a significant loss in milk, fat, and protein production of dairy cows with new lactational subclinical mastitis. The magnitude of the loss is determined by the definition of new subclinical mastitis and the SCC elevation.

5. ACKNOWLEDGMENTS

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MOLECULAR EPIDEMIOLOGY OF PSITTACOSIS IN PSITTACIFORMES BREEDING FACILITIES

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A population of 308 *Psittaciformes* from 39 breeding facilities as well as 46 the pet bird owners were tested for *Chlamydophila psittaci* infections. Fifty-nine (19.2%) birds were positive for *Cp. psittaci* by nested PCR/EIA detecting the chlamydial outer membrane protein A (*ompA*) gene in faecal samples. *Cp. psittaci* were isolated from 25 (42.3%) PCR- positive samples inoculating them onto BGM cells and subsequently identifying the bacteria by immunofluorescence staining. Twenty-eight of 71 tested *Psittaciformes* species were *Cp. psittaci* positive including 8 in which *Cp. psittaci* was detected for the first time. Eight of 39 (20.5%) tested breeding facilities were positive in both nested PCR/EIA and culture and respiratory disease was present at all eight. Five breeding facilities gave only positive results in the nested PCR/EIA and birds showed no clinical signs. Birds in one of these facilities were currently being treated with doxycycline, and the remaining four breeding facilities recently used doxycycline, oxytetracycline, or enrofloxacin. In 13 of 39 (33.3%) *Cp. psittaci* – positive breeding facilities a significant correlation between fecal excretion of viable Chlamydophila and respiratory disease was shown (odds ratio 14.5, 95% confidence interval 1.6-130.5, $p<0.05$). The remaining 26 breeding facilities with healthy birds had negative results for PCR and culture. Nested PCR/EIA showed the presence of *Cp. psttaci* DNA in pharyngeal samples of 6 on 46 (13%) pet bird owners. Interestingly, birds of those pet bird owners were positive in both PCR and culture. Viable organisms could be isolated from 4 out of 6 PCR positive human samples. Subsequent *ompA* genotyping by use of a genotype-specific real-time PCR revealed the presence of genotypes A (5 of 6) and E/B (1 of 6). Only mild clinical signs appeared in these persons and none were treated. In our study 18 (46.2%) of 39 breeding facilities had treated their birds with tetracycline, doxyclycline, or enrofloxacin in the past year. Four (10.2%) of 39 also used tetracyclines prophylactically. Thus, a *Cp. psittaci* vaccine for *Psittaciformes* and information on sensible use of antibiotics are needed to prevent psittacosis in humans and birds as well as possible development of drug-resistant bacterial strains.

EMERGING DISEASES: THE PROGRESSION OF ARBOVIRUSES

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1. INTRODUCTION

Although the term "arbovirus" refers to a group of viruses belonging to various families and genera, they all have one common characteristic: they multiply in blood-feeding arthropods on which they are dependent for transmission from host to host (Gubbler, 2001). Arboviruses are actually grouped into eight families and 14 genera. In general, the principal host for arboviruses is a vertebrate other than human beings but many arboviruses (approximately 25%) are transmissible and lead to the development of diseases that may be fatal among humans (zoonotic diseases). In recent years, several arboviruses have appeared outside the territories with which they have been historically associated. In 1999, the West Nile virus, WNV, belonging to the family *Flaviviridae* and the genus Flavivirus and transmissible to humans, was reported for the first time on the American continent. Within a few years, it became firmly established in the United States, where it has spread through some 20 states and into Canada (Marfin and Gubler, 2001; Gould and Fikrig, 2004). In Western Europe, WNV is progressing steadily towards the north, having reappeared in Italy in 1990 after an absence of more than 15 years (Hubalek and Halouzka, 1999) and in France in 2000 (Chastel, 2002).

In 2000, the Rift Valley Fever virus, RVF, a zoonotic disease caused by a virus of the family *Bunyaviridae* and the genus Phlebovirus, crossed for the first time the boundaries of the African continent into Saudi Arabia and Yemen (Shoemaker et al., 2002). Since then, 884 affected human cases were reported with 124 deaths in Saudi Arabia and 321 affected cases with 32 deaths in Yemen. More recently (December 2006 – May 2007) there was a very severe RVF outbreak in Kenya, Tanzania and Somalia after a period of massive floods resulting in 1062 human reported cases with 315 deaths (case fatality rate 23-45%) (*WHO, Programmes and projects, Epidemic and Pandemic Alert and Response (EPR), Disease Outbreak News, Rift Valley Fever in Kenya, Somalia and the United Republic of Tanzania, 9 May 2007*). The outbreak has forced the closure of livestock markets and slaughtering animals was forbidden as precaution measure, affecting seriously the economy of the region The safari tourism brings thousands of tourists to these areas with a high risk of rapid spread of this disease to all parts of the world. RVF can also hit regions already suffering very hard from natural or human disasters as now is happening in Sudan with 164 human deaths in less than a month (*Sudan Tribune, 23 November 2007*).

In 1998, bluetongue virus (BTV, an Orbivirus of the family *Reoviridae* that affects primarily domestic and wild ruminants) reappeared in Western Europe after having been absent for several decades. The current epidemic of BTV differs in several respects (examined by Purse *et al.*, 2005) from previous incursions of the BTV virus or of African horse sickness (a very similar Orbivirus that uses the same vector but affects only horses). BTV has not been present for seven years in the Mediterranean basin, although only sporadic incursions had been observed previously. As opposed to previous episodes, which involved only one serotype, five serotypes of the BTV have been implicated in the various epidemics affecting southern Europe since 1998. Appearance and expansion of another serotype of this virus in the Netherlands, Belgium and Germany is a completely new phenomenon, given that this virus has never before been observed at those latitudes.

Chikungunya fever is a viral disease transmitted to humans by the bite of infected mosquitoes. Chikungunya virus (CHIKV) is a member of the genus *Alphavirus*, in the family *Togaviridae*. CHIKV was first isolated from the blood of a febrile patient in Tanzania in 1953. The virus circulates throughout much of Africa, with transmission thought to occur mainly between mosquitoes and monkeys. Other countries that have experienced recent outbreaks of chikungunya include India, Sri Lanka, and the Indian Ocean Islands of Mayotte, Mauritius, Réunion (territory of France), and the Seychelles. In August/September 2007 some 160 people in Italy's northern Ravenna region were infected with the virus by the Asian tiger mosquito, resulting in one fatality. This is the first known example of chikungunya transmission outside the tropics (Watson, 2007).

The viruses causing Japanese encephalitis, dengue and yellow fever (three other Flaviviruses associated with zoonotic diseases) have also been progressing for some 20 years beyond the regions to which they were previously confined (Gubler, 1998; Reiter, 2001).

Reference has frequently been made to global climatic changes and, more particularly, global warming, to explain the recent evolution of these pathogens involving an arthropod vector. Although the data collected up to

the present time tend to confirm the impact of global warming, other observations suggest the considerable importance of anthropogenic factors in the propagation of arboviruses.

2. GLOBAL CLIMATE CHANGE

The existence of global climatic changes associated with anthropogenic activity was recognized for the first time in 1995 by a gathering of international scientists (the IPCC, the Intergovernmental Panel on Climate Change), who concluded as follows (Houghton *et al.*,1996): "the balance of scientific arguments suggests that human activities throughout the last century have begun to have a discernible effect on the world's climate, contributing to its warming".

Global increase in temperature is the most spectacular climatic change expected during the coming decades. The IPCC models predict an increase of global temperature of between 1.4°C and 5.8°C between 1990 and 2100 (Houghton *et al.*, 2001).

The intensity and distribution of precipitation are also likely to change during the next decade. On the global level, we can expect an increase in water-vapour pressure due to evaporation and to an increase in precipitation. Certain regions will likely experience a significant increase in precipitation, while others will be subject to increased risk of drought. The forecasts in this regard are, however, less reliable than those regarding temperature.

In line with this slow and continual progression of temperature and precipitation, the frequency and intensity of extreme climatic events (such as periods of severe cold or heat waves, episodes of drought, floods, storms and hurricanes), could also increase during the 21st Century (Sutherst, 2004). The extreme climatic events associated with El Niño, in particular, will likely increase in the coming decades (Houghton *et al.*, 2001).

3. INCIDENCE OF CLIMATE CHANGES ON THE DISTRIBUTION OF ARBOVIRUSES

3.1. The complexity of arboviral cycles

Arboviruses are generally associated with complex cycles, during which the virus intervenes on one or several arthropod vectors, reservoirs and hosts (principal or accessory). Climatic changes could have direct or indirect effects on the various players in these cycles. Thus, excessive temperature could lead to much higher vector mortality. On the other hand, significant drought could result in a subsequent reduction in predatory insectivore populations and indirectly favour the explosion of vector populations (Epstein, 2001). The indirect effects are difficult to quantify or foresee. They will not be dealt with here, even if they could be of considerable consequence.

3.2. Temperature

Temperature influences the distribution limit, competence and ability of vectors, as well as the speed with which arboviruses develop within their invertebrate hosts. Vector distribution is particularly limited by minimum and maximum temperatures, which prevent their survival from one season to the next (Mellor and Leake, 2000). Thus, the distribution of BTV and its principal vector in Europe, *Culicoides imicola*, are primarily limited by low winter temperatures (Sellers and Mellor, 1993; Ward, 1996; Wittmann *et al.*, 2001; Calistri *et al.*, 2003;Conte *et al.*, 2003). In Israel, the years during which there has been a high incidence of BTV have been preceded by significantly warmer winters than the periods characterised by a lower incidence of BTV (Braverman *et al.*, 2001). The distribution limit of the vectors of dengue and yellow fever (*Aedes albopictus* and *Aedes aegypti*) in North America is also negatively affected by the minimum temperatures observed during the coldest months (Nawrocki and Hawley, 1987; Reiter, 2001). This dependence on minimum temperatures suggests a possible progression of arthropod vectors to higher latitudes or altitudes subsequent to global warming. In Mexico, *Aedes aegypti* in particular has been found at much higher altitudes than those previously reported and it seems that this progression may be attributed to global warming (Herrera-Basto *et al.*, 1992; Reiter *et al.*, 1998a).

Temperature also influences the competence of certain vectors. *Culicoides nubeculosus* is a less competent BTV vector when the temperature at which the larvae develop is 25°C but its competence increases considerably when the immature stages develop at temperatures between 30°C and 35°C (Mellor and Leake, 2000). Brief exposure to high temperatures may also make competent a vector that is not originally so (Mellor *et al.*, 1998). Embrittlement of the digestive tube, facilitating the passage of the virus into the haemocoel and its progression towards the salivary glands could be the origin of this phenomenon, reported particularly with respect to BTV.

The ability of a vector population could be defined as its faculty to transmit virus effectively under given conditions. It depends especially on the competence of the vectors, the size of the population and the frequency with which the insects take a blood meal likely to transmit the virus. In temperate regions, where the maximum temperatures are relatively low, the capacity of vector populations increases generally with temperature (Mellor

and Leake, 2000). Although the insects' lifespans are reduced when the temperature increases, the reproductive cycle increases correspondingly, this leads to the development of larger vector populations. The transmission of the virus is also facilitated by increase in vector activity and in the frequency of blood meals. Temperature also influences the speed at which arboviruses develop within arthropod vectors whose temperature is directly influenced by climatic conditions (Paweska *et al.*, 2002). The development of the BTV virus is completely arrested when the temperature is below 15°C. It is relatively slow at 21°C but accelerates considerably when the temperature increases to 27°C or 32°C (Mullens *et al.*, 1995).

3.3. Precipitation

An increase in precipitation and relative humidity favours the development and propagation of populations of arthropod vectors. The immature stages (larvae, pupae) of the vectors are aquatic or semi-aquatic. The reproduction and development sites, which consist generally of humid areas or of those liable to flooding, multiply with increasing precipitation. Thus, *Aedes aegypti* finds favourable conditions for incubation of eggs and the development of its immature stages in water-filled cavities in hollow trees (Reiter, 2001). The epidemics of Rift Valley Fever generally break out after abundant rain, which creates conditions propitious to larval development in grassy depressions (Davies *et al.*, 1985). Immature forms of *Culicoides imicola* also require humid places rich in organic matter (Braverman, 1978). This vector is particularly sensitive to any precipitation deficits, as indicated by several models explaining its distribution (Ward., 1994; 1996).

Increasing precipitation and relative humidity also have a favourable effect on the vectors' adult forms. In general, insects have increased metabolism and extended life-spans in humid conditions. Humidity that is too low leads, on the other hand, to vector desiccation and precocious mortality (Mellor and Leake, 2000). In spite of this dependence of mosquitoes and other arthropods on water, epidemics of WNV in the United States are associated with periods of prolonged drought. Stagnant water in wastewater systems and collection basins is one of the principal reproductive sites for the *Culex pipiens* vector. During periods of drought, the organic material necessary for the development of the larval form is more concentrated in these places. Drought also causes a reduction in the populations of batrachian predators of *Culex pipiens* and forces birds (the principal hosts of WNV) to concentrate around residual watering points. Together, these conditions facilitate the circulation of the virus between the vector and the principal host and hence the increased incidence of the disease (Epstein, 2001). The gathering of animals around these watering points, which are rare in the event of drought, also favours transmission and circulation of the African horse-sickness virus in South Africa (Baylis *et al.*, 1999).

3.4. Wind

Powerful winds generally increase insect mortality (Baylis *et al.*, 1998). They accelerate desiccation and impede flight and the taking of blood meals (Meiswinkel, 1997). On the other hand, low to moderate winds contribute to insect dispersion. The theories put forward by Murray (1987) and Reynolds *et al.* (1996) suggest the transport of insect vectors for several hundred kilometres in the form of aeroplankton.

Several observations support the leading role of wind in the dissemination of BTV in Australia (Murray and Kirkland, 1995), Turkey (Sellers and Pedgley, 1985), and Israel (Braverman and Chechick, 1996), as well as on the Balearic Islands (Alba *et al.*, 2004).

3.5. Extreme climatic phenomena

The re-emergence of certain arboviruses has been attributed to episodes of drought or flood caused by the recurrent climatic phenomena. Baylis and his collaborators (1999), in particular, have demonstrated the link between epidemics of African horse sickness in southern Africa and abundant precipitation (creating sites favourable to the development of larval stages, followed by heat waves (increasing the ability of vector populations) associated with El Niño.

Significant correlation between extreme climatic phenomena linked to El Niño and epidemics of Rift Valley Fever in Africa (Anyamba *et al.*, 2001) or of dengue in the South Pacific (Hales *et al.*, 1996) have also been shown. In addition to an increase in the intensity and frequency of phenomena such as El Niño, IPCC climatologists also suggest intensification of storms and hurricanes. One example is Hurricane Mitch, which led to the outbreak of epidemics of dengue and malaria in Central America (Epstein, 2000). Devastating climatic events of this kind could also contribute to the spread of arboviruses.

4. OTHER FACTORS INFLUENCING ARBOVIRUS DISTRIBUTION

4.1. The development of means of transport

The considerable development of means of transport and their democratisation has resulted in an increase in business and pleasure travel and in the transport of animals and goods around the world. These displacements

have frequently led to the introduction of arboviruses and their vectors into previously disease-free regions (Sutherst, 2004).
Although this phenomenon is tending to accelerate, it is not new: *Aedes aegypti* and *Culex pipiens* were introduced into the United States more than 200 years ago in stocks of drinking water carried on large sailing vessels destined for the transport of slaves from Africa (Tabachnick *et al.*, 1985). The recent appearance of another vector of dengue and yellow fever (*Aedes albopictus*) in the United States (Hawley *et al.*, 1987; Reiter and Sprenger, 1987; Adhami and Reiter, 1998; Reiter, 1998b) and in Europe (Knudsen, 1995) has been facilitated by the trade in worn-out tyres for re-capping. The rapid appearance of populations of *Culex pipiens* resistant to organophosphorus insecticides around the world has been associated with air transport (Raymond *et al.*, 1998). Finally, the more northern localisation of *Culicoides imicola* in Europe (in the Canton of Ticino in Switzerland) (Cagienard and Stark, 2005) could also be the result of accidental importation.
Although this region has experienced particularly noticeable global warming during recent years (Purse *et al.*, 2005), it is most likely that the introduction of isolated individuals of this BTV vector was made possible by the Lugano airport, which is very nearby (Cagienard and Stark, 2005).
Several introductions of arbovirus by infected individuals have also been reported. The entry of viraemic persons or animals is probably the origin of episodes of Rift Valley Fever that broke out in Egypt in 1976 (Sellers *et al.*, 1982; Swanepoel and Coetzer, 1994) and Saudi Arabia in 2000 (Balkhy and Memish, 2003). The recent outbreak of Chikungunya in Italy follows the import of the virus one year before by travellers. Indeed, because the foci of Aedes albopictus, 1 of the 2 main vectors of CHIKV, were identified in 2006 in Italy and many travellers visit CHIKV-epidemic areas, surveillance for imported cases was mandatory in Italy at that time. From July to September 2006, a total of 17 confirmed cases of CHIKV infection were observed in travellers .Analogously, the illegal introduction of infected animals very likely contributed to the propagation of BTV in Europe (Mellor and Wittmann, 2002). The air transport of infected persons has also greatly facilitated the movement of different strains of dengue around the world (Reiter, 2001). The strain of WNV that was the origin of the epidemic in New York in 1999 probably originated in Israel or a nearby country (Jia *et al.*, 1999; Lanciotti *et al.*, 1999). Although the mode of introduction is unknown, the distance between the countries of origin and destination as well as the limited duration of the viraemia suggest accidental introduction by a bird or infected mosquito as the most probable cause of this strain (Gould and Fikrig, 2004). Certain hunting or fishing trips particularly favour contact between tourists and wild environments in which there are significant populations of vectors and reservoirs. Tourist trips of this kind led to the sporadic introduction of yellow fever to the United States and Europe (McFarland *et al.*, 1997; Monath and Cetron, 2002).

4.2. The adaptation of the virus to new vectors

Chikungunya was first described in Tanzania in 1953. The first outbreak in India was in 1963 in Calcutta. An outbreak of chikungunya was also discovered in Port Klang in Malaysia in 1999 affecting 27 people. Recent research by the Pasteur Institute in Paris has shown that a mutation enables the virus to be transmitted by Aedes albopictus (Tiger mosquito) (Vazeille *et al.*, 2007). This was the cause of the recent plague in the Indian Ocean and the spread to the Mediterranean coast with an outbreak in Italy late 2007, with 160 cases.
This transfer of the principal vector (*Culicoides imicola*) towards a new vector (*Culicoides nubeculosus*) is likely the origin of the spectacular propagation of BTV in Eastern Europe in recent years (Mellor *et al.*, 2002). A similar situation is observed last year in BTV epidemic in Northern Europe where the main vectors are resident mosquito's (*Culicoide dewulfi, chiopterus, punctatus and pulicaris).*

4.3. The use of soil and soil covering

The use of soil has a considerable influence on insect distribution.
Vegetable cover in particular creates a microclimate at the soil level and may thus enable vectors to survive in environments that would be inhospitable in the absence of vegetation. Agricultural practices such as irrigation may also create sites suitable for the reproduction and development of the immature stages of a vector, while the drainage of humid areas may lead to the disappearance of such sites (Reiter, 2001). As to deforestation, this practice may, depending on the case, decimate populations of vectors and host "reservoirs", or, on the contrary, lead to an explosion of vector populations when previously wooded areas are cultivated (Walsh *et al.*, 1993). Humid areas rich in organic material of animal origin constitute prime reproductive sites for *Culicoides imicola* (Braverman, 1978). The density of domestic animal populations and the methods used for stocking their dung thus also influence vector distribution.

4.4. Urbanisation and dwelling structures

Creeping urbanisation in developing countries is frequently accompanied by insufficient or inexistent drinking-water and wastewater collection and distribution networks. The irregular water supply forces inhabitants to set up drinking-water reserves that are easily accessible to insects, such as *Aedes aegypti*, for which they are ideal

reproductive sites (Zell, 2004). Places where stagnant wastewater accumulates are also ideal sites for the development of WNV vectors (Epstein, 2001).
Urban zones in developing countries generally have very high population densities, a factor that favours arbovirus transmission. Poorly-sealed dwellings enable vectors to enter and find favourable conditions for their survival during colder periods. Differences at the level of urban structures, dwellings and social behaviour may be particular causes of the low incidence of dengue in Texas with respect to nearby Mexican states (examined by Reiter, 2001).

4.5. Political instability

Similarly to devastating natural phenomena, wars lead to massive displacement of populations, who carry any production animals they may have with them. These populations sometimes reach densely-populated refugee camps in which the sanitary conditions create ideal reproductive sites for vectors. Political instability may also prevent the effective functioning of vaccination campaigns or of programmes to combat vectors.

5. DISCUSSION

The existence of global climatic changes has now been unanimously recognized. Although their evolution and future consequences are difficult to estimate, certain of their effects are already clearly perceptible. Thus the progression of BTV and its principal vector in the Mediterranean basin illustrates a possible consequence of global warming. The example of Switzerland, where a single specimen of *Culicoides Imicola* was found on one occasion only near the Lugano airport reminds us that prudence is necessary when interpreting data collected in the field. It also points out the influence of potential anthropogenic factors in arbovirus progression. Vector progression at higher and higher altitudes may be attributed to global warming whereas the mode of vector dissemination between continents underlines the considerable importance of human factors.
Whatever the origin, the progression of arthropod vectors into regions that were previously disease-free locates arboviruses in the proximity of the new arthropods. The latter may include possible new vectors likely to propagate the arbovirus widely beyond the distribution limits of the initial vector. This transfer of the principal vector towards new vectors that are endemic is likely the origin of the spectacular propagation of BTV in Eastern and Northern Europe in recent years.
Arthropod vectors frequently differ from one continent to another. Their biology may vary widely, as a function of species and climate, which makes comparisons difficult and necessitates case-by-case study. There may be many climatic and anthropogenic factors affecting the various players in arbovirus cycles. They are frequently dealt with in an isolated fashion even though they have differing influence (often antagonistic) on the components of arboviral cycles. Given this complexity, it is difficult to predict the evolution of arbovirus distribution in coming decades. Nevertheless, the examples given here lead to the conclusion that global climate changes contribute to the progression of several arboviruses.
The progression of several arboviruses could be slowed down by policies resulting in the limitation of global climatic changes and in the reduction of the incidence of anthropogenic factors particularly favourable to arboviruses or other vectors. This includes a reduction in greenhouse-gas emissions as well as care and attention to urbanisation phenomena in underdeveloped countries. Structural modifications in order to encourage the abandonment of soil covering and of cultural and housing practices that create ideal conditions for the development of vector populations would hinder the progression of several arboviruses.
In the short term, it would seem that it will be necessary to live with arboviruses at the same time as we attempt to limit their harmful consequences on human and animal health and on the economy. The establishment of monitoring networks is an indispensable phase in this process, in order to follow up the progression of arboviruses and their vectors. Networks of this kind would also make it possible to react rapidly in the case of individual progression of vector populations. When alert systems indicate that an epidemic is imminent, populations at risk must be vaccinated quickly (when appropriate vaccines exist, such as for yellow fever) and vector-control systems must be implemented rapidly.

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EVALUATING ECONOMIC PERFORMANCE AND NUTRIENT EFFICIENCY OF PIG FINISHING FARMS: CASE OF STRATEGIC DEWORMING

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1. INTRODUCTION

In the first instance, veterinary actions have a rational from curative and animal welfare perspective. Economic, or ecological, considerations may play a role in the choice between alternative actions, but rough estimates mostly suffice to steer a final, quickly to make, choice. However, the more the veterinary actions are oriented towards pro-active improvements of the animal production, the more a detailed analysis of possible economic and ecological outcomes becomes indispensable. This is in particular true when veterinary actions become part of a more holistic business plan, which is for example the case for strategic deworming. The question then arises what will be the impact of the veterinary component on the economic and ecological outcomes of the business plan? Current evaluation methods too often stick to a "quick and dirty" paradigm.

The paper analyses the effect of strategic deworming with flubendazole on the economic performance and nutrient efficiency of pig finishing farms. Strategic deworming with flubendazole is chosen as a case because it provides a variety of starting points for economic and ecological improvement. Strategic deworming with flubendazole aims at preventing Ascaris suum, the most important internal parasite in pigs worldwide, to reach its adult or patent stage. The strategic deworming consists of a five-day in-feed treatment at 30 ppm flubendazole or of a five-day in-water treatment at 5 mg flubendazole/ kg live weight every five weeks. It prevents physical damage and improves health status of the pigs. Physical damage results in liver rejection in the slaughterhouse and increased susceptibility for respiratory diseases. Improvement of health status leads to reduced mortality and medication costs and to improved lean meat percentage and carcass quality. Average daily weight gain increases and feed conversion rate decreases.

As such, strategic deworming with flubendazole thus influences manifold underlying drivers of economic and ecological performance. Some of these separate effects of strategic deworming on performance drivers seem low, at least at first sight (section 2), but nevertheless they have a pronounced synergetic impact on aggregate economic and/or ecological outcomes (section 3). The paper presents two techniques for evaluating economic and ecological effects: partial budgeting and a frontier benchmarking technique. Both techniques are applied based on a coherent production-theoretical system analysis. We conclude (section 4) that these approaches increase diagnostic power and that strategic deworming with flubendazole leads to economic-ecological win-win.

2. MATERIALS AND METHODS

2.1. Production-theoretical framework

The basic production entity is the farm, and within this entity we consider an average present finisher as production unit. In the short run, the total number of average present finishers is the limiting factor, this is the number of pig places corrected for the actual occupation. For example, production quota in Flanders is based on average present finishers. On one pig place, more than one pig per year can be finished. Looking for production optimization conform production theory, will necessitate to make input use and output generation explicit in a coherent way (production function). The main inputs for pig finishing are feed and piglets, or rotations. There is a clear substitution between both: the longer the finishing duration the more feed is consumed and the lower the rotation number will be. Main variable costs other than feed (mainly start-up costs and veterinary costs) can be linked to rotations (Lauwers *et al*., 1999). Rotation (or piglet) input will only be affected by daily weight gain:

the faster pigs grow the more rotations can be started. Feed use is influenced by daily weight gain, mortality and feed conversion. Output quantity is insensitive to feed conversion. Finally, input (rotation) price is influenced by medication costs and output price by carcass quality.

2.2. Partial budgeting

With a partial budget costs and revenues are calculated given a minor adjustment in the business. The net effect is the difference between the sum of positive and negative economic effects (Dalsted and Gutierrez, 1992). The production-theoretical system analysis allows for making changed inputs and outputs quantities explicit. Together with price information, they constitute the partial budget. As strategic deworming, through improved carcass quality, increases pig meat prices, output quantities obtained under a flubendazole treatment have to be linked to a higher pig meat price. Carcass quality data has to be linked with differential price setting according to lean meat classes. The budget can be completed by using average values for fixed costs and other variable costs per average present finisher. Finally, the change in labour income, as a result of strategic deworming with flubendazole, can be assessed.

A similar budgeting can be done for the nitrogen balance when nitrogen content information is available to be linked to the input and output quantities. Based on the materials balance condition (Coelli *et al*., 2007), the nutrient balance is calculated as the amount of nutrients that enters in inputs (feed and piglets) minus that which leaves bound up in useful output (marketable pigs). The changed quantities of inputs and outputs allow for assessing the changed nutrient balance, constituting a kind of ecological partial budget.

Finally, economic and ecologic outcomes are combined to assess eco-efficiency, which is defined as the ratio between an economic outcome, to be maximised, and an ecologic outcome, to be minimised (Huppes and Ishikawa, 2005). In this paper, eco-efficiency is calculated by dividing labour income by nitrogen excretion.

One of the pitfalls of partial budgeting is that only some major (e.g. statistically significant) technical-economic effects are retained for further calculation. But also small changes, in particular when they act synergetic, have to be taken into account because they also can entail a leverage effect on final outcomes.

2.3. Frontier benchmarking

Partial budgeting has some fundamental shortcomings. The input-output transformation is a non-linear production function on which, depending on the ratio of input and output prices, an optimum can be sought. The challenge is now to estimate the production function. There are basically two main ways for estimating production functions: econometric techniques and frontier function estimation (Coelli *et al*., 2005). The first assumes that all farms are efficient and differences are due to stochastic errors that have to be minimized. The second assumes that most farms are inefficient with respect to a best-practice benchmark. The benchmark itself is a frontier production function, which can be estimated with a broad range of mathematical techniques. We use the parametric stochastic frontier analysis originally and independently described by Aigner, Lovell and Schmidt (1977) and Meeusen and van den Broeck (1977).

The frontier benchmarking technique is based on the original work by Farrell (1957). Observed inefficiency is decomposed in two components: a technical one, which reflects the physical transformation of inputs into outputs, and an allocative one, which reflects whether the input allocation approaches the optimal solution. Technical efficiency is a measure of potential input reduction to reach a given output, or potential output increase given an input quantity. Allocative efficiency is a measure of potential criterion improvement (cost decrease, profit increase) of changing input mix from current to optimal proportions.

Given the production technology, there is not only an input proportion that minimizes costs, but also an input proportion that minimizes nitrogen excretion. Coelli *et al*. (2007) propose an ecological efficiency measure that can be decomposed into technical and ecological allocative efficiency measures, which is similar to the above mentioned allocative efficiency from cost-minimizing perspective. Based on the different efficiency measures, economic-ecological trade-off possibilities can be assessed.

2.4. Data sources.

To fill in the production-theoretical framework in a coherent way and make the PB or FB operational, following data sources are combined: ‘in situ’ experimental data, farm accountancy data, carcass quality data, forfeit nitrogen content coefficients. We dispose of data of three farm level experiments consisting of a five-day treatment every five weeks with 30 ppm flubendazole. Table 1 shows the relative change in main technical-economic key figures. For each field experiment (FE), the implementation of a strategic deworming program has a positive effect on feed conversion, average daily weight gain and mortality rate. Field experiment 1 (FE1) shows that also medication costs diminish.

Table 1. Relative technical-economic changes of the flubendazole trial outcomes with respect to pre-treatment

Key figure	FE1	FE2	FE2
Feed conversion (kg/kg)	-0.29	-3.36	-3.45
Average daily weight gain (kg/day)	+2.41	+2.15	+1.97
Mortality rate (%)	-27.5	-1.02	-22.8
Medication costs	-2.96	/	/

Farm accountancy data from the official FADN (Farm Accountancy Data Network) are used to link the experiment data to average day-to-day practical farms performances. We dispose of a sample of 117 pig finishing farms with data from three consecutive years 2001-2003. Average pig price during that period is about the long term trend price: 1.13 euro per kg live weight compared to the 1.03 euro per kg trend. Due to carcass quality improvement, strategic deworming results in higher pig meat prices. We dispose of average figures on the percentage of carcasses in the SEU classification for the pre-treatment and the strategic deworming trials (Table 2). In addition, we dispose of invoice figures of a Flemish pig farm that allow for assigning price differences to differences in lean meat class. Besides price information, we also use input- and output-dependent nitrogen content data, depicted from literature.

Table 2. Relative classification in the best lean meat classes (S, E and U)

Classification	Pre-treatment	Flubendazole treated
S	10.94	9.48
E	61.42	67.36
U	23.54	22.04
total	95.90	98.88

2.5. Sensitivity analysis.

Pig prices are highly variable. Therefore, sensitivity of our results is tested for several pig and feed price assumptions.

3. RESULTS

Table 3 shows the relative effects of strategic deworming with flubendazole on income components and nitrogen excretion. Impacts on the cost and revenues are still minor (only up to maximum 3%), but the leverage effect on income is substantial. Due to strategic deworming, income increases with 10 to 15 %. This corresponds to an absolute income increase that range from 5 to 10 euro per average present finisher. Also the effect on nitrogen excretion is beneficial (except for experiment 1). As an overall effect, the effect on eco-efficiency increase with 8 to 19%.

Table 3. Relative economic and ecological change of the flubendazole trial outcomes with respect to pre-treatment

Economic and ecological key figures	FE1	FE2	FE2
Costs (euro/APF)	+1.4	-0.1	0.45
Revenues (euro/APF)	+2.8	+2.0	+2.3
Labour income (euro/APF)	+10.1	+12.1	+14.8
Nitrogen excretion (kg/APF)	+1.7	-2.9	-3.3
Eco-efficiency (euro/kg)	+8.3	+15.5	+18.8

APF: average present finisher

The production frontier benchmarking analysis shows that the performance improvements are almost exclusively due to technical efficiency. The technical efficiency score improves with 0.01 to 0.02, indicating that strategic deworming with flubendazole constitutes a robust technological progress in pig finishing. The impact on economic and ecological allocative efficiency is minor. This means that strategic deworming with flubendazole leads to a more or less proportional reduction of input use per unit output (= kg live weight).

These results can be generalised. We first look to the robustness of the partial budgeting results to changes in pig prices. A sensitivity analysis, with statistical test is performed. For experiment 1, with a 10 to 20% decrease of pig price, the leverage effects on income become more substantial and gives rise to 22 to 35 % more income respectively. When assuming a 10 to 20 % price increase, the income increase become relatively smaller, 6 to 7 % respectively. The absolute income gain ranges from 4 euro per APF to 7 euro per APF. For experiment 2 an 3 this range is 6 to 9 euro/APF and 9 to 12 euro/APF respectively.
A similar sensitivity analysis can be done with varying feed price. As feed price trend bends upwards since 2006, we used a 10% and 30% increase while keeping the output unchanged to the observed 2001-2003 level. The absolute labour income gain due to strategic deworming with flubendazole is more or less the same as in the original partial budgeting and ranges from 5 euro/APF to 11euro/APF. As labour income become strongly under pressure with the high increases of input prices, the relative impact of flubendazole on the net income gain in crisis situation becomes substantial. With 30 % feed price increase and the FE3 conditions, labour income raises with 51% after strategic deworming.
Secondly, we look to the potential overall effect at sector level. As such, it might be dangerous to extrapolate the technical-economic results from the experiments to the situation in the field. The frontier benchmark analysis, which analyses the experimental data together with the real farm data, shows that also the pre-treatment data had already a very high efficiency score. This is not surprising, and may hamper a direct extrapolation to a larger sample of real businesses. However, the more or less robust technological improvement we observed argues for extrapolating this efficiency improvement to the real farm data set. We performed the extrapolation in two ways, first by extrapolating the relative change in the key figures, second by extrapolating the extrapolation in efficiency scores. Table 4 shows the results of the first type of extrapolation. Again, the net gain of strategic deworming remains in the same level of magnitude.

Table 4. Simulated income increase, in euro per APF, on a set of 117 pig finishing farms from the official Belgian FADN, according to the year and type of extrapolation

	Income change, euro per APF		
Year	FE1	FE2	FE2
2001	+7.16	+8.00	+10.2
2002	+5.41	+6.79	+8.66
2003	+5.18	+6.55	+8.37

APF: average present finisher

Thirdly, how can these results now be exploited at farm level? In the first instance, the farmer can realise a technical efficiency improvement. This leads to the major net gain in both economic and ecological performance. Given the results on allocative efficiency, he can further optimise his input allocation. Where the exact optimum will be, also depends on the internalisation (e.g. by taxing) of the nitrogen excretion. Anyhow, the optimisation has to be sought on the observed production function, and will lead to an income gain that goes beyond the above mentioned income effects as simulated by partial budgeting. Note that there might be other potential gains that are not discussed here, e.g. decrease of penalties due to liver rejections in the slaughterhouse.

4. DISCUSSION

Both based on a coherent production-theoretical framework, partial budgeting and production frontier benchmarking were used to estimate the economic and ecological performance of strategic deworming with flubendazole. Although the separate technical-economic effects are rather low, the improved feed conversion, increased daily weight gain lower mortality and better carcass quality act in a synergetic way. Based on the experimental results and with average prices, this finally results in a 10 to 15 % increase of labour income. The absolute income gain ranges from 5 to 10 euro per average present finishing and is rather robust, or insensitive to price fluctuations. This means that strategic deworming has a strong attenuation effect on farm income decrease during crisis periods.
The work presented in this paper shows the complementary worth of traditional partial budgeting and the frontier production function technique for analyzing the economic-ecological impact of introducing new technologies. With the frontier analysis, a robust technological improvement could be shown which facilitates the extrapolation of experiment results to the situation in the field. Moreover, the frontier results revealed additional improvement margins that goes beyond the experimental results.

5. ACKNOWLEDGMENTS

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OPINION OF THE BOERENBOND ON THE CONTROL OF EMERGING DISEASES

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1. INTRODUCTION

The Boerenbond is the most important Belgian association for Belgian professional agriculture. The organisation represents more than 40.000 farmers in Flanders and the East Cantons (German speaking part of Belgium).

To prevent and control diseases, Belgium has several instruments:

- National databases: identification & registration of farms, animals and their movements (SANITEL), slaughtering (BELTRACE), control activities (FOODNET) and results of laboratory tests (LIMS).
- European database of intracommunautary movements of animals and animal products (TRACES).
- FASFC (Federal Agency for the Safety of the FoodChain)
- National reference lab (CODA-VAR) and Animal Health Services (DGZ Vlaanderen, ARSIA)
- National legislation for animal disease control, in compliance with international standards
- Implementation of Codes of Good Practice on farms

In the recent past, outbreaks or threats of outbreaks of several diseases in Belgium have proven the performance of those instruments. Nevertheless, the threat of emerging or re-emerging diseases has led to redefining the needs. The launch of a new Animal Health Strategy for the European Union (2007-2013) can be considered as a first step. As a farmer organisation, we underline the view of COPA-COGECA (European farmer organisations) on the new Animal Health Strategy.

To be successful in the battle of emerging diseases, adaptation of international and national instruments to prevent and control emerging diseases will be very important.

2. GENERAL ASPECTS

Availability of well trained official veterinarians and veterinarians in the field.
Optimal cooperation between international reference labs to maximize knowledge and expertise.
Informing about and creating awareness of emerging diseases in livestock.
Improving the communication policy on the animal health situation in special regard to consumers and their organisations.

3. ABOUT THE EUROPEAN APPROACH

European legislation is complicated and should be simplified and especially become more flexible so it can be adopted fast to new emerging conditions.
Integrated approach in animal health policy making is necessary- avoid inadequate action by one member state.
Reduce the threats of certain diseases, by stepping up measures of biosecurity and disease surveillance in special regard to borders and import. All imports must be carefully monitored, restricted or banned when imports of livestock product or live animals do not conform to European legislation.
Improve international solidarity in the control of animal diseases.
Rapid and transparent notification by the member states.
Not to focus only on zoonotic diseases but also on different “economic” diseases.

4. ABOUT VACCINATION

Vaccination as an alternative to mass slaughtering of healthy livestock for reasons of prevention.
Use of vaccination when available and appropriate.
Investment in R&D on new vaccines-safe vaccines.
Free marketing and consumption of food products derived from vaccinated animals – no stigmatising in retail.
No limitations on trading “safe vaccinated animals” when possible.
Facilitate availability of vaccines from abroad.

In special regard to epidemiological research

Epidemiological research on emerging diseases should be encouraged and financed.
Create more epidemiological research groups on European level.
Access should be given to collect necessary information to set up monitoring systems and predicting the spread of diseases.

5. CONCLUSIONS

Through history, it has been natural for farmers to ensure the welfare of their animals, to keep them from suffering or pain, and to provide adequate conditions for the health of the animals. The last years, the agricultural sector delivered efforts in the field of “Codes of good practice on farm level”. Farmers cannot be left alone to deal with the responsibility for and the costs of measures to control animal diseases and especially their consequences. Despite appropriate preventive measures, farmers will be faced with threats over which they have little, or no control at all.

PREVALENCE OF ANTIMICROBIAL RESISTANCE AMONG *E.COLI* IN BELGIAN BROILERS

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1. INTRODUCTION

A survey was conducted on 20 Belgian broiler farms to estimate the prevalence of antimicrobial resistance in Gram negative bacteria at farm level. Also a follow up of resistance between different flocks reared on the same farm was evaluated.

2. MATERIALS AND METHODS

A total of 1425 individual cloacal samples were taken. The indicator bacterium *Escherichia coli* was recovered out of 1229 of the 1425 samples (= 86,25%). Susceptibility against 14 antimicrobial agents was tested by disk diffusion according to the CLSI guidelines using Rosco® Neosensitabs and Iso sensitest agar plates (Oxoid®).

3. RESULTS

Acquired resistance was mainly found against ampicillin (88%), tetracyclines (67%), nalidixic acid (64%) streptomycin (59%), trimethoprim/sulphomethoxazole (55%). Surprisingly there was an unusual high resistance against some important second line antibiotics as ceftiofur (34%) amoxicillin/clavulanic acid (11%) and enrofloxacin (19%). Against the fluoroquinolone flumequine resistance was rather high with 36% of the strains being resistant. Lower resistance percentages were found for the aminoglycosides apramicin (7%), gentamicin (2%) and neomycin (15%). Finally against the amphenicols chloramphenicol and florphenicol resistance was 20% and 1%, respectively. Less than 4% of the strains were fully susceptible. Half of the strains were at least resistant to 4 different antimicrobials, indicating a high level of resistance.
On 10 of the farms, sampling was repeated during the next but one consecutive production round to evaluate trends or persistence of resistance patterns. The second samples were taken 3 months after the initial visits of the flocks.
Highly similar susceptibility profiles were found between the two production rounds. Variation in the average resistance percentage for the different antimicrobial agents ranged between 13.1% and 0.7%.

4. DISCUSSION

Our results show a persistence of resistance against chloramphenicol, an antibiotic not in use anymore since mid nineties. Resistance against frequently used antibiotics like ampicillin and tetracycline is very high. What worries most is the emergence of resistance against newer and high-potential antimicrobials. A remarkable finding in this study is the high level of ceftiofur resistance, since no cephalosporins are currently registered for use in poultry in the EU. We could not yet find any reason for this high level of resistance, which was mainly associated with multiresistance. The repeated samplings indicate a stable resistance profile over time. As a consequence, resistance may be maintained for a long period in this ecosystem and seems not directly affected when a new production round is started. These preliminary data show that further follow up of antimicrobial resistance in poultry is imperative. Also detailed data on the origin of animals, drug use and the management and

hygiene strategies during and in between production rounds deserve further attention in the epidemiology of antimicrobial resistance.

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SIMULATING THE NUMBER OF INFECTED HERDS AND THE GEOGRAPHIC DISTRIBUTION AFTER INTRODUCTION OF CLASSICAL SWINE FEVER IN BELGIUM

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1. INTRODUCTION

In the nineties, Belgium has been confronted with several classical swine fever outbreaks (CSF) (Miry et al., 1991; Koenen et al., 1996; Mintiens et al., 2001). After these major outbreaks, the Belgium pig industry has altered regarding biosecurity habits and the degree of contacts through animal transports (Ribbens et al., 2006 & 2007). Additionally, the number of live animal imports from bordering countries (e.g. Germany) diminished, coupled with a higher level of sanitary measures and control (Anonymous, 2006). Because of earlier mentioned changes, the risk of a CSF introduction in Belgium is generally lower compared to earlier years. Nevertheless, the risk of CSF introduction remains present, illustrated by a recent CSF outbreak in Germany, year 2006. Wild boar and the unknown infection status in new EU member states and its bordering countries are nowadays considered to be the main risk factors for CSF virus introduction, so vigilance for appearance of disease remains appropriate.
Disease preparedness has shown to be crucial, as is the ability of farmers, veterinarians and other persons involved in disease control (state veterinary services) to recognise CSF infection in an early stage (Moennig, 2000). Disease recognition determines the duration of the 'high risk period' (HRP). This is the period of 'silent spread' of infection; its duration is related to the extent and consequences of a potential outbreak. For last EU CSF outbreaks, the HRP ranged from 3 up till 9 weeks (Elbers et al., 1999). Experts estimate this period most likely will last about 6-8 weeks (3 weeks at least, 14 weeks at most) (Anonymous, 2006). Early recognition and compliance of reporting suspected cases continue to be essential prerequisites for a successful CSF control. Mathematical models can be appropriate tools to help obtain disease vigilance and preparedness. Simulation models played an important role in guiding the development of the control policies in the 2001 foot-and-mouth disease epidemic in the United Kingdom (Kao, 2002). Apart from their use for evaluating control strategies of past outbreaks (e.g. InterCSF: Jalvingh et al., 1999; Nielen et al., 1999; Mangen et al., 2001), stochastic simulation modelling in pre-outbreak periods has several benefits in that repeated simulations allow the variability of predicted outcomes to be quantified (Taylor, 2003; Yoon et al., 2006; Karsten et al., 2005). If disease models use detailed information on routine animal movements and other forms of contact, they can be useful to understand the extent of spread in future outbreaks. Also, they can objectively support new measures that prevent extensive spread over a large geographical area, as this determines the global impact of disease outbreaks on the national pig industry.
For this paper, we used detailed movement data originating from a recent study of Identification & Registration data (I&R) (Ribbens et al., 2006) to adapt a generic simulation model (Interspread Plus, Massey University, New-Zealand). This model was used to make a careful estimate of the number of infected herds after incursion of an undetected foreign epidemic disease (i.e. CSF), and to make predictions of the expected geographic or regional distribution of infection. Simulated epidemics will be used to further evaluate alternative CSF control strategies.

2. MATERIALS AND METHODS

Actual Belgian pig herds (n=8510), each characterised by x and y coordinates, herd size and herd type were used by the model (Belgian I&R data, Sanitel-Pigs, 2006). We inserted three different transmission routes into the

model: (1) direct contacts (animal movements), (2) indirect contacts (persons & different vehicles) and (3) local virus spread (neighbourhood contacts). Parameterisation of the virus spread was based either on data of experimental infections, analysis of outbreak data or consultation of experts. The number and type of animals leaving (i.e. off-farm movements) per time period were known for each farm and used by the model; also it was known by the model which type of animals a farm could receive (onto-farm movements). Distances for between-farm movements were based on the type of animals transported and herd types of distributing and receiving herds. Animal movement data relied on a study of I&R data on livestock movements in 2006 (Ribbens et al., 2006) and were implemented in the model to generate direct contacts. Frequency and distances of indirect movements were based on a survey (Ribbens et al., 2006) and depended on herd size. As in history outbreaks neighbourhood infections showed to be of major importance, radial spread of infection (no further than 1 kilometre) was simulated by the model. Radial spread was different for different herd sizes.

To start an outbreak, we randomly selected index herds from different herd types (farrow-to-finish, breeding, piglet multipliers and finishing herds) and from different regions (DPLA-SPLA). Threshold for density was 300 pigs/square kilometre (calculated per municipality) (Michel and Windhorst, 2003). A total of 160 index herds were chosen ad random (20 herds/stratum). In this paper, only results of densely populated livestock areas (DPLA) are shown. For each of the randomly selected index herds 99 iterations of the model were performed for a fixed time-frame of 30 & 60 days ('High Risk Period' or HRP). Statistics of different outbreak scenarios were calculated per herd type and region (minimum, 25% percentile, median, 75% percentile, maximum) and locations of outbreaks were plotted (ArcMAP 8.1, ESRI, Redlands, CA, USA). In addition, trends in geographical distribution are displayed using kernel smoothing, which is useful for summarising the spatial distribution of infected farms from a large number of iterations (Wilesmith et al., 2003). Kernel smoothing was performed using R 2.0.1 and the sm package (Bowman and Azzalini, 2003).

3. RESULTS & DISCUSSION

Simulation exercises can help predict the magnitude and potential geographical spread of a CSF outbreak in Belgium, given certain circumstances (herd type & size, area of index herd, etc.). Exact predictive modelling is impossible as the outcome of an outbreak depends on a variety of unpredictable factors (virus strains …) and coincidences. Stochastic modelling generates a range of possible outcomes from 'best' to 'worst case'; this way it accounts for the variability and uncertainty. Earlier developed models simulating CSF outbreaks were 'fitted' to existing outbreaks (Jalvingh et al., 1999; Nielen et al., 1999). In this paper, we randomly started outbreaks without prior knowledge of earlier outbreaks. This was done by randomly selecting index herds.
In table 1, descriptive results of different outbreak scenarios in DPLAs can be found. In the initial phase of introduction of infection (HRP-1), small differences in spreading according to different herd types can be seen. This is explained by a higher number of contacts made by breeding herds and piglet multipliers. When infection remained undetected (HRP-2), certain densely populated areas were prone to neighbourhood infection through local spread.

Table 1: Descriptives of simulated outbreaks (99 iterations per index herd).

		HRP-1=30 days					HRP-2=60 days				
Category herd	N	Min	25%	50%	75%	Max	Min	25%	50%	75%	Max
Breeding	20	1	1	4	4	13	1	4	10	17	58
Farrow-to-finish	20	1	1	2	3	15	1	4	7	12	65
Finishing	20	1	2	3	4	14	1	7	10	12	50
Piglet multiplier	20	1	3	5	7	17	1	6	14	16	42

As can be seen in the results from the randomly selected herds, in many cases the infection stays confined in the index herd, and median results predict only 'small size' outbreaks in Belgium. A study on contact structure identified a small number of pig herds in Belgium are responsible for a large proportion of movements (Ribbens et al., accepted). Future research may benefit focussing on earlier identified risk 'spreading' herds, as these herds may be more likely to be involved. Modelling worst case scenarios can be an appropriate choice, as the impact and consequences of foreign contagious diseases is high. New surveillance programs may be developed to identify risk movements and alterations in movement profiles can be predictors of future outbreaks.
Partly shown in figure 1, the magnitude of geographical dispersal was different for different herd types (lowest for finishing herds & highest for breeding herds). Spatial modelling has the advantage that 'disease jumps' toward other regions can be modelled: if these 'geographical jumps' occur (as happened during the 1997/1998 Dutch outbreak), there is a significant impact on infection control in the country. Identification of high risk areas using spatial modelling was earlier performed by Le Menach et al. (2005). We used kernel smoothing (figure 2)

as suggested by Wilesmith et al. (2003) in a first phase to visualise areas which were more prone to infection; this technique will be further improved. It is likely these 'hot spots' correspond with DPLAs, but this has to be investigated.

Figure 1: Geographic distribution of simulated outbreaks (HRP: 30 days – 99 iterations – 20 randomly chosen index herds); from left to right, top to down: breeding, farrow-to-finish, finishing & piglet multiplying herds).

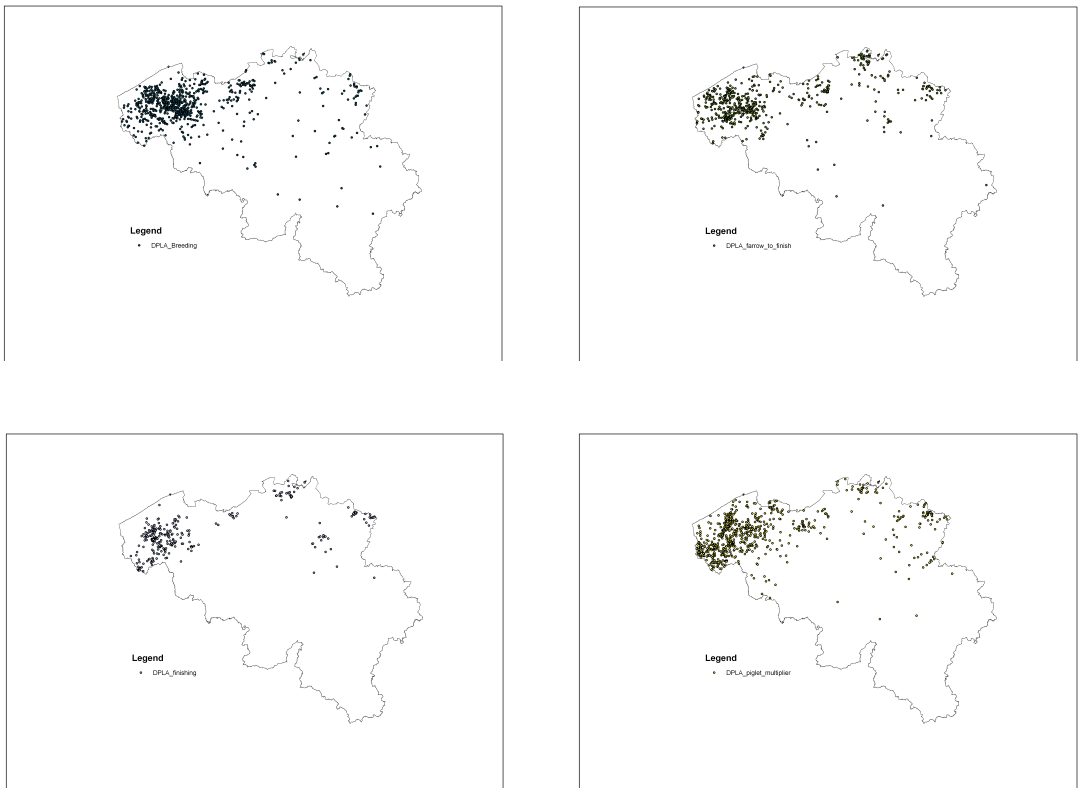
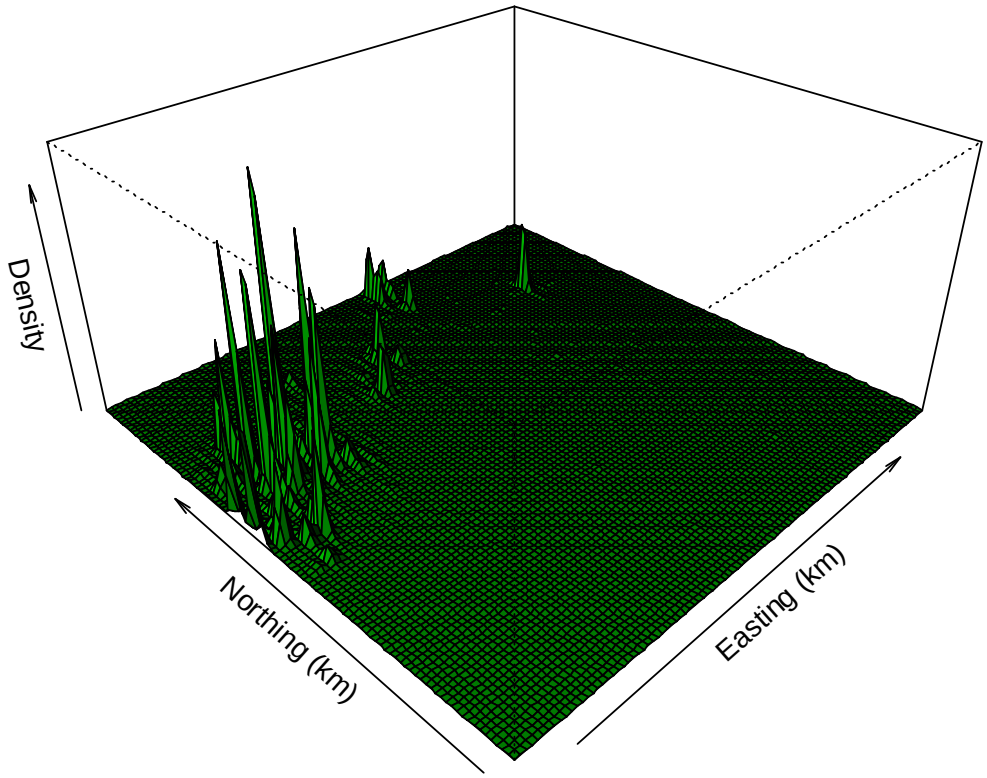


Figure 2: Example of kernel smoothing on simulated outbreaks (without index herds, HRP: 30 days – 99 iterations).



4. CONCLUSION

Stochastic and spatial simulation modeling with accurate information of the Belgian pig industry is a useful tool to gain knowledge on the size and geographical magnitude of future outbreaks in Belgium. Stochastic modeling gives a range of possible results regarding size and duration of an outbreak. Visual presentation of a large number of iterations using kernel smoothing is helpful to understand geographical spread and identify risk areas. Exact predictive modeling remains a difficult task as the outcome of an outbreak is influenced by a multitude of factors. The described model is currently used as a starting point to evaluate control strategies of future potential CSF outbreaks in Belgium.

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DESCRIBING BETWEEN-FARM MOVEMENTS IN BELGIAN PIG HERDS USING GRAPH DRAWINGS

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1. INTRODUCTION

The frequency and structure of different contacts between pig herds will to a large extent determine the spread of infection in different regions. This is especially the case during the period between the introduction of an epidemic and the first diagnosis of infection, also called the "high-risk period" (HRP). The importance of direct contacts for spreading of infections is clearly illustrated by the efficacy of movement restrictions in limiting outbreaks. Accurate knowledge on the structure and frequency of contacts between herds is both important for tracing purposes during contagious animal-disease outbreaks & for the understanding and prediction of the impact of particular infection-control strategies.

2. MATERIALS AND METHODS

We used data concerning animal movements from the I&R database for all Belgian pig herds for an 8-months period (2006). The information available comprised all recorded onto- and off-movements of pigs for each herd in Belgium, including the number & type of animals transported, & the individual identification of the transport vehicle. This information made it possible to link the onto- and off-movements on different herds for this time period. To demonstrate relationships through these between-farm movements, we constructed directed graphs using the Kamada-Kawai algorithm in the network software Pajek (2003). Farms are represented by a circle ('vertices'), which are connected through weighted lines ('edges'). Directed networks were constructed for a one month time period (April 2006).

3. RESULTS & DISCUSSION

In total 33,234 between-farm movements were made during the 8-month period. This corresponds with an average of 4,154.3 between-farm movements/month for the Belgian pig population. For the 8-month period, the median number of origin herds was 4 (Q1: 2; Q3: 8). In a study by Maes et al. (2004) mixing of animals from different origin herds was found to be a significant risk factor for mortality in finishers. Also for epidemic infections, mixing of animals is a clear risk. In figure 1 (left - Directed network of 1,493 piglet movements between 1,078 Belgian pig herds in April 2006 (929 source herds, 158 recipient herds with at least 5 purchases a month)) , piglet movements showed a typical structure, with piglet producers distributing towards a recipient herd making these herds some kind of 'collectors' of infection. In figure 2 (right - Directed network of 520 replacement stock movements between 570 Belgian pig herds in April 2006 (128 source herds, 465 recipient herds)), distribution movements of replacement stock is illustrated; some of these herds can potentially spread disease to a large number of recipients. If disease remains undetected in these 'spreader-herds', infection can disseminate towards several farms because different movements happen in a short time-frame.

MULTPLE-CORRESPONDENCE ANALYSIS & TWO-STEP CLUSTER ANALYSIS TO DESCRIBE BIOSECURITY PRACTICES IN BELGIAN PIG HERDS

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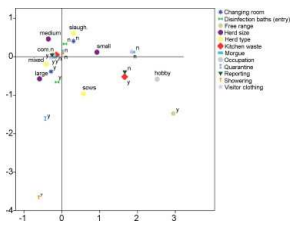
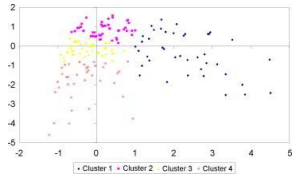
1. INTRODUCTION & MATERIALS AND METHODS

We used multiple-correspondence analysis followed by a two-step clustering procedure to describe biosecurity status in Belgian pig herds. Data was collected by means of a questionnaire, sent out to a stratified random sample of 609 pig farms. We achieved a 71.6% response, and 421/609 farmers (69.1%) returned questionnaires suitable for analysis. Multiple-correspondence analysis (MCA) is an exploratory technique used to describe the relationships between different independent categorical variables. The purpose of MCA is to find quantifications for different dimensions that are optimal in the sense that different variable categories are separated from each other as much as possible. The relationships between the different categories of the variables are shown by representing them as points in a multidimensional space. In this abstract only nine variables concerning biosecurity status are presented. Objects' scores derived from the MCA solution were used in a two-step clustering procedure to reveal populations of pig herds with similar biosecurity habits. The two-step cluster method is a scalable cluster analysis algorithm using a hierarchical clustering method.

2. RESULTS & DISCUSSION

Figure 1 (left) gives the MCA solution for the variables regarding biosecurity status. The location of the variable categories in the plot can be used to interpret the relationships between the different variables. We noted that the variables absence of feeding of kitchen waste, no free range, wearing of herd clothing obliged, existence of a morgue, reporting obliged and commercial herds are all closely correlated and plotted close to the origin of the axes, which represents the centre of gravity of all pig herds. Feeding of kitchen waste together with the variables absence of reporting, and no visitor clothing obliged together with no morgue are also correlated. Free range, quarantine period and showering obliged are all separately plotted and situated most far away from the origin of the axes. In figure 2 (right), object scores of the pig herds regarding variables on biosecurity status are presented. We differentiated four biosecurity groups, which we interpreted as indicating low- to high-biosecurity status. *Group 1* herds ('low-biosecurity status' (n = 49)) were small or hobby herds. All pig herds with "free range" husbandry were situated in this cluster. This cluster fed kitchen waste more than any other cluster did. Herds belonging to *group 2* ('low medium biosecurity status' (n = 138)) were mostly commercial, small to medium sized herds. Basic biosecurity measures (morgue, reporting and visitors clothing) were well practiced. Most responding herds were member of *group 3* ('high medium biosecurity status' (n = 172)): these were commercial, mixed herds. Apart from basic biosecurity measures, biosecurity related to entrance of persons was better practiced than in clusters 1 and 2. Pig herds in *group 4* ('high-biosecurity status' (n = 62)) had strict demands concerning entrance control (quarantine, showering).





ANTIBIOTIC RESISTANCE IN PATHOGENIC *E. Coli* FROM PIGS DURING 2005-2006

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E. coli is a major pathogen in swine causing neonatal and post weaning diarrhoea, or oedema disease. Resistance has frequently been reported among pathogenic *E. coli* from swine. Therefore, antimicrobial resistance in the different pathotypes of *E. coli* was investigated for the period 2005-2006.

A total of 541 *E. coli* strains were isolated from animals showing symptoms that might be associated with an *E. coli* infection. They were referred to the CODA for determination of the pathotype and antibiotic susceptibility testing. PCR was performed for the following virulence factors: F18, F4, F41, F5, F6, LT, STa, STb and VT2v. Susceptibility was tested by disk diffusion using Rosco^{R} tablets. The following antibiotics were tested: tetracycline, ampicillin, chloramphenicol, florfenicol, ceftiofur, amoxicillin+clavulanic acid, enrofloxacin, nalidixid acid, apramycin, gentamicin, florfenicol, neomycin and sulfonamides-trimethoprim.

In almost 90% of the strains haemolysis was seen, a trait associated with virulence. Few stains were non-haemolytic though carried virulence traits anyhow. This means that there is a disconcordance between virulence and haemolysis. A haemolytic strain does not necessary correspond to pathology, but a non-haemolytic strain will be most probably not related to the disease. In this study, a large portion of the strains (n= 228) could not be assigned to one of the virulence types, though they were isolated from diseased animals. They may be really non pathogenic and likewise regarded as indicator bacteria, or they may contain yet unknown virulence genes.

The majority of the strains were ETEC mostly associated with the STb toxin. F18 and F4 adhesion factors were almost equally represented. No strain was positive for the 987P and the K99 fimbriae. There were no major differences in the presence of the virulence genes compared between the two years. Only the colonizing strains seem to be increasing but this may be also a punctual event.

A small amount of strains (9,1%) were completely susceptible to all antibiotics tested. Most resistance was found against the antibiotics ampicillin (65,8%), tetracycline (80,6%) and trimethoprim+sulfonamides (65,9%). These are also the most used antibiotics in swine production in Belgium. Striking is that chloramphenicol resistance remains high (30%), since it has not been used anymore since mid nineties. Resistance against nalidixic acid was about 20%. The lowest resistance percentages were against ceftiofur (1,4%), amoxicillin-clavulanic acid (0,9%), enrofloxacin (3,5%), gentamicin (2,7%), florfenicol (1,8%), neomycin (5,3%) and apramycin (5,1%).

Differences between the susceptibility of the different pathotypes was assessed statistically using chi square test., A statistical significant higher resistance was found in the colonizing strains compared to enterotoxigenic *E. coli* for the antibiotics chloramphenicol and nalidixic acid. No other differences were seen.

Most strains displayed a multi-resistance phenotype. Eighty-eight percent of the strains were resistant to at least two antibiotics. On average a strain was resistant to nearly 3 antibiotics. One strain was found to be resistant to up to 10 antibiotics, being only susceptible to apramycin and amoxycillin-clavulanic acid.

In conclusion, the majority of the strains were ETEC, causing diarrhoea. Less than 10% of the strains did not carry any resistance and most strains were resistant against at least three antibiotics (mainly ampicillin, tetracycline et sulfa-trimethoprom) indicating a high level of multi-resistance. This may compromise future *E. coli* therapy in swine.

ANTIBIOTIC RESISTANCE IN PATHOGENIC *Escherichia Coli* FROM BOVINES DURING 2005-2006

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Esherichia coli is a major pathogen in bovines where it may cause neonatal diarrhea frequently evolving into septicemia. Therefore, antimicrobial resistance in association with the different pathotypes was investigated for the period 2005-2006. A total of 455 *E. coli* stains were isolated from animals showing symptoms that might be associated with an *E. coli* infection. They were referred to the CODA for determination of the pathotype and susceptibility testing.

Virulence genes were detected by PCR (CNF1, CNF2, eae, vt1, vt2, STa, F5 and F41) or colony agglutination (F111, CS31A and F17). Susceptibility was tested by disk diffusion using Rosco^{R} tablets. The following antibiotics were tested: tetracycline, ampicillin, chloramphenicol, florfenicol, ceftiofur, amoxicillin+clavulanic acid, enrofloxacin, nalidixid acid, spectinomycin, apramycin, gentamicin, kanamycin, neomycin, streptomycin, cefquinome, ceftazidime, tetracycline and sufonamides-trimethoprim**.**

In 133 of the 455 strains, no virulence factors were found. It should be noted that CS31A was only tested in 2006 and that strains from 2005, determined as no virulence factor detected might have been positive for CS31A. Two hundred and thirty-three stains were scored as potential colonizing strains (F111, F17, CS31a or F5 positive), 36 as NTEC (at least CNF1 or CNF2 positive), 9 as EPEC (eae positive), 27 as VTEC (at least vt1 or vt2 positive) and 17 as ETEC (an adhesion factor and STa positive). There were no major changes in the prescence of the virulence genes. F17 seems to be increasing but this may be also a punctual event.

Only few strains (8,8%) were fully susceptible. Most resistance was found against the antibiotics tetracycline (82%) and ampicillin (82,5%). Striking is that chloramphenicol resistance remains high (more than 60%), since it has not been used anymore since mid nineties. Alarming is the emerging resistance to ceftiofur and amoxicillin+clavulanic acid resistance (Approximately 5% of the strains). Cephalosporine resistance was clearly associated with multi-resistance. Resistance against the quinolones was evident in two-thirds of the strains, and fluoroquinolone resistance was present in nearly half of the strains. Resistance against the aminoglycosides spectinomycin and apramycin were the lowest (5 and 15% respectively). Against all other antibiotics resistance percentages attained more than 30%.

When taking all pathotypes together, most striking increasing in antibiotic resistance between these two years was the significant increase of resistance against florfenicol (from 19% in 2005, to 38,5% in 2006), nalidixic acid (from 48,8% in 2005 to 72,7% in 2006) and gentamicin (from 8,3% in 2005 to 32,9% in 2006). This increase was mainly due to increased resistance among colonizing strains, especially amongst CS31A strains. For the colonizing strains, resistance was also significantly higher for many antibiotics in comparison with the other pathotypes.

Most strains displayed a multi-resistance phenotype. A little more than half of the strains was resistant to at least 6 different antibiotics. Two strains, isolated in 2006, were resistant against up to 12 antibiotics. It must be noted that there is a shift towards more multi-resistance in the data. One should take into account that that new antibiotics were included in the year 2006. So, there is interference of increase in multi-resistance due to the increase of antibiotics tested.

In conclusion, resistance is at an alarming high point in *E. coli* strains from bovines. The resistances against cephalosporin, amoxicillin clavulanic acid, and marbofloxacin, all tree quite recent antibiotics is emerging or for fluoroquinolones already highly present and is clearly associated with multi-resistance. There is no specific evolution in the prevalence of virulence genes seen. These high resistance percentages may compromise therapeutic possibilities for bovine *E. coli* infections.

FIRST REPORT ON THE DIVERSITY IN BELGIAN BROILER FARMS OF BROAD-SPECTRUM-β-LACTAMASES AMONG CECAL *ESCHERICHIA COLI*

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Ceftiofur resistance is emerging in animal bacteria. Although ceftiofur is not allowed for use in poultry in Belgium, we investigated the presence of broad-spectrum β-lactamases (extended spectrum ß-lactamases (ESBLs) and class C β-lactamases (AmpC)) among ceftiofur resistant *E. coli* isolated from Belgian broilers. ESBLs confer resistance to most β-lactam antibiotics, but are not active against cephamycins and carbapenems, and can be inactivated by clavulanic acid. This is in contrast to class C β-lactamases that usually confer resistance to all β-lactams with the exception of dipolar ionic methoxy-imino-cephalosporins, such as cefepime, and the carbapenems. In *E. coli*, resistance provided by class C β-lactamases can be plasmid-encoded or due to the overexpression of the chromosomal *ampC* gene.

A total of 489 cloacal samples of chickens from 5 different Belgian broiler farms were examined for the present of ceftiofur resistant E. coli using McConkey agar (Oxoid) supplemented with 8 µg/mL ceftiofur (Pfizer). Strains were examined for resistance against β-lactam antibiotics and other antimicrobial agents by disk diffusion tests, using Neosensitabs (Rosco). Rep-PCR was used to exclude duplicate samples. Isoelectric focusing (IEF) was performed to determine the number of ß-lactamases present in a strain. PCR confirmed the presence of ß-lactamases and sequencing allowed us to determine the exact ß-lactamase present.

A total of 295 ceftiofur resistant commensal *E. coli* isolates were obtained from the 489 cloacal swabs. Fifty-one isolates were unique strains as determined by rep-PCR. No resistance to imipenem, apramycin and florphenicol was observed. Resistance to chloramphenicol, gentamicin, kanamycin, neomycin and enrofloxacin was below 10 % whereas 22% were resistant to trimethoprim-sulfonamides. In contrast, 64 % of the isolates were resistant to trimethoprim, 66 % were resistant to sulfonamides and nearly half was resistant to nalidixic acid and tetracycline. Nineteen percent of the isolates showed only resistance to β-lactams, 5% of the isolates were resistant to one additional antimicrobial agent and the other 76% were resistant to at least two or more antimicrobials agents. Some strains (4%) were resistant to up to 8 additional antibiotics.

IEF, PCR and sequencing revealed the following ESBLs among the 51 unique strains: TEM-52 (13.2%), TEM-106 (2%), CTX-M-1 (27.4%), CTX-M-2, (7.8%), CTX-M-14 (5.9%) and CTX-M-15 (2%). The only plasmidic AmpC β-lactamase found in this study was the CMY-2 enzyme in 41.2 % of the strains. The combination of an ESBL (CTX-M-1) with a plasmidic AmpC β-lactamase (CMY-2) was found in 7.8% of the isolates. TEM-1 as a narrow spectrum ß-lactamase was found in 38% of the strains, and was always in combination with an ESBL of AmpC. Mutations in the promoter and attenuator regions of the chromosomal *ampC* gene were found, but were not estimated of importance in resistance since they have never been described as being involved in over-expression and they are out of the important regions involved in over-expression. Moreover in the association with an ESBL gene, no phenotypic characteristics were present indicating AmpC mediated resistance. However, mutations were also found in association with bla_{CMY-2} gene and for these strains no phenotypic judgement could be made.

Our data show that ceftiofur resistant *E. coli* are often present in the intestinal tract of broilers at farm level in Belgium. These strains are frequently multi-resistant. The diversity of broad-spectrum β-lactamases among these isolates is high and they may act as a reservoir of ESBL genes. This finding necessitates a follow up evaluation of extended spectrum ß-lactam resistance in commensal *E. coli* of poultry in order to be able to estimate the public and animal health burden.

COMPARISON OF tDNA-INTERGENIC SPACER PCR AND RPOB-GENE SEQUENCING FOR SPECIES LEVEL IDENTIFICATION OF BOVINE COAGULASE-NEGATIVE STAPHYLOCOCCI

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1. INTRODUCTION

In many European dairy farms that have adopted the 5- and 10-point mastitis prevention programs, the relative importance of coagulase-negative staphylococci (CNS) has increased. They are the predominant pathogens found in milk samples and are causing the majority of intramammary infections (imi) in fresh dairy heifers. The increase in CNS prevalence and incidence relative to traditional major pathogens, combined with changes in limits for bulk milk somatic cell count penalties and the fact that CNS are causing clinical mastitis also, warrant reconsideration of their historical designation "minor pathogen". On the other hand, protective characteristics of CNS have been reported. The confusion can partly be explained by the lack of (accurate) species identification. Accurate and low-cost identification is a prerequisite for epidemiological studies aiming at elucidating the relevance of the different CNS species in bovine mastitis. Current identification methods are largely phenotypic and based on reference strains of human origin. These methods may not be suitable for isolates of bovine origin. In this study, we have updated tDNA-intergenic spacer PCR (tDNA-PCR) for identification of bovine CNS species by extending the current library of the technique, followed by comparing the results with sequencing of the *rpo*B-housekeeping gene.

2. MATERIALS AND METHODS

2.1. Isolates

2.1.1. Updating tDNA-PCR library using bovine field isolates.

Ninety-four CNS-isolates originating from milk and 52 from teat apices were available. Both tDNA-PCR and gene sequencing were performed on all isolates. Peak patterns obtained with tDNA-PCR were added to the existing database, which consisted of reference CNS-strains. Gene sequencing was used as gold standard, implying that when tDNA-PCR identification was uncertain or did not correspond with sequencing identification, the latter was considered correct.

2.1.2. Comparison of tDNA-PCR and gene sequencing for the identification of bovine CNS.

One hundred CNS-isolates originating from milk and 48 from teat apices were available. All isolates were subjected to tDNA-PCR and gene sequencing. tDNA-PCR was performed using the updated library as described before.

2.2. Techniques

DNA-lysates were prepared by alkaline extraction (Baele *et al.*, 2000). tDNA-intergenic spacer PCR was performed as described (Vaneechoutte *et al.*, 1998; Baele *et al.*, 2000, 2001). The length of the PCR-products was analysed with capillary electrophoresis using an ABI-Prism™ 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Vaneechoutte *et al.*, 1998; Baele *et al.*, 2000, 2001) and a software program was used for interpretation (Baele *et al.*, 2000). Sequencing of the *rpo*B-gene was performed as described (Drancourt and Raoult, 2002) with small modifications. Results were compared to online reference data using nucleotide-nucleotide BLAST. When there was no amplification with the *rpo*B-primers or less than 97% homology with reference strains was seen, additional *cpn*60-sequencing and if not sufficient *16S*-sequencing, was performed.

3. RESULTS

3.1. Updating tDNA-PCR library using bovine field isolates.

Nine isolates (6.2%) were unidentifiable with gene sequencing and were therefore temporarily ignored for further study. Of 21 (15.3%) of the isolates identified with gene sequencing, there was doubt about the tDNA-PCR identification. The peak patterns of these isolates were added to the library based on the sequencing identification. The updated library was used for the identification of the CNS-isolates in step 2.

3.2. Comparison of tDNA-PCR and gene sequencing for the identification of bovine CNS.

The results are presented in table 1 (milk samples) and 2 (teat apices). Overall, 12 isolates (8.1%) were unidentifiable with gene sequencing. The overall agreement (isolates from milk and teat apices) between tDNA-PCR and gene sequencing was 97.5%. When focussing on milk samples and teat apices separately, 96.6 and 100% of the identifications agreed in both tests, respectively.

Table 1: Comparison of tDNA-PCR and gene sequencing for identification of CNS-isolates originating from bovine milk samples.

	gene sequencing identification																	
tDNA-PCR identification	no identification	*S. capitis*	*S. caseolyticus*	*S. chromogenes*	*S. cohnii*	*S. epidermidis*	*S. equorum*	*S. fleuretti*	*S. haemolyticus*	*S. hyicus*	*S. nepalensis*	*S. saprophyticus*	*S. sciuri*	*S. simulans*	*S. succinus*	*S. warneri*	*S. xylosus*	Total
no identification	2			3		1			1	1	1					1	1	11
S. capitis		1																1
S. caseolyticus			0	1														1
S. chromogenes				42						1								43
S. cohnii					1													1
S. epidermidis						11												11
S. equorum							4											4
S. fleuretti								2										2
S. haemolyticus									2									2
S. hyicus				1						5								6
S. nepalensis											0							0
S. saprophyticus												1						1
S. sciuri													1					1
S. simulans														3				3
S. succinus															2			2
S. warneri																3		3
S. xylosus																	8	8
Total MILK	2	1	0	47	1	12	4	2	3	7	1	1	1	3	2	4	9	100

Table 2: Comparison of tDNA-PCR and gene sequencing for identification of CNS-isolates originating from bovine teat apices.

tDNA-PCR identification	gene sequencing identification																	
	no identification	*S. capitis*	*S. caseolyticus*	*S. chromogenes*	*S. cohnii*	*S. epidermidis*	*S. equorum*	*S. fleuretti*	*S. haemolyticus*	*S. hyicus*	*S. nepalensis*	*S. saprophyticus*	*S. sciuri*	*S. simulans*	*S. succinus*	*S. warneri*	*S. xylosus*	Total
no identification	7		1										1					9
S. capitis		2																2
S. caseolyticus			3															3
S. chromogenes	1			4														5
S. cohnii					2													2
S. epidermidis						0												0
S. equorum							5											5
S. fleuretti								2										2
S. haemolyticus	2								5									7
S. hyicus										2								2
S. nepalensis											0							0
S. saprophyticus												0						0
S. sciuri													7					7
S. simulans														1				1
S. succinus															1			1
S. warneri																0		0
S. xylosus																	2	2
Total MILK	10	2	4	4	2	0	5	2	5	2	0	0	8	1	1	0	2	48

4. DISCUSSION

When studying the impact of different CNS species on performances (udder health, milk production…) in dairy cattle, an accurate identification technique is required. Although no single test can offer fully reliable identification of bacterial species, gene sequencing is often seen as the gold standard. Unfortunately, the high cost and its labour intensiveness limit its use in large field studies for most routine laboratories. Phenotypic methods on the other hand are usually cheaper but lack accuracy. The results of this study show that tDNA-intergenic spacer PCR could be a good alternative for gene sequencing. It's a rapid, low-cost and easy to perform technique that has a high reproducibility if capillary electrophoresis is available (Baele *et al.*, 2001). The overall agreement between tDNA-PCR and gene sequencing was high.

Overall, a high number of CNS-isolates could not be identified with the gold standard (gene sequencing: *rpo*B, *cpn*60, *16S*), especially isolates originating from teat apices (20.3%). Possible explanations could be the presence of undefined species on teat apices, or strain differences between isolates from different origins. Still, availability of a complete reference database is a prerequisite and could be the bottleneck. Additional sequencing of the *tuf*-gene might give a definite answer (in progress).

To conclude, tDNA-PCR will be a useful tool for our field study aiming at elucidating the relevance of CNS imi in dairy cattle.

5. ACKNOWLEDGEMENTS

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IMPLICATIONS OF FSO FOR *SALMONELLA* IN THE BROILER SUPPLY CHAIN

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1. INTRODUCTION

In order to increase the safety of food products, the concept of Food Safety Objectives (FSO) has been developed (ICMSF, 2002). An FSO is defined as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption (or at retail) that provides or contributes to the appropriate level of human protection. Once such an FSO has been established at the end of the production chain, Performance Objectives (PO) can be derived for preceding stages of the chain. The aim of this research was to determine PO in the broiler supply chain based on a preset FSO at the end of the chain, using a model based on data from the chain.

2. MATERIALS AND METHODS

An analytical model has been developed based on *Salmonella* prevalence data collected by the broiler industry in the Netherlands. Data covers the period 2002-2005 and six sampling points in the chain, including: departure from hatchery, arrival at and departure from the farm, arrival at and departure from the slaughterhouse, and end of processing. Based on an FSO at end of processing, the model estimates PO for the five preceding points in the chain. Model parameter values were estimated based on the monitoring data.

3. RESULTS AND DISCUSSION

Results from scenario analyses, using various levels for the FSO, showed the current FSO (2.5% Salmonella positive) could be reduced by spreading the PO along the production chain. Sensitivity analysis using the model parameters indicates that interventions at the end of the supply chain are more effective than at the broiler farm. In general, a decrease in contamination is more effective than a increase in Salmonella reduction, implying contamination of flocks should be prevented rather than treated. A combination of both gains most results.

4. CONCLUSIONS

Scenario analyses using the FSO-PO model developed could be helpful in the discussion between authorities and industry on the feasibility of setting an FSO on broiler meat. Furthermore, the project is a practical application of the international theoretical framework of FSO-PO. As such, it contributes to the implementation of this framework.

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IDENTIFICATION OF *SALMONELLA* RISK FARMS BY SEROLOGICAL SURVEILLANCE AT PRE-HARVEST LEVEL: MISSION IMPOSSIBLE!?

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1. INTRODUCTION

The EU Zoonoses Regulation Nr 2160/2003 (Anonymous 2003) requires Member States to take effective measures to detect and control *Salmonella's* of public health significance. Although the Commission provides directives, it is likely that National control programs will vary to some extent between the Member States. The Belgian Federal Agency for the Safety of the Food Chain (FASFC) installed a National *Salmonella* surveillance and control program in pigs (SAP) in January 2005 which became compulsory by means of a Royal act in July 2007(Anonymous 2007 a,b) and which is outlined in Figure 1. In a first stage of the SAP, the FASFC aimed to identify maximum 10% of the herds which keep pigs with high levels of *Salmonella*-specific antibodies (risk herds) detected by a commercial indirect serum ELISA (SP ratio's). Since July 2007, the FASFC decided to identify risk farms as farms with a mean SP ratio equal or higher than 0.6 for 3 successive sampling rounds.

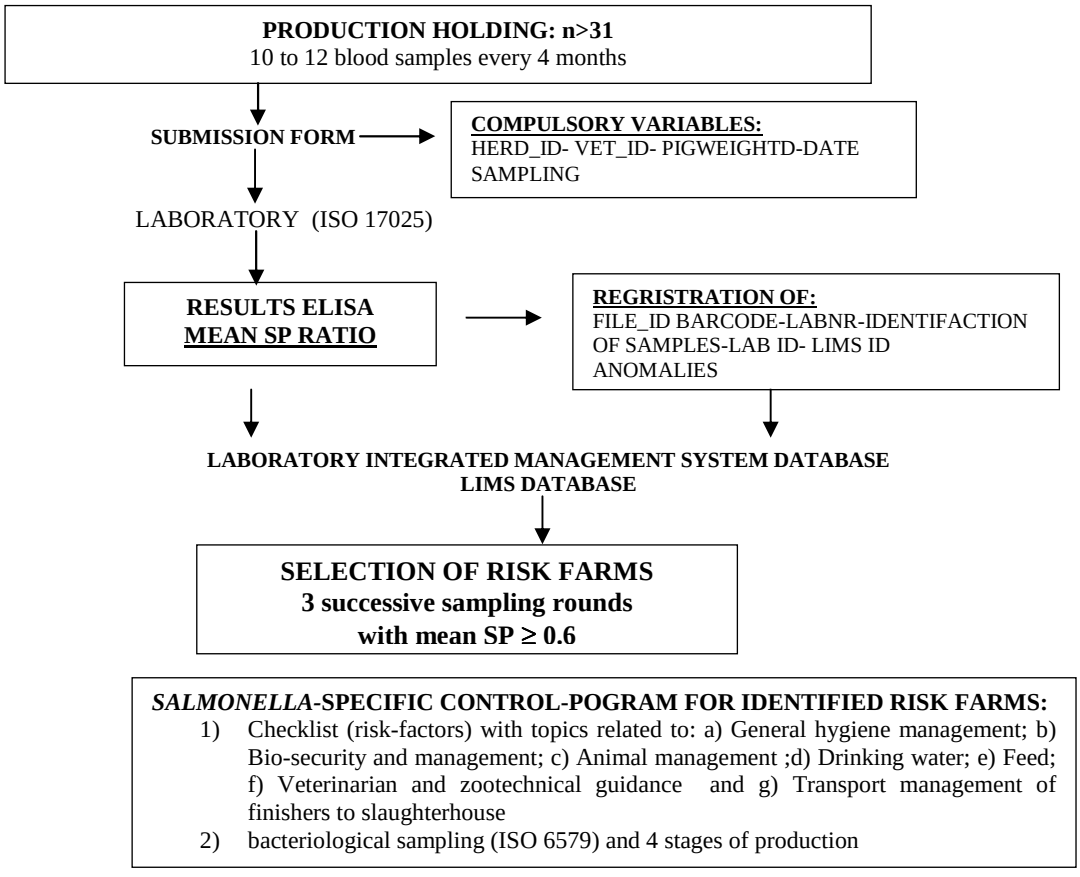

Figure 1: The *Salmonella* Surveillance program in Belgium (2007)

It is well agreed that serological testing is a good method for screening at herd level with an acceptable correlation with bacteriological isolation. However, in order to classify herds as risk herds based on the ELISA results it is necessary to obtain insight about the serological data obtained within the SAP since January 2005. A detailed analysis of the available serological data was performed in order to obtain population-averaged and herd-specific (sero)prevalence estimates of *Salmonella* on Belgian pig herds. In addition, variability between and within herds as well influential factors associated with increased levels of *Salmonella*-specific antibodies were investigated in order to assign risk herds.

2. MATERIALS AND METHODS

The study population consisted of pigs which were considered in the Aujeszky disease monitoring program in Belgium (= weaners, growing pigs and finishing pigs from all production holdings (farrow to finisher herds, grower herds and weaner to finisher herds)). A two-stage sampling was carried out as most of the Belgian production pigs are kept in clusters (herds, pens), having the same characteristics such as nutrition, housing and exposing to various infectious agents. Since January 2005, 10 to 12 blood samples (~herd size) of different weight categories (<40 kg, 40-59kg, 60-79 kg and ≥ 80kg) were collected in each pig herd every 4 months by the herd veterinarian in order to confirm the Aujeszky free status.
These blood samples were also tested with an indirect enzyme linked immunosorbent assay (ELISA) for the detection of antibodies against Salmonella (Idexx Laboratories, HerdChek* Swine Salmonella Antibody Test Kit). The presence of antibodies against Salmonella in each sample was determined by relating the optical density values (OD) to the mean positive (ELISA kit) control by calculating the sample to positive ratio (SP ratio = OD_{sample} - $OD_{neg\ kit\ control}$ / $OD_{pos\ kit\ control}$ - $OD_{neg\ kit\ ontrol}$).
The estimation of the within-herd-prevalence with 95% confidence intervals was based on a logistic-normal regression model assuming a normal-distributed random intercept for each herd. The intraclass correlation coefficient (ICC) was calculated to establish the correlation between two animals within a herd. The herd-prevalence's computations were based on a logistic regression model. For the purpose of this study, a herd was considered as positive if at least one of the sampled animals were tested positive by the indirect ELISA test (0.25 was used as a cut off value).
It was necessary to determine the number of sampling rounds that had to be taken into account when identify risk herds with high mean SP ratio and a stable within-herd variability (ensuring a stable predictive interval for the mean SP ratio's/herd), and a stable between-herd variability (to determine a cut off value for identifying a problem herd at risk). Several linear mixed models-capturing the herd effect, season and pig weight - were fitted based on data obtained from different (successive) sampling rounds since January 2005. The general form for these fitted models can be expressed as:

$$SP\_ratio\_transformed = \beta_0 + \alpha_1 + \beta_1\ days1\_std + \beta_2\ days2\_std + \beta_3\ days3\_std + \beta_4\ category\_pig\ weight + \varepsilon_{ij}\ .$$

β_0 β_1 β_2 β_3, β_4 were the population level parameters of interest, α_1 was the random intercept of the specific herd i. The models were build on the assumption of normality with respect to the error term ε_{ij} and the random intercept α_1. Therefore, the SP ratio's were transformed into SP-ratio_transformed (= $1/(SP\ ratio+2)^2$). The variable days was standardised (days1_std) to avoid computational problems. Also quadratic (days2_std) and cubic terms (days3_std) were standardised to avoid multicollinearity and possible computational burden.

3. RESULTS

More than 475,000 samples (January 2005 until April 2007) from more than 7,000 production holdings were examined. More than 95% of all samplings were performed with a sample size of 12 at each sampling round. Around 92% of all samples were collected from herds located in the northern part of Belgium (region of Flanders) represented by the provinces Limburg, Vlaams-Brabant, Antwerpen, Oost en West-Vlaanderen. Nearly half of the samples collected in Belgium came from the latter province. Significant differences ($P<0.01$) were found between provinces and all weight categories, with the highest mean SP ratio in the provinces of Antwerpen (mean SP ratio in 2006 of 0.606 [95%CI = 0.57-0.64] and Limburg (= mean SP ratio of 0.54 [0.50-0.57] in 2006 and the lowest mean SP ratio's in West-Vlaanderen (=0.275 [0.27-0.28]. The mean SP ratio's from finisher and slaughter pigs (= 0.372 [0.37-0.38] and 0.483 [0.48-0.50] respectively) were significantly higher compared with those of weaned piglets below 40 kg (= mean SP ratio 0.223 [0.22-0.23]). Besides spatial differences and weight effect also significant seasonal effects were observed with higher expected SP ratio's when sampling occurred during summer (=mean SP ratio of 0.401 [0.397-0.404]) and autumn (= mean SP ratio

of 0.380 [0.376-0.384] compared to the winter months (=mean SP ratio of 0.354 [0.35-0.36]. In addition, significant higher SP ratio's were observed in breeder, mixed and sow herds in comparison with piglet and reproduction herds but not with specialist finishing herds.
The overall apparent herd seroprevalence in Belgium was estimated at 94.6% [95% CI = 93-96], 72.5% [71.6-73.4], 66.1% [65.2-67] and 60% [59-61] using a cut off value of respectively 0.25 (table 1), 0.50, 0.75 and 1.00 (not shown). Province-specific apparent herd prevalence (=apparent seroprevalence) and province-specific apparent within-herd prevalence are shown in Table 1.

Table 1: Within-herd and herd seroprevalence in Belgium stratified by province. A SP ratio of 0.25 was considered to classify a sample positive. A herd was assigned to be seropositive if at least one sample on that herd was positive

Region / country	**Province**	**Within herd prevalence% + 95% CI**	**Herd prevalence% + 95%CI**
Belgium		36.8 [36.0-37.0]	94.60 [93.0-96.0]
Flanders	West-Vl	31.7 [31.0-32.3]	97.86 [97.3-98.2]
	Oost-Vl	39.0 [37.7-40.2]	96.63 [95.5-97.4]
	Antwerpen	46.0 [44.5-47.6]	99.02 [98.1-99.5]
	Limburg	44.9 [43.1-46.7]	98.00 [96.7-98.8]
	Vl-Brabant	40.2 [37.3-43.1]	94.24 [90.9-96.4]
Wallonia	Waals-Brabant	41.2 [34.8-47.9]	88.24 [78.2-94.0]
	Luik	41.7 [38.9-44.5]	92.17 [88.8-94.6]
	Henegouwen	30.2 [27.7-32.8]	86.06 [81.8-89.5]
	Namen	39.3 [33.0-45.9]	78.38 [67.6-86.3]
	Luxemburg	35.3 [31.5-39.3]	75.78 [69.7-81.0]

Higher prevalences were observed in Flanders compared to Wallonia and in the provinces of Antwerpen, Limburg and Vlaams-Brabant compared to West-Vlaanderen. Using all data until April 2007, the overall within-herd prevalence is 36.8% [95% CI = 36-37]. The within-herd prevalence in Belgium decreased gradually from 40% [38.9-40.1] in 2005 to 35% [34.8-36] in 2006 with provincial differences. In the provinces of Antwerpen, Limburg and Luik the highest within-herd prevalences were observed. The within herd prevalence in Belgium was 20% and 11% using a cut off value of respectively 0.50 and 1.00. The ICC was found to equal 0.38 (0.37-0.39) in 2005, 0.39 (0.38-0.40) in 2006 and 0.54 (0.52-0.56) in 2007 which reflects that the correlation between two animals within a herd with respect to the presence/absence of *Salmonella* is important.

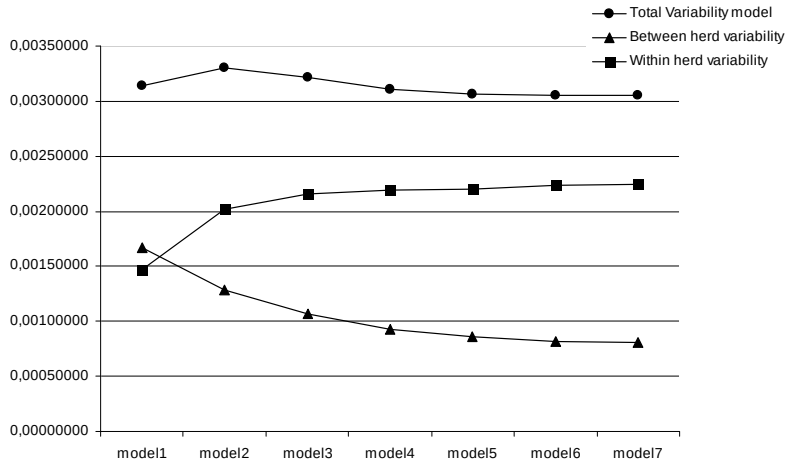

Figure 2: Development of variability according different linear models based on successive sampling rounds since January 2005.

Seven different models were used to fit data obtained form 7,702 herds sampled 7 times since January 2005. The first model was fitted by using data obtained from the first sampling round. The second, third, fourth, fifth, sixth and seventh model were fitted by the first 2, 3, 4, 5, 6 and 7 sampling rounds correspondingly. The models clearly show (figure 2) that the variability between the herds becomes smaller and smaller when more data were used to fit the model with a decreasing intra class correlation going from 53% in model 1 towards 26% in model 7. This process becomes stable after 3 successive sampling rounds. So, the more data used to fit the model the more information is obtained by the specific herds. In contrast, the within-herd variability increased across the 7 models but the pace to reach the largest value becomes smaller and smaller across these models. This indicates that the true variability, due to a different animal serological profile and/or measurement error, is closely captured when more and more data are used. The mean squared predicted error and its variance in the succeeding models stay unchanged or changed very slightly after three successive sampling rounds. From these models it was concluded that it is necessary to include data of 3 successive sampling rounds in order to classify herds at risk.

As outlined in Figure 1, *Salmonella* risk herds are defined as herds where a mean SP ratio higher than 0.6 is reached in 3 successive sampling rounds. A sub sample of 5,113 herds, all sampled 6 times (sampling rounds) since January 2005, was selected to demonstrate the stability of the identification of risk herds according to this criterion. For these herds, four overlapping time period windows (= sampling round 1,2 and 3; sampling round 2,3 and 4 ; sampling round 3,4 and 5 and sampling round 4,5 and 6) could be considered to assign risk herds. The distribution of the (non)-selected herds according this criterion is outlined in table 11. Four to six percent of these herds, depending on the time period window, were identified as risk herds. In general, about 89% of the herds (4551/5113) are never selected as a herd at risk. Subsequently, about 11% of these herds in Belgium will be selected at least at once within the SAP. In addition, 53 to 62% accordance was calculated between the identified risk herds from 2 successive time period windows which make the classification of herds at risk rather stable (Table 2).

Table 2: Distribution of herds (sampled at least 6 times since January 2005) according to the criterion within the Belgian *Salmonella* surveillance program (SAP).

profile	Sampling Round	Number of herds	Herds selected with mean-spratio ≥ 0.6 (%)	Number of herds in accordance with previous selection (%)
1	1-2-3	5113	330 (6.0)	-
2	2-3-4	5113	270 (5)	175 (53)
3	3-4-5	5113	229 (4.1)	168 (62.2)
4	4-5-6	5113	178 (3.2)	129 (56.3)
5	5-6-7	2454*	85	50 (60.2)
6	6-7-8	42*	3	3(¶)

*not all herds were sampled 7 or 8 times which make it less likely to be selected in windows 5 and 6. 2454 and 42 herds were sampled 7 and 8 times respectively ; ¶ to few observation to make a valid estimation

4. DISCUSSION AND CONCLUSION

Since July 2007, the FASFC decided to identify risk farms as farms with a mean SP ratio equal or higher than 0.6 for 3 successive sampling rounds. Once a herd is identified as 'risk herd' they will be subsequently invited to participate in a herd-specific *Salmonella* support and control program to reduce the risk for *Salmonella* infections on these herds. The choice for the mean SP ratio at herd level as a cut off level has the advantage that there is no need for taking diagnostic sensitivity and specificity of the ELISA at animal level into consideration. The cut off value of 0.6 was chosen in order to ensure that the most affected herds were assigned as herds at risk. Although, it is clear that a calculated mean of individual SP ratio's might be inflated due to a low number of animals having a high individual SP ratio, we believe that a high mean SP ratio (=0.6) might predict a previous and even current *Salmonella* infection. Previously, it was shown that a strong association was observed between serology and microbial testing (faeces) when the mean SP ratio was considered as 'definition' for a sero-positive herd (Laevens et al., 2005). However, bacteriological methods has been shown to have a low sensitivity which make it very likely that on an assigned risk farm , based on its serological results, no positive bacteriological result can be obtained as a 'firm evidence' of being a risk farm. In that way, it is very important for decision makers to explain and to communicate the test results of the Belgian SAP with the whole pig sector and its stakeholders.

The identification of risk farms based on serological data is still a difficult task as many (un)known factors should be considered to install a credible *Salmonella* surveillance program at pre-harvest level. Some of these factors were identified. Even though more than 50 % of the samples are obtained from pigs >60kg, allowing sampling from young weaners and growing pigs can lead to an underestimation of the seroprevalence. In that way, the Belgian SAP differs from that with other countries like Germany and Denmark (Osterkorn et al., 2001; Alban et al., 2005) where antibody levels are exclusively measured in meat juice samples from finishing pigs (80kg-110kg). A seasonal behaviour of *Salmonella* antibody levels was also observed in UK and Danish pig herds with the most pronounced peak in autumn (Clough et al., 2007; Hald and Strodl Andersen, 2001). An other study (Bollaerts et al., 2008) had taken these factors into account. In a study, in which different- more complex-methodologies to assign risk herds based on serological data were compared, it was shown that there was reasonably good agreement among the methods (Cortinãs Abrahantes et al., 2007).

Until now (October 2007) more than 60% of the selected risk herds had at least 1 positive faeces sample during the herd-specific *Salmonella*-control program within 2 months after the assignment. This suggests that the assignment of risk herds throughout the mean SP ratio is a practical method which may predict the real *Salmonella* 'status' on a herd. However, continuously monitoring of all possible influential factors and risk factors is still necessary.

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IMPROVEMENTS OF HEALTH STATUS ON PIG BREEDING FARMS SURPLUS VALUE OF SURVEILLANCE

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1. INTRODUCTION

These days improving health status becomes more and more important for the pig sector. Thereby animal disease surveillance is in a phase of rapid evolution and innovation. Worldwide animal health status is pictured. Disease surveillance is a management tool in which activities undertaken to measure disease events are encompassed in order to obtain value information to decision makers (e.g. producers, veterinarians, government).Surveillance information is applicable to support decisions about for instance health status certification. Fundamental objectives of surveillance are to establish the qualitative status of animal populations (e.g. groups, farms) with respect to the presence or absence of a specific disease or agent, or to quantify frequency of disease in affected populations and its distribution in space and time (determining disease trends) [Davies and Stärk, 2006; Salman, 2003].

Surveillance programs are primarily focussed on agent-defined infectious diseases, such as diseases listed by the Office International des Épizooties (OIE), or zoonotic or other diseases targeted in national control programs [Davies and Stärk, 2006]. Industry, corporate and commercial producer learned the surplus value of surveillance for managing health risks.

In 2005, TOPIGS and the Animal Health Service (GD) launched a surveillance program to improve overall health status at breeding farms by monitoring endemic production diseases on one side and supporting farmers with health management decisions on the other side. TOPIGS is an international pig breeding organisation and has many years of experience with specific pathogen free (SPF) production at the highest level. However, SPF production has been reserved to a small group of (nucleus)breeders. GD is a market-oriented organisation primarily for the improvement of animal health and safety of animal products. Veterinary specialists from GD provide farmers and veterinary practitioners with assistance and advice not only for the control of infectious diseases but also regarding aspects of animal husbandry and animal welfare.

This project is designed to picture health status of breeding farms and their offspring, the latter in order to improve health on multiplier sow farms. For improving health status infection rate should be reduced. This can be achieved by means of management improvements, minimizing the contact of non-infected pigs with infected pigs. Therefore farms are visited by a GD specialist. Together with TOPIGS, the local veterinarian and the feed consultant the farm is inspected and based on the findings and technical results a management health plan is presented. To visualise effects of improvements, pigs on breeding farms are sampled every four months by examination for three respiratory pathogens and three intestinal pathogens in blood and faeces, with special emphasis for agents causing high economic damage.

Aim of the study is to improve health status of breeding farms by specific recommendations on management.
Improvements are visualised by monitoring of endemic production diseases.

2. MATERIALS AND METHODS

2.1. Participants

TOPIGS provided GD a list of breeding farms that subscribed for the surveillance. GD sent participants and their local veterinarian instructions and materials for sampling. All samples are tested on GD's laboratory and results are processed. After each sampling the farmer and their local veterinarian have received an overview of the test results and an explanation in an added letter.

After the 1st sampling, farmers are phoned by a GD veterinary specialist to discuss the results by phone. After the 2nd sampling a farm visit by the GD veterinary specialist is strongly recommended.

The first visit were focused on biosecurity management. After farms inspection the GD specialist, together with TOPIGS, the farmer, the local veterinarian and the feed consultant, evaluates available monitor results and technical index figures. From all this information a health management plan is formulated. In this plan health management with special emphasis on internal and external biosecurity, vaccination and/or treatment programs is discussed. Farms receive a farm-specified advice. A subsequent visit or phone call was focused on reducing or eliminating specific farm agents. Close corporation between the farmer and specialists is inevitably.

Mean farm size of participating farms is about 400 to 500 sows and 1000 to 1500 gilts. Minimal farms size is around 150 sows and 400 gilts and maximum is 2500 sows and 1600 gilts farms.

2.2. Sampling scheme

Approximately every four months six causative agents, namely Porcine Reproductive en Respiratory Syndrome virus (PRRS), *Actinobacillus pleuropneumoniae* (App), *Mycoplasma hyopneumoniae* (Mhyo), *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*, *Lawsonia intracellularis* (Laws) and *Salmonella spp* (Salm) are examined in blood and faeces [table 1]. To asses health on breeding farms as accurate as possible, samples are taken in various age groups, namely piglets of 9 weeks old, sows 1st or 2nd parity, sows 3rd parity or higher, gilts 3 to 4 month in age and gilts 5 to 6 month in age.

Because the monitor should be affordable for the farmer, sample sizes to monitor the diseases are minimized. Minimal detectable prevalence is calculated with .Win Episcope version 2. Hence test characteristics are supposed to be 100%. To estimate the minimal detectable prevalence per sampling herd size has been determined on 200 sows per herd. For Brachyspira one pool is perceived as one sample.

Table 1. Sampling scheme

Age group	**Blood samples** *PRRS*	*App*	*Mhyo*	*Laws**	*Salm**	**Total number of blood samples per age group**	**Faecal samples** *Brachyspira***
Piglets (9 weeks old)	5	5	-	5		**5**	-
Sows (1st or 2nd parity)	5	-	5	5		**5**	-
Sows (≥ 3rd parity)	-	-	5	-		**5**	-
Gilts (3 to 4 months old)	5	5	5	5		**5**	6
Gilts (5 to 6 months old)	5	5	5	5	12	**12**	6
Total number of samples	**20**	**15**	**20**	**20**	**12**	**32**	**12**
Minimal detectable prevalence per sampling	14%	18%	14%	14%	22%		40%

* Laws (=Lawsonia) and Salm (Salmonella) are optional

** Samples are pooled per 3

2.3.Diagnostic tests

Diagnostic test used for examination of samples, test kit and producer of the kit are mentioned in table 2. Also used cut-off values are showed. For analyses of Salmonella, the cut-off value of Dutch Salmonella surveillance program of the Product Boards for Livestock, Meat and Eggs (PVE) has been used.

Table 2. Diagnostic test, test kit and cut-off value per examined agent.

Agent	**Diagnostic test**	**Test kit**	**Producer**	**Cut-off value** Marked positive if:
PRRS	antibody ELISA	HerdCheck* PRRS 2XR Antibody Test Kit	IDEXX	S/P ratio*≥0.40
App	antibody ELISA	CHEKIT APP-ApxIV Antibody Test Kit	IDEXX	OD-%**≥40
Mhyo	indirect antibody ELISA	HerdChek *Mycoplasma hyopneumoniae* Antibody Test Kit	IDEXX	S/P ratio*≥0.30
Lawsonia	antibody ELISA	Enterisol® Ileitis-ELISA	Svanova Biotech, AB	PI***≥30
Salmonella	B, C, D, LPS antibody ELISA	HerdChek* Swine *Salmonella* Antibody Test Kit	IDEXX	OD-%**≥40
Brachyspira	PCR	Adiavet®	Adiavet S.A.	****

* S/P ratio=Sample to Positive ratio
** OD-%=Optical Density percentage
*** PI=Percentage of Inhibition
**** For Brachyspira only species *hyodysenteriae* and *pilosicoli* are marked as positive.

2.4. Statistics

Monitor data are accumulated in a database and are evaluated every quarter of a year by performing descriptive analysis. For calculating differences in prevalence between samplings on population level proportion test is used, whereby all positive samples are added and divided by total number of samples. This test is preferred above calculating mean prevalence, because not all farms examined as much samples. By this all samples are taken into account in proportion. Statistical analyses were carried out using SAS® version 9.1 and Statistix version 8.0. Results with $p \leq 0.05$ are considered significant.

3. RESULTS

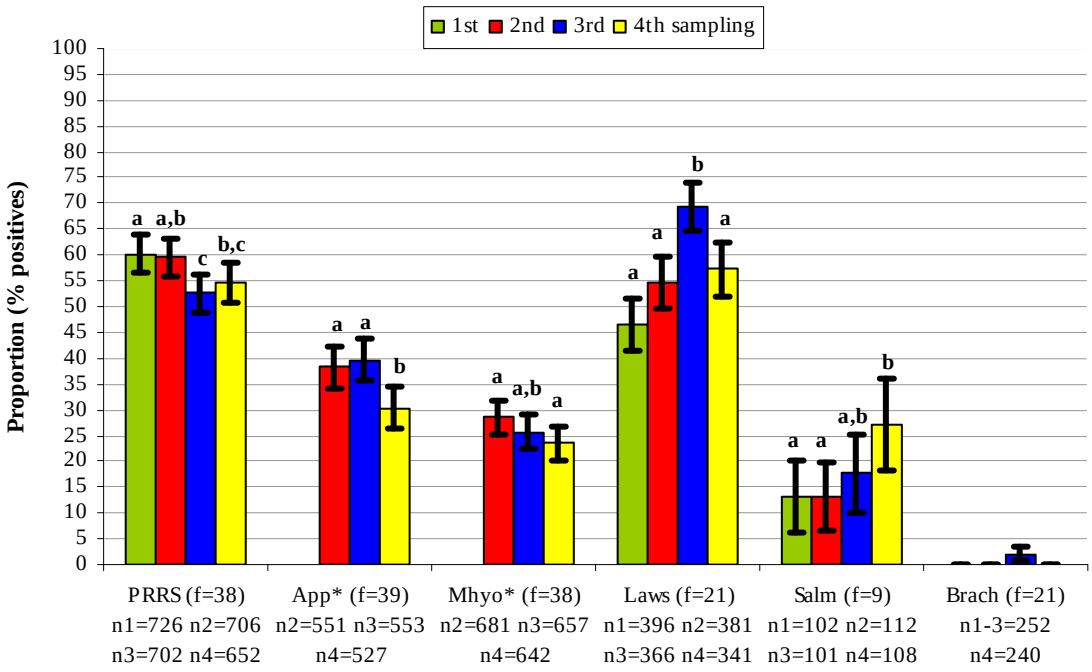
3.1. Participants

Until mid November 2007, around 150 breeding farms completed the 1st sampling, 118 the 2nd, 83 the 3rd and 42 the 4th. At some farms (n=6), the 5th sampling is already completed. Albeit new participants still apply, some farmers already signed out for different reasons (for instance closing their farm, excessive surveillance costs).
It should be noticed that not all farmers consistently sample every four months. So period between samplings varies between farms and samplings. Moreover, sampling scheme is not always followed up, meaning that not all six causative agents are examined every sampling.
The GD veterinary specialist visited over 65 farms, some farms already twice. A few breeding farms are supported intensively.
At farm level, first improvements are seen in reduction of clinical aspects of the diseases and improved technical en economic results. Measurements like improved hygiene management, changed transferring policy for piglets in the farrowing house, making use of drying time after cleaning the pens, strict all in all out, less pigs mixing around and after weaning, well considered and structured route are carried out. From conversations, improvements like increased feed intake, better health of sows and piglets and subsequently less treatments are mentioned. In short, farm visits and advises given at these visits seem to result in improvement of pig health and satisfied farmers. Scientific analyses of these data are not yet available.

3.2. Monitor results, prevalence

In figure 1 proportions per agent in all examined pigs are shown. Per agent significant differences between samplings are indicated by different letters above the bars. Only results from farms that accomplished 1st, 2nd, 3rd and 4th sampling were used. Most of these farms were visited by a GD veterinarian specialist.

The prevalence of the respiratory pathogens decreases significantly in time. Progress of Lawsonia is unpredictable, but changes in time are significant. Development of Salmonella shows a significantly increasing proportion. Brachyspira is only seen once in a farm until now, at least the pathogen specie *hyodysenteriae* [figure 1].



* because of changes in sampling scheme first sampling of App and Mhyo are not taken into account

Figure 1. Proportion in all sampled pigs, upper and lower CI on farms that accomplished 1st, 2nd, 3rd and 4th sampling (f=number of farms, n=number of samples). Per agent significant differences between samplings are indicated by different letters above the bars.

3.3. Monitor results, number of positive farms

Table 3 illustrates the numbers of farms that are positive 0, 1, 2, 3 or 4 times out of four samplings. For instance concerning PRRS, after four samplings three farms are still low in prevalence (no positive samples found yet), on three farms there were found positives in one of the four samplings, on one farm positive samples were found in two out of four samplings, on one farm there were found positives for PRRS in three out of four samplings and in thirty farms in all four samplings positive samples for PRRS were found. So some farms are still low in prevalence for PRRS, App, Mhyo and Salmonella after three or four samplings. Almost all farms (20 out of 21) that accomplished the 1st to the 4th measurement are negative for Brachyspira. On the other hand all farms are positive for Lawsonia in all four samplings.

Table 3. Number of farms that are positive 0, 1, 2, 3 of 4 times out of 4 samplings. All farms accomplished 1st, 2nd, 3rd and 4th sampling.

	Number of times farms were positive					**Total number of farms**
	0	*1*	*2*	*3*	*4*	
PRRS	3	3	1	1	30	**38**
App*	1	1	6	31	-	**39**
Mhyo*	2	2	7	27	-	**38**
Lawsonia	0	0	0	0	21	**21**
Salmonella	3	0	2	2	2	**9**
Brachyspira	20	1	0	0	0	**21**

* Because of changes in sampling scheme 1st sampling of App and Mhyo are not taken into account.

4. DISCUSSION AND CONCLUSIONS

Aim of this project is improving pig health on breeding farms. For this purpose a monitor system was contrived to visualise effects of improvements and to control health status on breeding farms. Because the monitor should be affordable for the farmer, sample sizes to monitor the diseases are minimized. A consequence of minimal sample size is low power. On the basis of these monitor data it can only be reported whether agents are present on the farm or just present in low prevalence on farm level. Especially farms that do not sample consequently are hard to evaluate.

Though it is not confirmed yet, some first improvements are mentioned in reduction of clinical problems and improved technical results. On *farm level* changes in serology are not seen immediately. After some more samplings it is expected that trends over time will be visible for each farm separately. Interpreting the test results it should be taken into account that results can be influenced by vaccinations. More data are needed to determine the influence of the season on test results.

Even though judgements at farm level are hard to make at this stage of the project, on *population level* some (significant) trends in serology are already showing. Most reliable judgements can be made for respiratory agents, because most of the samples are analysed for these agents. It should be noticed that only farms that accomplished 1st, 2nd, 3rd and 4th sampling were used in analysis. Apparently, improvement of biosecurity management has contributed to lower prevalence, regarding to the respiratory agents. The question, why these better management has not lead to lower prevalence for the gastro-intestinal agents has to be answered. Further analyses of data, with respect to the different age groups will possibly help to answer this question.

After four samplings, some farms are still not positive for some agents. Although no free status can be given based on these monitor results, more indications for a low prevalence or maybe absence of agents are present. However, absence of a specific agent can only be guaranteed when more animals are sampled.

In this paper only preliminary analysis are presented. Momentary further possibilities for analysis on the monitor data and questions related to heath improvement are lined up, e.g. calculating exact herd sensitivity and herd specificity using test characteristics, or a comparison between production data of Dutch breeding farms participating in this surveillance and Dutch breeding farms not participating in the surveillance can be made. Request for technical and economical results is preferably. Effects of participation, farm visits, management improvements, vaccination, season and so on can be analysed in a multivariate regression.

In conclusion, preliminary results indicate that the health status on farms participating in this surveillance program improves. These improvements can be visualised by results from the monitor.

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SAMPLING SCHEME FOR PREVALANCE ESTIMATION OF *SALMONELLA* IN LAYING HENS IN BELGIUM

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1. INTRODUCTION

From 2012 onwards, housing laying hens in conventional battery cages will be forbidden in the EU. They will be replaced by different types of alternative housing systems such as: enriched cages, aviary systems, floor-raised, free-range and organic systems. In the EU research project "Safehouse" the potential effect of the transition towards alternative housing systems on the occurrence of *Salmonella* is studied. In this framework a cross-sectional field study is performed in Belgium to asses the between and within herd prevalence of *Salmonella* in different laying hen housing systems. In this abstract a comparison between different sampling procedures for the assessment of the within herd prevalence is described.

2. MATERIAL & METHODS

Herds were selected based upon a list of contact addresses of registered laying hen farms (provided by the official Belgian I&R authorities). All farms with more than 1000 laying hens were contacted by telephone; participation was voluntary. Participating laying hen farms were sampled in the week prior to depopulation.
On each farm, following samples were collected: (1) 40 cloacal swabs of 40 randomly selected laying hens, (2) 5 pooled faeces samples, (3) 1 mixed dust sample and finally (4) 1 red mites sample. Subsequently, 100 randomly selected hens were transported to the Faculty of Veterinary Medicine (UGhent). All possible hygienic measures were taken during transportation of hens, to avoid contamination of the hens by the environment. After arrival, from each hen an individual blood sample was taken to evaluate the level of antibodies against *Salmonella*. After this, each hen was swabbed (cloaca) and euthanized. Both caeca of each hen were removed and pooled for further processing.

3. RESULTS & DISCUSSION

Table1: Preliminary results of *Salmonella* screening in 10 Belgian laying hen herds (2007).

Farm	Housing system	Pooled faeces	Mixed dust	Red mites	Cloacal swabs	Cloacal swabs after transport	Caeca after transport
1	Battery	0/5	0/1	0/1	0/40	**3/100**	**6/100**
2	Battery	0/5	0/1	0/1	0/40	0/100	0/100
3	Battery	0/5	0/1	0/1	0/40	0/100	0/100
4	Floor-raised	0/5	0/1	0/1	0/40	0/100	0/100
5	Floor-raised	0/5	0/1	0/1	0/40	0/100	0/100
6	Floor-raised	0/5	0/1	0/1	0/40	0/100	0/100
7	Free-range	0/5	0/1	0/1	0/40	**3/100**	**10/100**
8	Free-range	0/5	0/1	0/1	0/40	**1/100**	**14/100**
9	Free-range	0/5	0/1	0/1	0/40	0/100	0/100
10	Organic	0/5	0/1	0/1	0/40	**4/100**	**7/100**

All of these farms were screened negative for *Salmonella* by the official monitoring program. Using the on-farm sampling as described above, we could not detect any *Salmonella* in the samples taken on the farm. However, after transportation of the animals, *Salmonella* was detected in laying hens of 4 farms, both in cloacal swabs as in the caeca (Table 1). This suggests that on farms which are 'apparently *Salmonella*-free', a relatively large proportion of the hens can carry the pathogen without shedding. Possibly, the stress caused by the transport makes the hens go from the 'carrying' state to a 'shedding' state. The prevalence of *Salmonella* in the cloacal

swabs was never above 4%, where the prevalence in the caeca varied between 6 and 14%. These preliminary results clearly illustrate that depending upon the sampling procedure different estimates of the within herd prevalence can be obtained.

THE STORY OF MRSA, A DESCRIPTION OF A PLAY

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it is a tale
Told by an idiot, full of sound
and fury,
Signifying nothing.

1. PREFACE

Methicillin-resistant *Staphylococcus aureus* (MRSA) has since the 1970's been an important cause of nosocomial disease worldwide. Recent reports indicate that the epidemiology of MRSA is undergoing a major change through the emergence of community-acquired MRSA (CA-MRSA) (Herold *et al.*, 1998; Vandenesch *et al.*, 2003; Kluytmans-Vandenbergh *et al.*, 2006). CA-MRSA can cause serious infections in otherwise healthy people and has in some instances even surpassed methicillin susceptible *S. aureus* as a pathogen (King 2006).

In the Netherlands, the prevalence of MRSA in clinical isolates of *Staphylococcus aureus* has been <1% over the past decennia (Voss *et al.*, 1994; Tiemersma *et al.*, 2004) and is at present with 1.0%, still one of the lowest in Europe (Anonymous , 2004). This low prevalence is best explained by a restrictive use of antibiotic in combination with the national "search and destroy" policy, that demands isolation and screening of patients at risk of MRSA carriership/ carriage on admission to health care facilities. So far, the "at-risk" patients mainly consisted of patients who have been admitted to and/or treated in foreign hospitals.

In 2004, three patients with no relation to foreign hospitals or exposure to other known sources of MRSA, were unexpectedly found to carry MRSA. The patients were a pig farmer, a pig farmer's child, and a child of a veterinarian. (Voss *et al.*, 2005).

2. LEADING ACTOR: nt-MRSA

2.1. Typing

A special type of MRSA is the leading actor in this play, also called the non typable MRSA. MRSA are normally typed by Pulsed Field Gel Electroforesis (PGFE), but the MRSA described above was non-typable by that method, i.e. the DNA cannot be cut by the frequently used restriction enzyme (Sma I). Alternative typing methods was performed, namely spa-typing, a method that use the gene for protein A, a surface protein of S. aureus. This method showed that all pig associated strains belonged to a number of closely related spa-types. On the isolates with different spa-types, multi locus sequence typing (MLST), a method that sequences 7 household genes was done, which showed that all strains belonged to MLST 398, a hitherto uncommon strain in humans, Once it is determined to what MLST a spa-type corresponds, spa-typing is sufficient. This is convenient since MLST is much more labour intensive and relatively expensive.

Resistence

By definition, MRSA are resistant to methicilline. This resistance is encoded by the mec A gene and can be detected by PCR. The hospital-acquired MRSA is mostly multi resistant sometimes leaving only vancomycin or one of the newer anti-staphylococcal drugs such as linezolid or daptomycine as treatment options. Generally, the nt-MRSA are resistant to lesser antimicrobials, but they are always resistant to tetracyclines. In addition resistance to clindamycin/erytromycine, cotrimoxazol and gentamycine has been found. In pig husbandry, oxytetracyclines are widely used, but the role of tetracyclines in the evolution and spread of MRSA in the pig population remains to be elucidated.

3. SUPPORTING ACTORS

3.1. Humans

Carriership for MRSA can be detected by a nose and/or throat swab. Thereafter the swab is cultured in a selection broth and typed as described above.

In 2004 human carriers with a nt-MRSA were detected for the first time(Voss , 2005). These human carriers all had a relation to pig farming. To investigate if those in professional contact with livestock are at higher risk of MRSA carriership, 53 Dutch veterinary students who had contacts with farm animals and 99 farm animal veterinarians were screened and questioned about animal contacts and known MRSA risk factors. Two out of 53 students were carriers and 5 out of 99 veterinarians, leading to an overall prevalence of 4.6% in this study (Wulf *et al*., 2006).

To investigate if this is also true for professionals in contact with pigs in an international setting, a convenience sample of 272 participants attending an international conference on pig health in Denmark were screened for MRSA carriage (Wulf *et al*.2007[a]*)*. The overall prevalence of carriers at this conference was 12.5%, which is remarkably higher than the prevalence under Dutch veterinarians. However, the prevalence under the Dutch veterinarians in this study was 23 % (6/26). Furthermore, the conclusions from this study were that there was an association between the mean hours a veterinarian spends between the pigs and the probability of being a carrier, supporting the role of pigs in transmission. Interestingly, the use of protective measures like clothes, mask and gloves could was even correlated with a higher rate of carriage of MRSA.

Because it is thought that the use of antibiotics is also correlated with the prevalence of MRSA, organic farmers were screened. The prevalence under organic farmers was 11% (3/27), while Wulf et al. (2007[b]) found a prevalence of 50% (13/26) under regular pig farmers. Moreover, one organic farmer also had a regular farm and one farmer had recently switched from regular farming to organic pig farming.

The first outbreak in a hospital of the nt-MRSA was reported by Wulf *et al*.(2007[c]) The index case of this outbreak was not determined, but most probably this was a health care worker living on a pig farm, although the health care worker never came in contact with pigs.

Van Rijen *et al.* (2007) found a enormous increase in prevalence of nt-MRSA during the past years and this was correlated to persons in contact with pigs and in contact with veal calves.

Last year, MRSA was also found in persons working with poultry. When sampling the faeces of the poultry farm only 1 out of 16 samples was positive. Because the poultry farms were in Noord-Brabant, the region with the most intensive pig farming, the source of this MRSA outbreak is still to be elucidated.

En extensive Belgian study (Denis, 2007) revealed that 38 % (48/127) of persons related to pig farms was colonised with the nt-MRSA. Furthermore, with one exception, the pigs on the farms with the colonised persons were also colonised. Also other animals (dogs and horses) may play a role (adjusted OR= 11). In agreement with the findings of Wulf et al.(2007a), MRSA carriage was associated with higher levels of personal protection and hygiene (e.g. wearing of masks or gloves or reported hand hygiene.) However, confounders, like antibiotic use, could not be assessed.

A survey in Canada among veterinary personnel revealed that the prevalence of MRSA was high, 7% of the veterinarians was carrier, while 12% of the technicians was carrier (Hanselman, 2006). Remarkably, large animal practice was associated with a higher risk of colonization (OR=2.9, 1.2-6.6)

3.2. Pigs

Several studies have been conducted to estimate the prevalence of MRSA in pigs. Neeling et al. (2007) conducted a study in finishing pigs. They screened 540 pigs in 9 slaughterhouses (from 54 farms) and found a prevalence of 39% (209/540). On a farm basis, 44 batches had 1 or more positive samples, so 81% of the farms could be carrier. This figure is probably an overestimation, because during the waiting period in a slaughterhouse, the microorganisms can be transmitted from pigs of one farm to pigs of another farm.

Another Dutch study (Van Duijkeren *et al*., 2008) sampled 10 pigs per farm of a total of 31 farms. The prevalence between farms was 23% (7/31). In a Belgian study, the pig prevalence was 44% (663/1500) and the farm prevalence 68% (34/50), which resembles the data of de Neeling *et al.* (2007). Furthermore, the farms which purchased pigs had a higher probability of harboring the nt-MRSA than closed farms.

It is still not clear why the nt-MRSA has disseminated so fast among pig farms. One of the main routes of dissemination is the trade of animals between farms. Van Duijkeren et al. (2008) have clearly demonstrated that the status of the pigs in a farm is strongly related to the status of the pigs at the farm delivering pigs to that farm (OR= 34, 95% CI: 3.8-1480). Moreover they found a strong relationship of using standard batch treatment of antibiotics and being carrier of nt-MRSA (OR=33, $p<0.01$).

Recently there was a report that nt-MRSA was cultured from a pig with lesions of greasy pig disease (van Duijkeren *et al*., 2007). The MRSA was not only found on the farm itself, but also on the farm that delivered pigs to this farm. The clinical relevance of this finding is not clear.

A recent study in Canada on 20 farms showed that 25% of the sampled pigs were carriers. These pigs were housed on 9 farms. Moreover, 20% of the pig farmers were also carriers. In Denmark, Guardabassi *et al* . (2007) have found the first pigs that are carrier of MRSA These findings illustrate that MRSA in pigs is not only a national or European but a worldwide problem.

3.3. Veal calves

A large study is undertaken at the moment in farms with veal calves to estimate the prevalence not only of veal calves but also of other species present on the farms, including people.

3.4. Other species

Weese (2004, 2005) has published several studies where MRSA was found in horses and small animals. This is however another CA-MRSA and not an nt-MRSA.

4. DÉCOR

We live in a world, where people are more demanding. Not only do they not want to be at risk when eating (food safety), but also they put conditions for medical care and are willing to sue the medical professionals. So, hospitals are diminishing the risk on nosocomial infections. The animal industry is faced with the challenge to improve internal and external biosecurity measures, and reduce the use of antimicrobials. Only then has this play a happy end for the animal industry.

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A CROSS-SECTIONAL SEROLOGICAL STUDY TO DETERMINE THE BLUETONGUE SEROTYPE 8 PREVALENCE IN CATTLE IN THE NETHERLANDS

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1. INTRODUCTION

In August 2006, an outbreak with bluetongue virus serotype 8 (BTV-8) was confirmed in the southern part of the Netherlands. Soon, the infection was confirmed in other areas and in the surrounding countries (Germany, Belgium, Luxembourg and France) (Elbers et al., 2007; Mehlhorn et al., 2007; Toussaint et al., 2007). As a part of a European Commission decision, a cross-sectional serological study was carried out in the first half of 2007 to determine the extent and geographical spread of BTV-infection in the Netherlands. This period was chosen because it would likely be a period with limited activity of the vector Culicoides and thus with a stable infection status of the ruminant livestock population. Cattle were the target species because they were presumed to be the preferred species by biting Culicoides.

BTV-8 is thought to give limited clinical signs in cattle (Bartels et al., 2007; Elbers et al., 2007; Darpel et al., 2007). However, Darpel et al. (2007) also found more severe pathology in cattle than suggested by the mild clinical signs. A study by the Animal Health Service in 2006 on 30 infected and 30 non-infected dairy herds showed fairly limited effects on health and production (Bartels et al, 2007). However, in 2007 farmers report more and more severe clinical signs in cattle than the year before. This paper presents the seroprevalence of BTV-8 on animal, herd, and regional level in cattle in the Netherlands. In addition, herd characteristics and production losses of infected herds are described.

2. MATERIALS AND METHODS

A cross-sectional serological study was carried out between January and June 2007 to determine the extent and geographical spread of BTV-infection in cattle in the Netherlands. The epidemiological units for monitoring and surveillance purposes for bluetongue in the Netherlands are 20 geographical units called compartments as proposed in Commission Decision 2005/393/EC (Figure 1). Blood samples from Dutch cattle submitted to the laboratory of the Dutch Animal Health Service in the framework of voluntary and obligatory health programmes were serologically tested (competitive ELISA, Institute Pourquier, Montpellier, France) anonymously and reported per compartment. This ELISA has a high sensitivity (~100%) and specificity (>99.8%) (Toussaint et al., 2007). The number of cattle to be tested per compartment was dependent, amongst others, on the number of cattle farms, the number of cattle, the number of cattle on bluetongue infected farms and the presumed expected prevalence of infection per compartment, which was calculated to be between 770 and 2040 cattle per compartment.

Figure 1. The 20 compartments for the cross-sectional seroprevalence study for bluetongue serotype 8 in the Netherlands.

In this study, the within-herd seroprevalence was calculated for herds that had tested at least 50% of their cattle. Analyses were carried out on the infected herds to determine whether the within-herd seroprevalence was significantly different ($P \leq 0.05$) between herd sizes, geographical location and the type of herd. The definitions for the herd types were based on Identification and Registration data (I&R) of the Dutch cattle sector and these are also used in the Cattle Health Monitor in the Netherlands (Velthuis and Mourits, 2007; Bartels et al., 2006). Briefly, we distinguish dairy herds, suckling cow holdings, traders, beef farms, young stock raising herds and small scale herds (<20 cattle).
In addition, a comparison was made between infected and non-infected herds for several production parameters such as net returns for 305-day milk production for an average cow in a herd (a parameter for the herd productivity), somatic cell count (SCC), culling and mortality in dairy herds and mortality and culling in small-scale farms (i.e. non-milkproducing farms). In a multivariate regression model the bluetongue infected herds were compared with the non-infected herds with respect to production parameters before the bluetongue period and in the bluetongue period. An effect of bluetongue was assumed when there was a significant in- or decrease of the production parameter in bluetongue herds in the bluetongue period.

The analyses were carried out with a generalized linear model procedure with a correction for repeated measures in STATA 10.0 (XT GEE). The within-herd seroprevalence was modelled as a Poisson distribution (the number of seropositives relative to the number of cattle that were tested) with a log-link function. The production parameters were the outcome variables and, dependent on the distribution of these parameters (normal, count, binary), the best fitting distribution (Gaussian, Poisson, Binomial) and link-function (identity, log, logit) were chosen.

3. RESULTS

3.1 Seroprevalence

Table 1. The distribution of number of samples tested for antibodies against bluetongue virus serotype 8 per herd in the Netherlands.

# of samples	# of herds	% of herds
1	2476	45,5
2-10	2297	42,3
11-25	368	6,8
26-50	140	2,6
>50	155	2,9
Total	5436	100

In total, 37,073 samples originating from 5,436 herds were tested for antibodies against BTV-8 with a range from 1 to 296 samples per herd. In Table 1 the distribution of the number of samples per herd is provided. Forty-five percent of the herds submitted only one sample to the Animal Health Service.

The seroprevalence and the true prevalence (test sensitivity of 100% and specificity of 99.8%) in the compartments are shown in Table 2.

Table 2. The seroprevalence for bluetongue virus serotype 8 in 20 compartments in the Netherlands.

Compartment	# seropos.	Total # tested	Seroprevalence	True prevalence (Se=100%, Sp= 99.8%)
1	0	4,113	0.0	0.0
2	4	1,989	0.2	0.0
3	0	2,073	0.0	0.0
4	0	2,121	0.0	0.0
5	1	2,090	0.0	0.0
6	0	1,197	0.0	0.0
7	2	2,012	0.1	0.0
8	1	2,043	0.0	0.0
9	3	2,099	0.0	0.0
10	8	2,031	0.4	0.2
11	2	2,093	0.1	0.0
12	10	2,096	0.5	0.3
13	18	2,021	0.9	0.7
14	14	547	2.6	2.4
15	75	789	9.5	9.3
16	52	2,047	2.5	2.3
17	84	2,053	4.1	3.9
18	33	2,042	1.6	1.4
19	36	776	4.6	4.5
20	316	841	37.6	37.5
Total	659	37,073	1.8	1.6

Table 3 shows the distribution of samples across the different herd types. A large part of the samples (48%) came from dairy herds, 26% of the samples came from suckling cow farms and 15% of the samples from small-scale farms. Hundred and forty seven herds could no be typed in one of the six categories. The number of samples from dairy herds and traders was as expected. The number of samples from suckling cow herds and young stock raising herds was higher than expected and for the other herds it was lower than expected.

Table 3. Distribution of cattle holdings in the Netherlands in 2006 and the distribution of holdings that were tested for antibodies in cattle against bluetongue virus serotype 8.

	Distribution in 2006		Cross-sectional bluetongue	
Herd type	N	%	N	%
Traders	290	0.64	41	0.75
Young stock raising farms	1,153	2.54	235	4.32
Small-scale farms	14,286	31.42	819	15.07
Dairy herds	20,897	45.96	2,602	47.87
Non-identifiable herds	338	0.74	127	2.34
Beef herds	3,355	7.38	197	3.62
Suckling cow farms	5,153	11.33	1,415	26.03
	45,472	100.00	5,436	100.00

In 340 herds more than 50% of cattle was tested and these were mainly dairy herds (n=62) and small cattle holdings (n=205). In 37 herds (11%), at least one test-positive cow was detected. Eleven of these herds were

dairy herds (22%) and 20 herds were small-scale farms (11%). The other infected herds were 4 suckling cow herds (11%), one veal calf farm and one beef farm (22%) (Table 4).
Figure 2 contains the distribution of the within-herd seroprevalence for infected herds in which at least 50% of the cattle was tested (N=37). Forty percent of the herds had a within-herd seroprevalence below 10% and about 20% of the herds had a seroprevalence above 90%.
In Table 4 the within-herd seroprevalence for the different herdtypes is provided. The average within-herd seroprevalence in the 37 herds was 39.3%, 2.2% in the 11 seropositive dairy herds and 68.4% in the 20 small-scale herds and 14% in the four suckling cow herds.

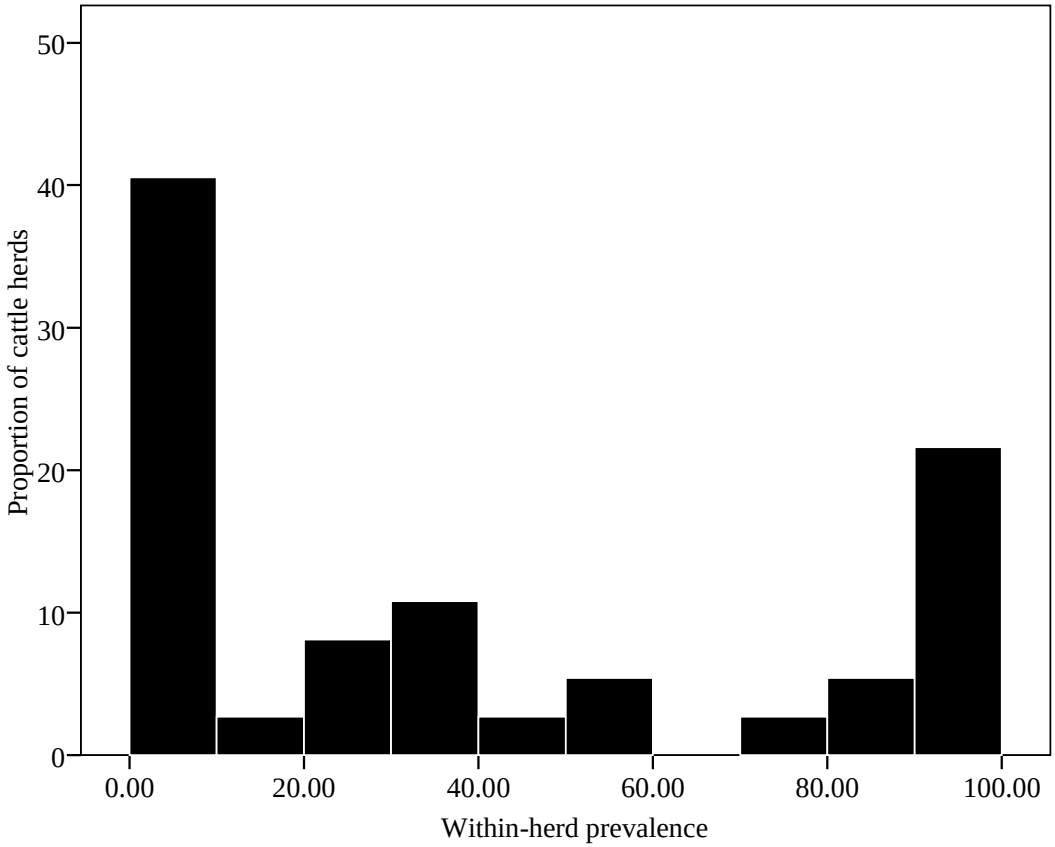

Figure 2. Within-herd seroprevalence for bluetonque virus serotype 8 in infected Dutch herds in which at least 50% of the cattle were sampled.

Table 4. Number and proportion of cattle herds test-positive for bluetongue virus serotype 8 and the mean within-herd prevalence in infected Dutch herds that sampled at least 50% of their cattle.

Herd type	# free	# seropositive	% seropositive	Within-herd seroprevalence in seropositive herds (SD)
Traders	13	0	0	-
Young stock raising farms	2	0	0	-
Small-scale farms	185	20	11	68.4 (31.5)
Dairy herds	51	11	22	2.2 (1.9)
Non-identifiable herd	7	0	0	-
Beef herds	9	2	22	2.9 (2.8)
Suckling cow farms	36	4	11	14.0 (12.0)

Table 5 shows the within-herd seroprevalence for herds that tested at least 50% of their cattle for the testpositive compartments in the Netherlands (compartment 10 to 20). In contrast to the cow-prevalence that showed a clear trend (increasing from north to south; Table 2) the within-herd seroprevalence did not show this trend.

Table 5. The within-herd seroprevalence for bluetongue virus serotype 8 in the testpositive compartments in the Netherlands.

Compartment	# herds that tested>50% of their cattle	# of seropositive herds	Within-herd seroprevalence
10	28	2	1.4
11	22	1	2.3
12	18	0	-
13	19	4	0.3
14	3	1	8.3
15	7	3	31.3
16	9	3	1.0
17	24	12	27.4
18	16	5	2.9
19	2	1	0.2
20	8	5	50.0
Total	340	37	39.3

The results of the statistical analysis on within-herd seroprevalence are shown in Table 6. The within-herd seroprevalence differed significantly between herd types, region and by herd-size.

Table 6. The results of the generalized linear model for the within-herd seroprevalence of bluetongue virus serotype 8 in herds (n=307) in the Netherlands.

Variables	β	SE	*P*	Exp(β)	Lower limit	Upper limit
Intercept	-6.54	0.90	0.00	.	.	.
Small scale farms	1.89	0.76	0.01	6.60	1.50	29.07
Suckling cow farms	0.44	0.67	0.51	1.55	0.42	5.68
Dairy farms	0.00	.	.	1	.	.
Herdsize	-.008	0.003	0.05	0.99	0.99	1.00
South	3.57	0.45	0.00	35.55	14.83	85.22
North and middle	0.00	.	.	1	.	.
DScale (based on the Deviance)	0.68					

3.2 Production losses

The average net returns for 305-day milk production amounted €2,417/cow and it significantly decreased with €48/cow (95%CI: €3-€94) in bluetongue infected dairy herds in the bluetongue period. The other production parameters were not significantly different.

In small-scale farms the incidence rate of mortality increased 3.2 (95%CI: 1.2-9.1) times in bluetongue infected herds in the bluetongue period while the voluntary culling rate decreased with a factor 2.3 (95%CI: 1.1-4.8).

4. DISCUSSION

The cross-sectional sample was fairly representative for the distribution of cattle herd types in the Netherlands. Small-scale herds were slightly underrepresented and suckling cow farms were overrepresented. The bluetongue prevalence in 2006 was low both on compartment as well as herd-level. Although the number of infected herds was limited, the results indicated that infected dairy herds had a lower within-herd seroprevalence than small-scale and suckling cow herds. In addition, smaller herds had a lower within-herd seroprevalence than larger herds and the within-herd seroprevalence was significantly higher in the southern part of the Netherlands compared with the middle and northern part. The latter was to be expected because the outbreak started in the south and thus infection pressure was higher in the southern parts. The higher seroprevalences in the small-scale and suckling cow herds may be a result of differences in housing and grazing management relative to dairy

herds. Most dairy herds graze their cattle a limited period during the day (between milkings) and keep their cattle in the barn overnight, while cattle from small-scale and suckling cow herds mostly graze outside for 24 hours a day. Furthermore, the grazing areas may be different between the herd types. Cattle from small-scale herds or suckling cow herds are more likely to be grazed in more extensively kept areas that are more favourable for the vector. Thus, the exposure to the vector may have been higher for small-scale and suckling cow herds than for dairy herds.

In 2006, bluetongue seemed to have had an effect on production parameters in cattle. In dairy herds, the net returns decreased with 2% but for the other parameters no effect was seen. The external validity of the results may be limited because of the small number of infected dairy herds in the study (n=11) and the low seroprevalence in the dairy herds (2.2% on average). In the twenty infected small-scale herds, mortality was increased in the bluetongue period compared with the non-infected herds. However, the number of cattle in these herds is small (<20 head). Thus, the absolute number of cows that may have died in these herds as a result of bluetongue will be fairly limited.

5. ACKNOWLEDGEMENTS

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MRSA IN PIGS IN BELGIUM

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Stapylococcus aureus and especially the methicillin resistant (MRSA) variant is an important human pathogen. MRSA has until recent been restricted to the hospital, though recently community acquired MRSA (CA-MRSA) is rising. In veterinary medicine, MRSA has not been a problem until only very recent. Most reports were dealing with infections in companion animals (mainly dogs and horses), though these are mainly infections caused by human contamination during surgery. In 2004 however, MRSA was detected in pigs in The Netherlands. This was discovered by the fact that pig farmers were positive for MRSA, and this in contrast with the general situation in The Netherlands where MRSA is very rare. The prevalence in pigs was extremely high, especially since MRSA is nearly absent in the Dutch hospitals. Subsequently, MRSA were discovered in pigs and other animal species in Germany, Austria, and other European countries.Upon the warnings of the presence of MRSA in animals in other countries, the Belgian government asked a small cross sectional study on the prevalence of MRSA in pigs in Belgium.

Fifty farms were randomly chosen from the nearly 8800 pig farms registered in Belgium, ensuring representativity for region. In each farm, thirty animals were sampled by swabs of the anterior nose. In farrow-to-finish farms 10 piglets, 10 sows and 10 fattening pigs, and in finishing farms 30 fattening pigs were sampled. Swabs were immediately inoculated into Stuart transport medium (Copan, Italy) and sent to the laboratory for culture. Swabs were placed in enrichment broth medium made of BHI broth supplemented with NaCl 7.5%. After 24h incubation, broth was sub-cultured for another 16 h onto a selective chromogenic agar for MRSA (MRSA-ID, BioMérieux, France). All S. aureus suspect colonies were plated on a blood agar plate for phenotypic characteristics (colonymorphology and typical double heamolysis) and the tube coagulase test was performed. Susceptibility to oxacillin was tested by the cefoxitin disk diffusion method. Identification was confirmed by multiplex PCR for nuc, mecA and 16S rRNA genes.

Susceptibility to gentamicin, tobramycin, kanamycin, neomycin, streptomycin, erythromycin, tylosin, lincomycin, clindamycin, ciprofloxacin, tetracycline, minocycline, sulfonamides, cotrimoxazole, rifampin, mupirocin, chloramphenicol, linezolid and fusidic acid, Quinupristin/dalfopristin (pristinamycin) was determined by the disk diffusion method using Rosco NeoSensitabs tablets on Mueller-Hinton II agar under test conditions and breakpoints according to CLSI. Glycopeptide susceptibility will be determined by teicoplanin screen agar method (5 mg/l).

MRSA strains were genotyped by PFGE after SmaI macrorestricion and DNA sequence analysis of the polymorphic repeat region of the protein A gene (spa typing). A subset of MRSA clones were genotyped by multi-locus sequence typing (MLST). Staphylococcal cassette chromosome mec (SCCmec) were determined by two multiplex PCRs. Resistance genes encoding for tetracycline efflux pump system tetK or for ribosomal protection protein tetM, aminoglycoside modifying enzymes (AME) encoded by aac(6')-Ie + aph(2"), ant(4')-Ia and aph(3')-IIIa genes, ribosomal methylases encoded by ermA and ermC and the macrolide efflux pumps encoded by msrA and msrB genes were tested by multiplex PCR.

Sixty-eight percent of the farms were positive, nearly all fattening farms were positive while farrow-to-finish farms were less positive (56%). No regional difference could be found. A total of 663 strains were isolated (44% of the samples were positive). Twenty-six percent of the sows and fattening pigs of farrow-to finish farms were positive, while 41% of the piglets were positive on these farms. In fattening farms, 71% of the pigs were positive. Fattening pigs were significantly more MRSA positive when reared in finishing farms than in farrow-to-finish farms. In the latter farms, the piglets were significantly more positive than the other age groups. There was no resistance against the antibiotics linezolid, muirocin, rifampicin and fucid acid. Resistance against sulphonamides, quinupristin/dalfopristin and chloramphenicol was low (2, 5, and 5% respectively). One third of the strains were resistant to ciprofloxacin. Nearly 50% of the strains were resistant to gentamicin (43%), kanamycin (47%) and tobramycin (47%). The resistance genes found were mainly aac6'-aph2" (aacA-aphD) (88%), ant4' (aadC) (50%) and few aph3' (aphA3) (2%). Resistance against the macrolides erythromycin and tylosin was 56 and 53% respectively, indicating that a small % was inducible resistant. Only in a bit more than

3% of the strains, this resistance was mediated by the ermA gene, mainly in combination with the ermC gene. This latter gene was present in 65% of the strains tested. The msrA/msrB gene was not found in any strain tested. In 27% of the strains, no resistance macrolide resistance gene was found. Resistance against lincosamide was 73%. Nearly all strains were resistant to trimethoprim and all were resistant to tetracycline. This resistance was mainly caused by the tetM gene (90% of the strains tested). The tetK gene was present in one third of the strains, and all but one was in combination with tetM. In approximately 9% of the strains, no tet gene was detected. PFGE showed that the strains were untypable. Only one MLST type was found (ST398). Two spa types were detected; t011 was present in all 34 farms while t034 was found in only two strains from one farm. The SCCmec types associated were type IVa, V or untypable.

It can be concluded that MRSA is highly prevalent in Belgian pigs distributed over the whole country. The prevalence is depending on the age of the animals and type of farms. MRSA in pigs are genetically similar, representing one MLST type (ST398) and two spa types (t011 and t034), and differ mainly in their mobile resistance genes.