NUSAP: A TOOL TO EVALUATE THE QUALITY OF ASSUMPTIONS IN QUANTITATIVE MICROBIAL RISK ASSESSMENT

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ABSTRACT

Quantitative microbial risk assessment (QMRA) is a scientific tool that inherently involves the formulation of assumptions. The NUSAP method was implemented to evaluate 13 key-assumptions in a Belgian QMRA model for *Salmonella* in pork meat. A workshop with experts was organised to assess the subjective component of assumptions using four pedigree criteria: the influence of situational limitations, plausibility, choice space and the agreement among peers. Points of disagreement between the scoring experts were highlighted, and the importance of the assumptions on the output of the model was estimated. Four key assumptions with low pedigree scores and a high expected influence on the model results were considered as problematic, whereas three assumptions were characterized by a lower degree of subjectivity and a weak influence on the results. The proposed NUSAP method resulted in an enhanced debate on the quality of assumptions. This is helpful for redesigning critical modules in the QMRA model, and favours a more transparent quality assurance in QMRA.

1. INTRODUCTION

The overall quality of quantitative microbial risk assessment (QMRA) largely depends on the assumptions made in the model. A rigorous evaluation of assumptions is therefore of paramount importance. The approach known as the Numeral Unit Spread Assessment Pedigree (NUSAP) notational system was chosen to evaluate assumptions (1). Within the NUSAP acronym, the Pedigree qualifier is what is most innovative for assessing assumptions. Crucial for a pedigree evaluation is the use of a pedigree matrix, which is expressed by scores for a set of pedigree criteria. The aim of the present study was to evaluate the assumptions made in the METZOON model, a model that aimed to assess the risk of human salmonellosis through consumption of pork meat in Belgium (2). The NUSAP methodology was used to pinpoint the strengths and the weaknesses in the METZOON model, and to enhance the quality assurance in the QMRA process.

2. MATERIAL AND METHODS

The assumptions in the METZOON model were identified, prioritized and critically reviewed during a workshop, as described in Kloprogge et al (3) with small modifications adapted for QMRA. The pedigree matrix contained four

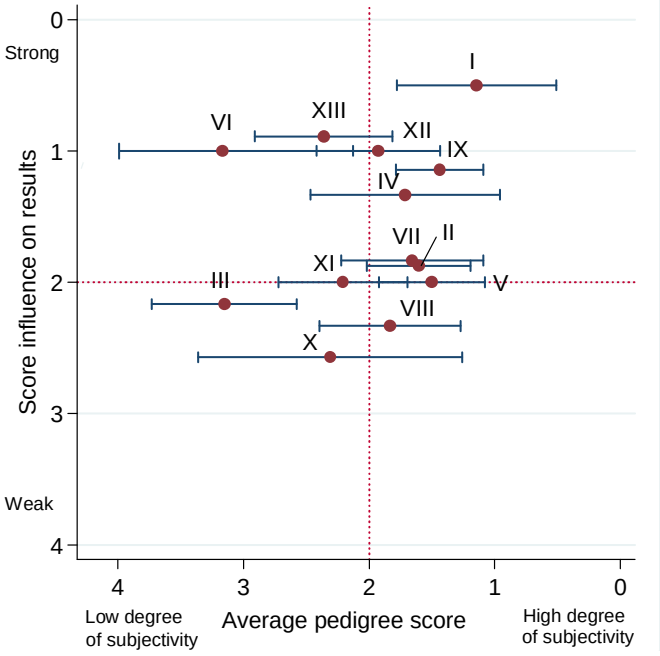
pedigree criteria: influence of situational limitations, plausibility, choice space and agreement among peers (Table 1). The lower the scores in the pedigree matrix the higher the subjective component of the assumption. In addition, the pedigree matrix contains a fifth criterion to assess the influence of the assumptions on the outcome of the model. An overall pedigree strength per assumption is obtained by averaging the mean scores for situational limitations, plausibility, choice space and the agreement among peers over the scoring group members.
From a list of 39 assumptions identified by reviewing the risk model, 13 assumptions were prioritized and evaluated in a workshop. Assumptions were analysed in a diagnostic diagram (3). In this diagram, assumptions with low overall pedigree strengths and having a strong estimated influence on the outcome of the QMRA are considered as weak links in the model. Overall kappa values were computed to determine the inter-rater reliability between scoring experts. Wilcoxon Mann-Whitney test were used for pairwise comparisons in strengths among the pedigree criteria.

Table 1: Pedigree matrix (adapted with small modificatons from Kloprogge et al. (3) and Craye et al. (4))

Criteria→ Score ↓	**Influence situational limitations (time, money, human resources,…)**	**Plausibility (accordance with reality)**	**Choice space**	**Agreement among peers**	**Influence on results risk assessment**
4	Choice assumption hardly influenced	Assumption very plausible (based on established theory, verified by peer review)	Hardly any alternative assumption available	A large majority among peers would have made the same assumption	The assumption has little or no impact on the results
3	Limitedly influenced	Plausible (based on model with theoretical basis, empirically verified data)	Very limited number of alternatives	Many experts would have made the same assumption	The assumption has only a local impact
2	Choice assumption moderately influenced	Acceptable (based on a simple model, extrapolated data)	Limited choice from alternative assumptions	Several experts would have made the same assumption	The assumption greatly determines the results in a major step in the calculation
1	Choice assumption importantly influenced	Doubtful (based on not verified empirical data)	Moderately large number of alternatives	Few experts would have made the same assumption.	The assumption has a moderate impact on the end result
0	Totally different assumption had there not been limitations	Fictive or speculative	Ample choice from alternative assumptions	Controversial assumption, hardly any expert would have made the same assumption	The assumption greatly determines the end result

3. RESULTS

The overall pedigree strength of the assessed assumptions varied in scores between 1.1 and 3.2 (Figure 1). Assumption I obtained an overall strength < 1.4 (highly subjective), whereas assumptions III and VI were given scores > 2.6 reflecting a low degree of subjectivity. The remaining ten assumptions were given scores ranging between 1.3 and 2.6. No significant differences were observed between the average scores of the four assessed pedigree criteria.
None of the overall kappa's exceeded 0.2, indicating a poor concordance between the attributed scores of workshop participants. The diagnostic diagram (Figure 1) identified four assumptions located in the upper right quadrant (I, IV, IX and XII) that were judged as problematic by the panel of experts. On the other hand the assumptions III, VIII and X, were located in the lower left quadrant of the diagnostic diagram indicating relevant and appropriate assumptions for the QMRA model. The low overall pedigree score of assumption I was due to the fact that situational limitations played a large role in the choice for this assumption, the assumption was largely implausible, and there was a large disagreement among peers. Workshop participants disagreed on their score for the criterion "choice space". Assumption III, VI and XIII corresponded to a low degree of subjectivity for the pedigree criteria assessed. Assumption VI obtained the highest overall pedigree score and the experts agreed that the assumption should be very plausible (scores "3" or "4"). Experts disagreed however on attributing scores on the influence of situational limitations and the choice space (scores ranging from score "1" to "4").



	Key assumptions
I	Assumption regarding the *Salmonella* concentration at the begin of the slaughterhouse.
II	Assumption that the *Salmonella* seroprevalence represents the infected (excreting pigs) + carriers (infected / not excreting situation).
III	Assumption that during the cutting & mixing process *Salmonella* cells are not mixed homogeneously in meat mix
IV	Assumption that *Salmonella* cells inside the minced pork are exposed to temperatures of 60-70°C during 0.5-1.5minutes
V	Assumption that the odds-ratios for external prevalence of *Salmonella* versus internal prevalence is 1.16 (70% of cases) and 0.6 (30% of cases).
VI	Assumption that adequate cooking of minced meat destroys *Salmonella* spp. cells.
VII	Assumption that the concentration of *Salmonella* spp. per 100 cm² is homogenous over the entire pig carcass.
VIII	Assumption regarding the effect of transport & lairage on exterior contamination of carrier pigs.
IX	Assumption that transport & lairage increases the bacteriological *Salmonella* prevalence.
X	Assumption that no growth of *Salmonella* Typhimurium occurs below 10°C in minced pork meat.
XI	Assumption that the *Salmonella* serologically positive pigs represent the excreting pigs (infectious) + carriers (infected / not excreting) + the immune.
XII	Assumption regarding the *Salmonella* starting prevalence at the slaughterhouse.
XIII	Assumption regarding the used dose-illness model.

Figure 1. Diagnostic diagram for key assumptions in the METZOON model. Low pedigree scores and strong expected influence on the results indicate weak links in the model. I-XIII: key assumption assessed during a NUSAP workshop.

4. DISCUSSION

The NUSAP approach was already successfully tested in environmental models (5-7) but not in QMRA before. NUSAP, aiming to score the criteria within the Pedigree matrix requires training for the participants. With respect to the pedigree matrix, workshop participants found it was hard to score the criterion “agreement among peers” and argued it was difficult to imagine if peers would have made a different choice in assumptions as compared to the analysts’ choices in the assumption of the METZOON model.

Unlike in other NUSAP studies where the evaluation of assumptions was carried out for finished models (7, 8), the present one was undertaken during the model building process. However, NUSAP clearly helped to redesign parts of the QMRA model before the end of the project. The diagnostic diagram identified in a clear and transparent manner that the use of the data related to concentration of *Salmonella* CFU numbers on pig carcasses in different processing steps at the slaughterhouse (Assumption I) was inappropriate for the model. Assumptions having obtained low pedigree scores for the criterion “influence of situational limitations” is an indication that lifting the limitations can lead to a different assumption. If the assumption is at the same time highly implausible, carrying a high degree of expert disagreement, and has a large expected influence on the results, the assumption needs reconsideration and new research could be stimulated.

In the present study, only experts from the METZOON consortium were involved in the selection and analysis of assumptions. As a part of an extended peer-review process, peers and stakeholders (veterinary services, farmers’ organisation, slaughterhouse and processing industry, consumers’ organisations,…) can also be included in the analysis of assumptions. Involving the stakeholders in the debate on assumptions will be beneficial for the transparency and acceptance of management decisions based on the QMRA model.

The proposed NUSAP method resulted in an enriching debate among participants with different background and disciplines. In addition, the diagnostic diagram will certainly increase the communication of assumptions, used in the model, towards the decision makers.

5. ACKNOWLEDGEMENTS

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THE DIRECT COSTS OF INFECTIONS WITH GASTROINTESTINAL NEMATODES AND LIVER FLUKE IN THE FLEMISH DAIRY POPULATION

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ABSTRACT

The objective of this study was to estimate the direct costs of gastrointestinal nematode and liver fluke infections in the Flemish dairy population. First, the population at risk, the prevalence of production-limiting infections, the effects on animal production, the monetary value of animal products and the frequency and cost of an anthelmintic treatment were defined through a study and interpretation of specialist literature. Next, these elements were incorporated in a spreadsheet and the annual costs were assessed for each infection. The annual cost of gastrointestinal nematode infections was estimated at € 11,118,289 or € 40 per adult cow. The annual cost of liver fluke infections was estimated at € 8,916,800 or € 32 per adult cow. These figures do not present the average cost of an infected animal, but reflect the relative importance of the disease. The results of this study emphasize the value of incorporating both infections in animal health programmes.

1. INTRODUCTION

An important evolution in veterinary medicine has been redefining disease more broadly, to include subclinical conditions. This expansion has resulted both from improved diagnostic technology as well as from te evolution of health management in which any factor that limits animal or herd performance can be considered as a component of disease (LeBlanc et al., 2006). Subclinical infections with gastrointestinal nematodes and liver fluke are considered as an important cause of production loss in grazing cattle worldwide (Corwin, 1997; Dargie, 1987). Allthough an exentsive compilation of literature exists of the effect of these infections on animal performance (growth, fertility indices and milk production), only few attempts have been made to convert these production losses to an economic cost, expressed in a monetary value.

Several methods exist to assess the direct costs of a disease in animal production and the obtained results can vary to great extent depending on the used methodology (Morris, 1999). In response, Bennett et al. (1999; 2003) developed a simple spreadsheet model that can be used for different animal diseases and allows comparison of the direct costs associated with different diseases.

The objective of this study was to use this previously described model to assess the direct cost associated with 2 important helminth infections (gastrointestinal nematodes and liver fluke) in the Flemish dairy population.

2. MATERIALS AND METHODS

According to the method of Bennett et al. (1999), the different loss-making outputs were identified, calculated and summed to obtain the total cost of a disease. For both helminth-infections, following steps were performed separately: (1) identification of number of animals at risk and an estimation of the prevalence of production-limiting infections in this population; (2) identification of the different effects on production that are associated with the considered infections and an estimation of the mean magnitude of these losses; (3) determination of the monetary value (€) of each production effect; (4) estimation of the mean frequency and cost of anthelmintic treatment for the herd owner. Because it is considered that reduced appetite is responsible for an important part of the observed production losses caused by gastrointestinal nematodes (Forbes et al., 2004), we also took into account saved costs by reduced roughage intake during the stabling period (151 days) for this infection. The final sum is an estimation of

the total cost of the infection over the period of one year in the studied population. To obtain a concrete image of this cost, the final sum was divided by the total number of adult cows in the studied population.

3. RESULTS

The whole dairy population in Flanders was considerd at risk for infection with gastrointestinal- and liver fluke infection and was subdivided in replacement stock (n= 262,313) and adult dairy cows (n= 280,720) (Anonymous, 2008). The used data for the prevalence of production-limiting infections and the frequency of anthelmintic treatments are given in Table 1. The fysical effects of these infections on production and roughage uptake and their cost (€) per measurement unit that were used in the calculations are given in Table 2. The cost attributed to an anthelmintic treatment for a replacement heifer and adult cow was € 3.5 and € 10.9 for gastrointestinal nematodes and € 2.5 and € 8.0 for liver fluke.
The total estimated cost of gastrointestinal nematode infections was € 11.118.289 or € 40 per adult cow. These costs were caused both by infections in the replacement stock (estimated at € 2.201.488 or € 8 per replacement heifer) and infections in the adult stock (estimated at € 8.916.800 or € 32 per adult cow). The largest loss-making activity was loss in milk production, followed by costs spent on anthelmintic drugs. The total estimated cost of liver fluke infections was € 8.925.112 or € 32 per adult cow. These costs were caused by both infections in the replacement stock (estimated at € 3.615.985 or € 14 per replacement heifer) and infection in the adult cows (estimated at € 5.309.127 or € 19 per adult cow). The largest loss-making activity was loss in milk yield, followed by costs due to increased number of inseminations and delayed onset of puberty.

4. DISCUSSION

In this study, the direct costs associated with 2 common helminth-infections of cattle were estimated for the first time in the Flemish dairy population. Although lungworm infection are also very common in this population, they could not be taken into account because very few literature exists on the prevalence of the infection and the effect of subclinical infections on productivity.
For interpretation of the obtained results it is important to consider the objectives and limitations of the current approach. The obtained figures do not present the average cost of an infected animal nor can they be used to estimate the cost of disease in an average dairy herd or the costs that can be recovered through anthelmintic control. Rather, they reflect the relative importance of the disease in the current situation and could be compared with the figures of other infectious diseases obtained by the same methodology.
The costs of increased labour, veterinary services of increased susceptibility to other diseases due to infection with the studied parasites were not taken into account in the present calculation. Therefore the obtained figures can be considered as a conservative estimation and are probably an underestimation of the true monetary cost.
Despite these limitations the used methodology provides a simple approach that can be easily updated each year according to actual prices of animal products and recent prevalence estimates. Moreover, with a few adaptations it can be used to calculate the cost of helminth infections on the herd level and become a tool in farm health management.

5. ACKNOWLEDGEMENTS

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Table 1. Estimation of the prevalence of production-limiting infections with gastrointestinal nematode and liver fluke infections and the frequency of anthelmintic treatments in Flemish dairy population.

	Replacement heifers	Adult cows
Prevalence of production-limiting infections		
Gastrointestinal nematodes	5 %	50 %
Liver fluke	15 %	15 %
Proportion of population receiving anthelmintic treatment		
Gastrointestinal nematodes	70 %	30 %
Liver fluke	12 %	12 %
Average number of anthelmintic treatments per animal		
Gastrointestinal nematodes	1.4	1.0
Liver fluke	1.5	1.5

Tabel 2. Estimation of the average effects on production parameters and roughaghe intake associated with gastrointestinal nematode and liver fluke infections in dairy cattle in Flanders.

Production parameter (measuring unit)	Average effect		Cost (€)
	Gastrointestinal nematodes	Liverfluke	
Replacement stock			
advent of puberty (day)	10	25	1.33 / dag
milk yield in 1st lactation (kg)	-331	-159	0.35 / kg
roughaghe intake (kg/day)	-2.2	0.0	0.09 / kg
Adult cows			
milk yield (kg/day)	-0.9	-0.7	0.35 / kg
nr of inseminations per conception	0.10	0.75	49 / inseminatie
intercalving interval (day)	0.0	4.7	1 / dag
roughaghe intake (kg/day)	-3.1	0.0	0.09 / kg
liver condemnation in abattoir (kg)	0	6	3 / kg

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IMPACT OF ENDEMIC DISEASES ON DAIRY FARM PROFITABILITY

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ABSTRACT

In order to support decisions in the field of animal health on dairy farms, knowledge of the impact of diseases on farm profitability is important. Basically, endemic diseases associated with dairy production (production diseases) are decreasing the efficiency of milk production, requiring a higher level of input to produce the same amount of milk. The optimal production with and without disease will differ for a specific farm. To estimate the economic effect of a production disease the following cost factors should be taken into account: decreased (milk) production, veterinary services, diagnostics, drugs, discarded milk, labour, decreased product quality, increased risk of new cases of the same disease or of other diseases, increased risk of culling, and materials and investments for prevention. In a recent Dutch study, the costs for mastitis (clinical and subclinical), was estimated to be € 78 per average cow present on a farm per year. However, these costs could differ largely between farms (€ 17 - € 198 per average cow on the farm per year). Costs for ketosis on a Dutch dairy farm were estimated to be € 27 per average cow on the farm per year, varying from € 24 to € 54 per average cow per year, because of natural variation. Because of the large differences between and within farms, a good understanding is important to translate generic cost calculations to a farm specific situation.

1. INTRODUCTION

Some endemic diseases are implicitly associated with dairy production. These, so called, production diseases do cause large economic effects. In fact, the most expensive disease on dairy farms is mastitis, one of these production disease. Because of the chronic nature of production diseases, economic damage is spread out over the year, and the economic damage of certain factors, such as milk production decreases, cannot directly be seen. Farm accounting reports give all kinds of detail about the costs of production but these are in terms of feeding costs, machinery costs, costs for animal improvement, etc. The factor health costs only comprise costs for drugs and the veterinarian, which is only a small proportion of the total economic damage of a production disease (as will be shown later in this chapter). The total costs of disease can be large. For instance, for the Dutch dairying situation, it was estimated that the costs of health and fertility problems accounted for 10 % of the gross production value (Dijkhuizen, 1990).

A good understanding of the costs of a disease is important to support decisions of farmers with regard to animal health. It is important that this understanding goes beyond the knowledge of costs of a disease as it is given by calculations of others. All calculations of costs of disease and cost-effectiveness of preventive and curative measures can be regarded as averages for a certain situation. Costs of disease vary from farm to farm. This is not only dependent on the incidence of disease but also on the level of cost factors (Huijps et al., 2007). In order to support decisions of farmers, the advisor must be able to interpret such published data to translate them to the specific situation of an individual farm. Therefore, insight in the theories behind economic calculations in the

field of animal diseases is necessary. Therefore, in this chapter, first a generic framework showing the principles behind animal health economics is described, including the cost factors making up costs of a disease. Finally, a few recent examples of economic calculations of costs of disease will be described.

2. ANIMAL HEALTH FROM AN ECONOMIC PERSPECTIVE

Basically, work in the field of animal health economics is dedicated to support decisions. Although we often focus on the dairy farm when discussing production diseases, diseases should be looked upon from a broader perspective. A good background of this perspective is given by McInerney (1996). In a livestock production system, resources (input) are processed on a farm into several products. The main product of a dairy farm is obviously milk. These products are useless when they do not improve the welfare of the society by increasing human benefits. Therefore, society is willing to pay a price for these products (Figure 1). Diseases may affect this process in different ways (Figure 1):

1. Lower the efficiency of the production process, which leads to a lower productivity of the resources, either by a lower level of output but also by a higher need for resources to maintain the same level of output.
2. Lower the suitability of products for human benefit, either by a lower quality of the product, or by a lower suitability to process the product
3. Affect the human well-being directly by, for instance, zoönoses.
4. Reduce the total value a society gains from livestock. This is an indirect economic effect or instance because people loose trust in milk or beef due to diseases. Another example is constraints in trade because of animal disease.

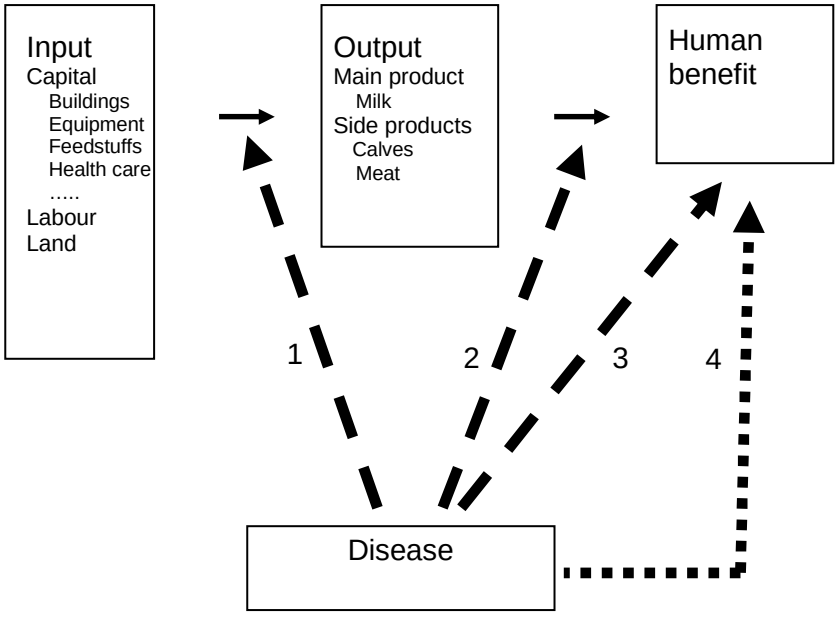

Figure 1. Pathways through which disease affects the dairy production system (after McInerney, 1996).

All of these 4 pathways do affect the dairy farmer directly through a lower production or a lower price for lower quality products, or indirectly through a lower demand for dairy products, which affects the price. It also implies that decisions with regard to diseases can be taken at different levels. Decisions can be taken at the farm level, either with regard to individual animals (do I treat this animal or not), the herd (do I improve the level of prevention or not), the sector (do we as dairy farmers improve the bulk milk somatic cell count to improve the image of dairy products) or at society level (do we make laws to reduce the level of *Escherichia coli* VTEC in beef).

2.1. Effects of disease on farm profitability

In Figure 1, the production processes on the dairy farmer are represented by an arrow between input and output. Figure 2 zooms in on this “black box” and represents the three main areas of dairy farm management. The “core business” of a dairy farm is the husbandry of dairy cows. Per definition, a dairy farm must have dairy cows. The

management areas young stock rearing and grass and crop growing are auxiliary management areas. Although not common, a dairy farm can do without any young stock rearing and grass and crop production. Fresh or pregnant heifers can be bought elsewhere, just as feedstuffs. There can be advantages by keeping these two areas on one farm, but those are business decisions of the dairy farmer. Within the husbandry of the dairy cows, a number of management areas can be distinguished, such as housing, feeding, hygiene, health and reproduction. Please note that this list does not intend to be complete and only provides examples of management areas.

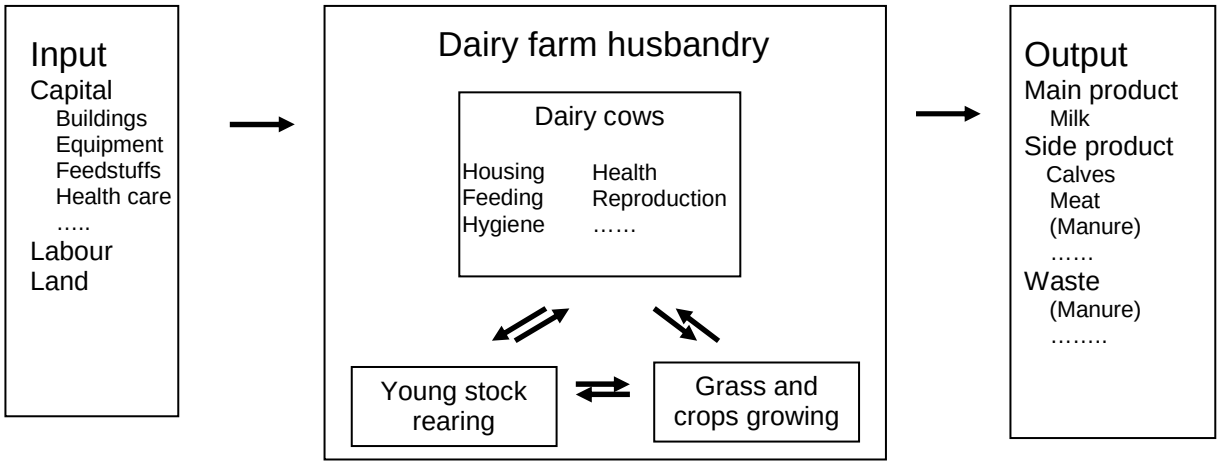

Figure 2. Production processes on a dairy farm.

When evaluating the economic effects of a disease, knowledge is necessary on the basic resource-using process of the dairy farm, a process which is very well described by McInerney (1996). This resource-using process can be represented by a production function (Figure 3). This function represents the efficiency in which output (milk, calves and meat) is derived from the use of variable resources such as feedstuffs and health care (input), within the constraints of the farm structure (for instance the available land, buildings and labour). This process is more efficient (in terms of resources needed for a certain amount of output) for a farm without diseases (the top curve in Figure 3) and with diseases (the bottom curve in Figure 3).

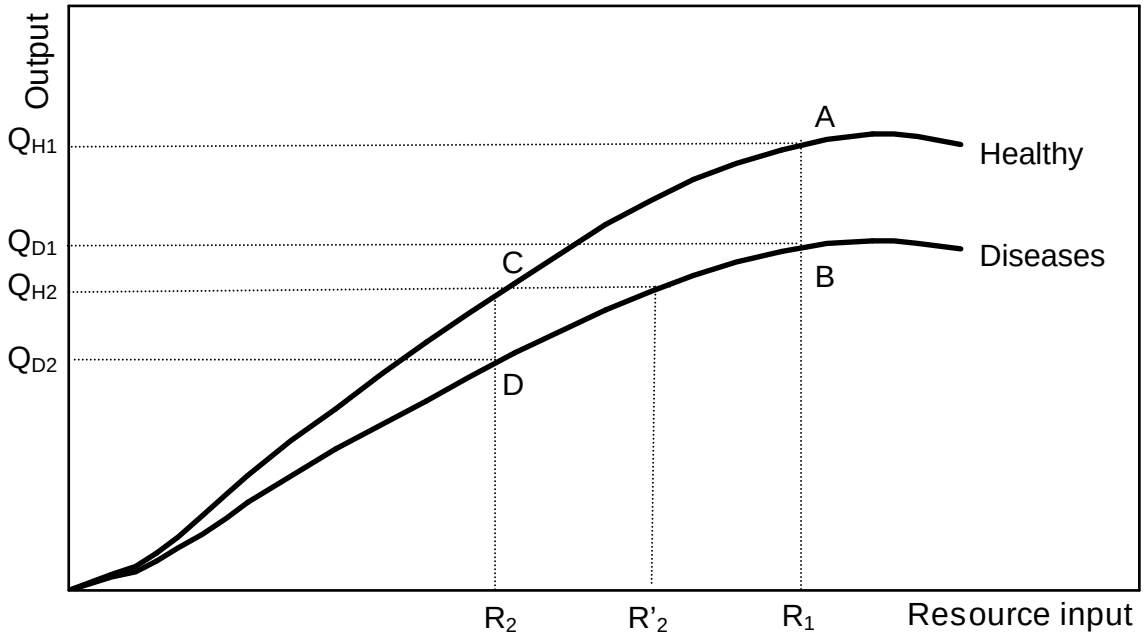

Figure 3. Effect of disease on the dairy farm production curve (after McInerney, 1996).

Suppose 2 farms, producing on different points of the production curve. The occurrence of disease has the first farm move from point A on the healthy curve to point B on the disease curve. While the second farm moves from point C on the healthy curve to point D on the disease curve. It is obvious that the damage, in terms of a

decreased output with the same level of input, is lower for the first farm ($Q_{H1} - Q_{D1}$) than for the second farm ($Q_{H2} - Q_{D2}$). From Figure 3, it can also be seen that the damage of disease can be looked at differently. In order to maintain a same level of production Q_{H2} farm 2 can opt to increase the resource input from R_2 to R'_2. Depending on this choice, the damage of disease for farm 2 is either $Q_{H2} - Q_{D2}$, multiplied by the price of output, or $R'_2 - R_2$ multiplied by the costs of resources.

This latter example reflects a decision problem often faced on dairy farms because production diseases are per definition present: Should I accept a loss in output or should I increase the level of input (more drugs, more time of the veterinarian, more hygiene etc.)? Under a quota situation, this question can even be more complex. When there is a milk quota, farmers produce a certain level of milk (output is constant). That means that the decision problem has the form of: Should I maintain the same level of output by increasing the number of cows on m y farm (and thus increasing the input of feedstuffs, breeding, bedding material, labour etc.) or should I increase the level of health input?

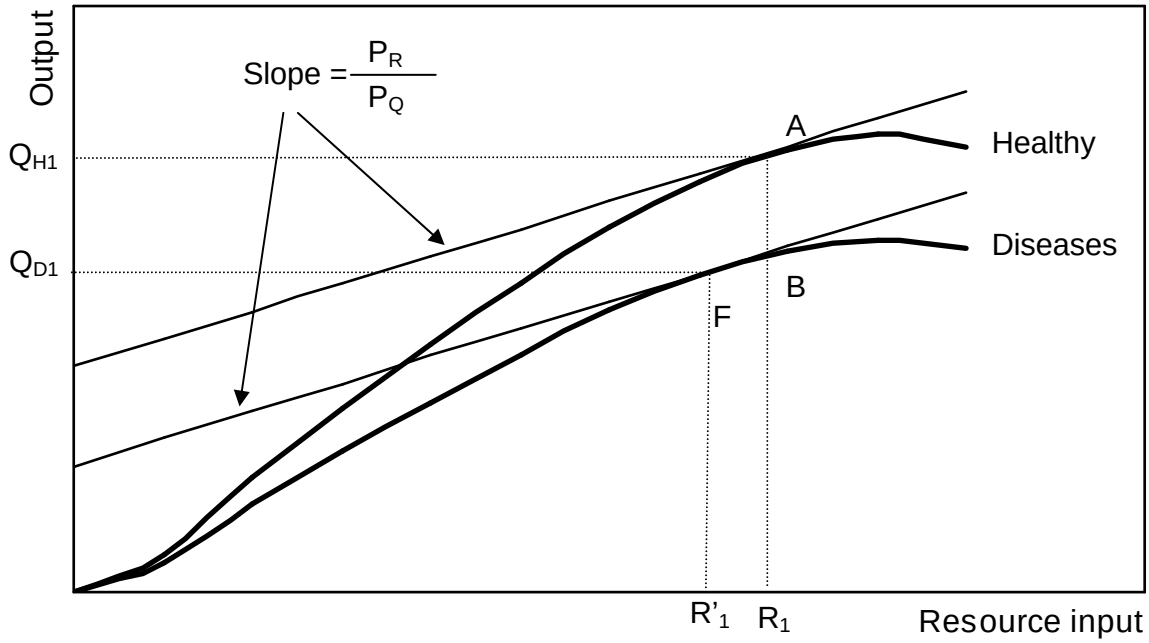

Figure 4. Effect of disease under optimal economic management (after McInerney, 1996).

From economic theory, the situation is even more complex. A very basic economic rule regarding the maximizing of profit, states that the optimal level of input R, given a certain production function is that point where the change of output, relative to input is equal to the relative prices of input P_R and output P_Q (McInerney, 1996). In Figure 4 the relative prices of input and output are represented by the slope of a line. For farm 1, the optimal production level is at point A. When disease occurs, from this theoretical point of view for farm 1, the economic rational thing for to do is to move production to point F and not to point B. This would minimize the economic consequences of disease.

One final element to keep in mind is that changes in production efficiency of a single farmer will not affect the market supply. Additional profits of this improved production will be completely for the dairy farmer. However, when a large part of the sector improves the production efficiency, for instance by improving the disease situation, this will affect the market supply of milk. Under “perfect” market conditions this change in supply, will eventually have an effect on the price of milk. Part of the profit of this improved efficiency will therefore be for the consumer and not for the producer. Under milk quota circumstances, the supply of milk is set constant, and then the pricing mechanism will not work. The profit of a more efficient milk production will therefore, under quota circumstances, be complete for the dairy farmer.

2.2. Factors that determine the cost of disease

Because the production functions differ from farm to farm and because many farmers do not optimize the production level according to the rule described above, there are hardly any publications on cost of disease

applying this production theory as described in the foregoing paragraph. Moreover, under practical circumstances it is very difficult to make an estimation of the costs of disease. In this section, we will therefore be pragmatic and give the factors that determine the cost of disease as they are described by Halasa et al. (2007). In their paper, economic consequences of mastitis (clinical or subclinical) were described. The following cost factors can be distinguished:

- Decreased (milk) production
- Veterinary services
- Diagnostics
- Drugs
- Discarded milk
- Labour
- Decreased product quality
- Increased risk of new cases of the same disease or of other diseases
- Increased risk of culling
- Materials and investments for prevention

Although the relative cost of the factors might differ between countries and between regions, the economic principles behind them are the same. A more detailed description of these factors is given elsewhere (Halasa et al., 2007).

3. EXAMPLES OF COST CALCULATIONS

There is a wide arrange of methods available to calculate the costs of disease and the economic efficiency of disease control measures (Dijkhuizen et al., 1991). In this section these methods will not be further explained. Moreover, in the scientific literature numerous papers have been published on the economic effects of disease and the cost-effectiveness of disease prevention. It goes too far to give a complete review of the costs associated with all diseases in dairy production. In this section we will give a two examples of recent calculations around animal diseases: the costs of mastitis on a dairy farm and the costs of ketosis on a dairy farm.

3.1. Mastitis

In a recent study (Huips et al., 2007), costs of mastitis were calculated for average Dutch circumstances. The average costs for a case of clinical mastitis were estimated to be € 210, varying from € 235 for clinical mastitis in the first month post partum to € 164 for clinical mastitis in the last part of lactation. The costs for subclinical mastitis were dependent on the number of cows with an increased somatic cell count and were due to milk production losses. For a farm with an average production of 8,500 kg per 305 days and a bulk milk somatic cell count of 200,000 cells/ml, these costs were € 20 per average cow on the farm per year. Using an average incidence for clinical mastitis (30 %) the total costs of mastitis for a Dutch dairy farm with 65 cows were calculated to be € 78 per average cow on the farm per year. Costs for production losses are the largest proportion of these costs. Some of the assumptions made for this basic calculation can be found in Table 1.

Table 1. Costs of mastitis calculated for the average Dutch situation (Basic) and according to data collected on 64 Dutch dairy farms. The mean, minimum and maximum values are given (source: Huijps et al., 2007).

		Farmers data		
	Basic	Min	Mean	Max
Farm size (nr cows)	65	28	83	160
Farm size (kg quota)	650,000	195,000	702,621	1,500,000
Yearly mastitis incidence (%)	30	6	29	100
Bulk milk somatic cell count (cells/ml)	200,000	60,000	178,484	300,000
Costs milk production losses (€/kg)	0.12	0	7.47	12
Costs visit of veterinarian (€/visit)	20	0	23.50	100
Costs of drugs (€/treatment)	20	5	33.18	110
Value of farmers labour (€/hour)	18	0	18.83	200
Costs of culling (€/culled cow)	480	0	382.50	750
Total costs for mastitis (€/cow present)	78	17	78	198

As stated before, the economic consequences of disease may differ between farms. Moreover, the incidence and severity of disease may also differ. To illustrate this, data have been collected on 64 dairy farms. As can be seen

in Table 1, the incidence of clinical mastitis differed largely between farms. Also the bulk milk somatic cell count and thus the number of cows with an increased somatic cell count, differed also largely between farms. From an economic point of view the variation in costs of for instances milk production losses, labour and culling is much more interesting. The costs associated with a decreased milk production due to disease differed from 0 to 12 cents per kg (under quota circumstances). Also the costs for labour differed largely between farms (0 – 200 € per hour). In these costs for labour, some farmers did not look at opportunity costs per se, but took also the willingness to pay to prevent the labour associated with clinical mastitis into account. Also a large variation could be seen in costs for culling. Under practical circumstances, the costs per cow present on a farm for mastitis varied between € 17 and € 198.

3.2. Ketosis.

An interesting aspect of ketosis is that it is clear that ketosis does increase the risk of clinical mastitis and left displaced abomasum. In a recent Dutch study, costs of ketosis, clinical as well as subclinical were calculated using a Monte-Carlo simulation model to simulate a herd with 65 dairy cows (Shrestha et al., 2008). Costs for ketosis were calculated for a situation with and without a milk quota (Table 3). Incidence of clinical ketosis was 3.5 %, while the incidence of subclinical ketosis was 6.7 %. The resulting yearly costs due to ketosis were estimated to be respectively € 1,778 and € 2,353 for a situation with and without a milk quota. As can be noticed from Table 3, natural occurring variation did give a large difference in costs per year. The largest proportion of costs is caused by milk production losses. However, culling give the highest risk of high costs. The costs due to increased risk of other diseases as mastitis, left displaced abomasums and decreased fertility are substantial, but in relation to the costs due to milk production losses and culling relatively low.

Under a non-quota situation, costs for milk production losses are higher than under a quota situation. This can be seen in Table 2.

Table 2. Dynamics of ketosis and other disease events caused by ketosis and resulting economic effects for a Dutch dairy farm with 65 cows under quota and non-quota circumstances. The mean, 5 % percentile and 95 % percentile are given (source Shrestha et al., 2008).

	Quota			Non quota		
	5%	Mean	95%	5%	Mean	95%
Dynamics						
Probability of clinical Ketosis	1	3.5	7	1	3.4	7
Probability of Subclinical ketosis	3	6.6	11	3	6.8	11
Probability of Culling (%)	0	2.0	6	0	2.0	6
Proability of mastitis (%)	0	0.6	4	0	0.6	4
Probability of LDA (%)	0	0.13	0	0	0.15	0
Probability of Cystic Ovary (%)	0	0.16	0	0	0.15	1
Costs						
Costs of Milk Losses (€)	405	807	1,267	678	1,366	2,149
Costs of Culling (€)	0	751	2405	0	1,172	3,902
Costs of Mastitis (€)	0	120	840	0	115	840
Costs of Treatment (€)	0	78	300	0	73	250
Costs of Left Displaced Abomasum (€)	0	16	0	0	18	0
Costs of Prolong Calving interval (€)	0	6	26	0	6	25
Costs of Feed (€)	-	-	-	-624	-396	-197
Total costs (€)	1,588	1,778	3,506	702	2,353	5,170

CONCLUDING REMARKS

On a dairy farm, production diseases are responsible for a large part of the cost price of milk. To support decisions around diseases, understanding the economics of diseases is important. It is not enough to use an average cost calculation per case of disease and multiply the number of cases with that average cost figure. It is important to understand the principles behind the farm economics so that farm-specific calculations can be made. Knowledge about basic economic principles such as the production function are therefore important. On the

other hand, economics are not the only factor influencing the behaviour of dairy farms. A recent study showed that economics are only 30-40 % of the motivation of dairy farmers to change mastitis management (Valeeva et al., 2007). This might also be the case for other diseases. However, in European dairy farming, the forces of the free market are going to play an increasingly important role in the income of the dairy farmer. Therefore, the costs of production and thus the animal disease status will become more and more important. In this respect the goal with regard to animal health should not be a maximum level of animal health, but an optimal level of animal health.

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BLUETONGUE IN BELGIUM: EPISODE II

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ABSTRACT

In 2006, ruminant populations in Belgium became infected with Bluetongue virus (BTV). A second wave of BTV outbreaks started in July 2007. The objective of this study was to describe the evolution and the clinical impact of the second episode. Both outbreak and rendering plant data were analysed. Overall cumulative incidence at herd level was estimated at 11.5 (11.2-11.8) and 7.5 (7.3-7.8) % in cattle and sheep populations, respectively. A high level of correlation was demonstrated between BT incidence and small ruminant mortality data when shifting the latter of one week backwards. This result supports the hypothesis that the high increase in small ruminant mortality observed in 2007 was the consequence of the presence of BT. For cattle, the correlation was not as high. An increase in cattle foetal mortality was also observed during the year 2007 and a fair correlation was found between BT incidence and foetal mortality.

1. INTRODUCTION

Bluetongue (BT) is an arthropod-borne viral disease of ruminants. In August 2006, domestic ruminant populations in Northern Europe became infected with BT virus (BTV). In Belgium, the first case of BT in 2007 was suspected in July. This case was the starting point of a new wave of BT outbreaks. Farmers suggested that BT induced more severe clinical signs in 2007 than it did the previous year. The main objective of this study was to describe the evolution and the clinical impact of the 2007 BTV episode in Belgium. In addition, the main differences with the previous epidemic (19 August to 15 December 2006) are reported.

2. MATERIAL AND METHODS

Livestock density data was extracted from the Belgian animal identification and registration system to provide estimates of the population at risk at the start of the 2007 episode. The cumulative incidence (probability that a herd at risk developed BT during the year 2007; based on outbreak data) and 95% confidence intervals were estimated.

In 2006-2007 outbreak herds, the total numbers of animals that developed BT clinical signs and that were slaughtered/died due to BTV infection were recorded. The mean morbidity and mortality rate were estimated. Simple linear regression models were used to assess the difference in morbidity/mortality rates between the 2006 and 2007 episodes (SAS®).

Rendering plant data (Rendac) from 2002 onwards were acquired. To study the impact of BT on ruminant mortality, the BT weekly incidence curve for 2007 was compared to rendering plant data for the same time period. The correlation between BT incidence curve and the curve of weekly differences was evaluated using the Pearson correlation coefficient (SAS®). The same comparison process was followed for dead cattle foeti, but, in this case, over both 2006 and 2007 calendar years.

3. RESULTS

Overall cumulative incidence at herd level based on confirmed outbreaks was estimated at 11.5 (11.2-11.8) and 7.5 (7.3-7.8) % in cattle and sheep populations, respectively.
In cattle herds, mean morbidity rate in 2007 was estimated at 9.3% (95%CI 8.6-9.9%). The linear regression model showed that the impact of year was significant ($p<0.0001$) with an increase in morbidity rate in 2007 when comparing with 2006 (β= 4%; 95%CI 2.4-5.6%). The same significant impact was observed for sheep flocks, when comparing morbidity rate in 2007 (25.7%; 95%CI 24.4-26.9%) with 2006 (β= 13.4%; 95%CI 10.9-16%)) ($p<0.0001$). In 2007, in cattle outbreaks, mean mortality rate was estimated at 0.6 % (95%CI 0.5-0.8%). The impact of year was statistically significant and mortality rate was higher in 2007 compared to 2006 (β= 0.4%; 95%CI 0.1-0.6%; $p=0.01$). Mean mortality rate in sheep flocks was also found to be significantly higher in 2007 (12.9%; 95%CI 12-13.8%) than it was in 2006 (β= 4.7%; 95%CI 2.6-6.8%); $p<0.0001$).
Figure 1 shows the BT incidence curves along with the rendering plant data curves. The visual analysis of the cattle curves (Figure 1a) shows mortality differences were positive from week 35 onwards. This positive mortality pattern seemed to follow the increase of the BT incidence curve for cattle. For sheep (Figure 1b), the two curves clearly follow the same trend with a delay of more or less one week between them. The Pearson correlation coefficient was shown to be the highest for adult sheep/goats when shifting back the rendering plant data for one week with $r=0.98$ ($p<0.001$). Correlation between BT and mortality was not as good for cattle as it was for sheep/goats ($r=0.54$;$p<0.001$). For dead foeti, correlation was found to be the highest when shifting back the data for 3 weeks ($r=0.57$; $p<0.0001$).

4. DISCUSSION

Death may occur in 8-10 days in BT diseased animals. Mortality usually ranges between 10 and 20 percent, but can reach 70 percent in individual flocks (Breard et al., 2004). The analysis demonstrated a strong and significant correlation between the incidence of BT and adult sheep and goat mortality. However, this high correlation does not necessarily induce a relation of causality between the two events. Some factors such as the breeds of the infected animals or the presence of concomitant diseases may here interact.
Many reproductive disorders in cattle have been reported during the year 2007 in Belgium. Recent laboratory analyses on aborted foeti, newborn calves and dam-newborn pairs provided evidence of the capacity of BTV serotype 8 to pass the placental barrier (De Clercq et al., 2008). The present study indicated an increase in foetal mortality in 2007, as well as a fair correlation between the incidence curve of BT and that of abortions when shifting back the mortality data for 3 weeks. The association between BT and abortion is however difficult to objectify using this type of study design, since various impacts on embryo/foetus may have occurred according to the gestation stage the dam was infected.
The objective of this paper was to describe the evolution and the clinical impact of the BT 2007 episode. Even though further validation is needed, some conclusions can be drawn:

- The 2007 episode had a more severe clinical impact compared to the situation in 2006, which seemed to justify the higher level of reporting in 2007.
- The clinical impact was higher in sheep flocks compared to cattle herds.
- A high level of correlation was demonstrated between small ruminant mortality and BT incidence data when shifting back the mortality data for one week. This finding supports the hypothesis that the high increase in small ruminant mortality in 2007 was the consequence of the presence of BT. For cattle, the correlation was not as high.
- An increase in cattle foetal mortality was observed during the year 2007 and a fair correlation was found between BT incidence and foetal mortality.

5. ACKNOWLEDGEMENTS

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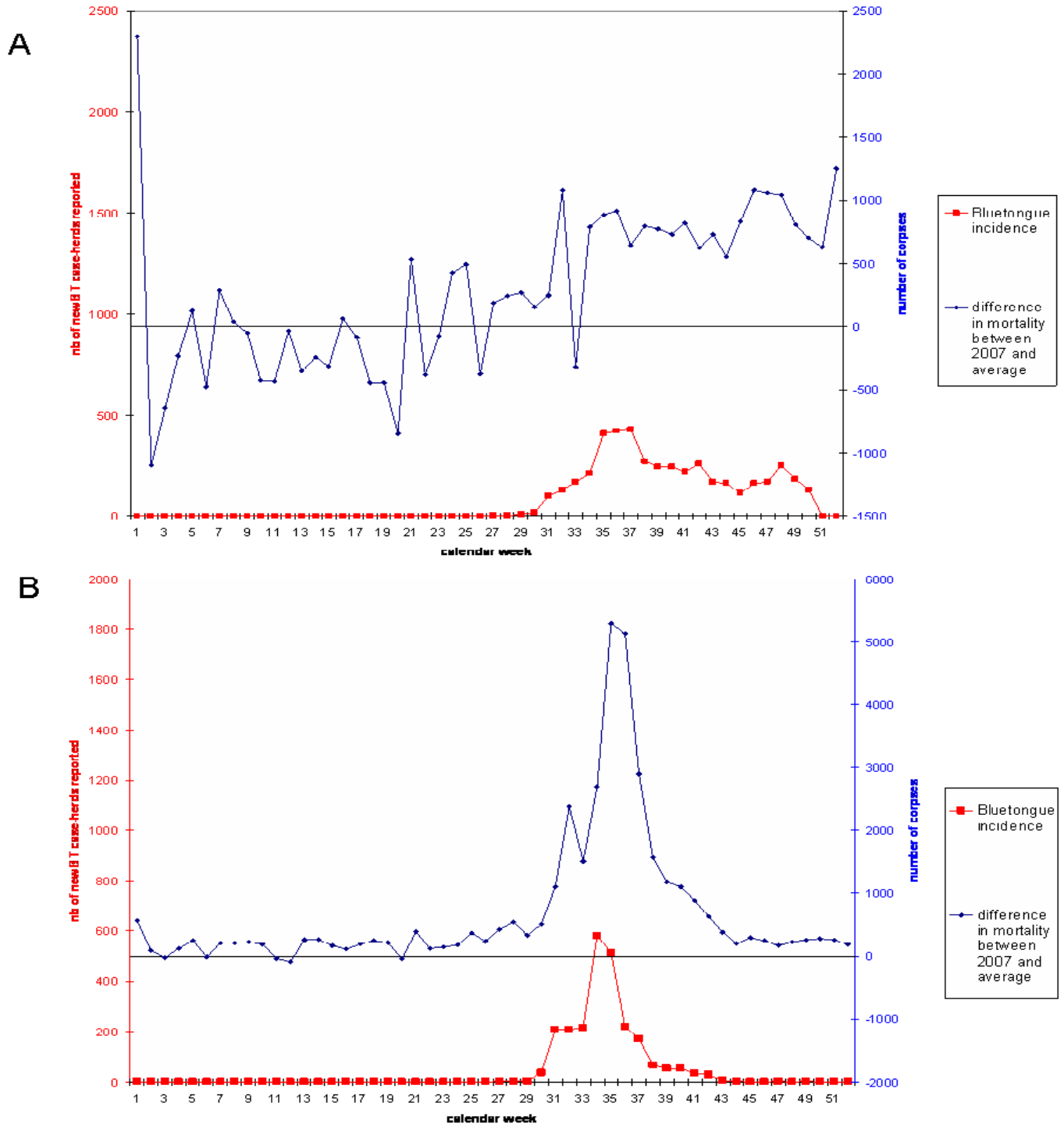

Figure 1. Bluetongue weekly incidence curve and difference in number of carcasses collected per week between 2007 and average (2002-2005) for cattle in Belgium in 2007.A. cattle. B. sheep and goats.

EXPLORING SAMPLE SIZE FOR ANTIMICROBIAL RESISTANCE PROFILING OF *E.COLI* IN BROILER CHICKENS

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ABSTRACT

To gather data on herd level antimicrobial resistance in broiler chickens for Belgium in an efficient way, without oversampling and thus spilling of valuable research money and time, a suitable sample size is imperative. A study was conducted to investigate the precision in estimating herd-specific antimicrobial resistance profiles as a function of sample size. Basis for all analyses was the bootstrapping method. The antimicrobial resistance profiles are calculated based on random subsets of varying sample size n_1 (animals) and cluster size n_2 (isolations per animal) of the original data set containing the test results on antimicrobial resistance in *E.coli* from broiler chickens. This way, insight is gained in estimation precision as a function of sample size and cluster size taking into account the between- and within-animal variability. Results show that the within-animal variability was very low compared to the between-animal variability and hence, the gain in estimation precision is negligible when increasing the number of isolations per animal. Focus in sampling strategy should therefore be on the number of animals per flock rather than on the number of *E.coli* isolations to test per animal. Finally, the economic aspects of antimicrobial resistance profiling are evaluated as well to determine the sampling strategy having the best trade-off between precision and sampling costs.

1. INTRODUCTION

Few studies on sample size assessment have been published, although it is one of the key aspects in epidemiologic research. In contrast to most national monitoring programmes, where one animal is sampled per flock, for herd level antimicrobial resistance profiling more animals will have to be tested. The question is: how many animals? And do we need to test more than one isolate per animal? One of the major issues that arise when trying to tackle sample size with the classic statistical formulas is that no single overall prevalence is by hand when a range of antimicrobials is to be tested. Going for the maximum sample survey assuming a prevalence of 0.50 could be an option, but will most definitely result in spillage of valuable research funding and time. Moreover, to our knowledge, statistical formulas are lacking when sample sizes n_1 and cluster sizes n_2 are to be determined jointly.
The objective of this study was to develop a methodology which makes it possible to interpret, challenge and optimize sample size when setting up an antimicrobial resistance profile of *E.coli* in broilers at flock level, taking into account both epidemiological and economical evaluations based on herd level data collected from broiler chickens.

2. MATERIAL AND METHODS

Five Belgian broiler flocks were randomly selected from the Belgian identification and registration system for livestock (SANITEL, 2005). The only selection criterion was that the flock needed to consist of more than 10,000 animals to be representative for the average Belgian broiler flock. All flocks were sampled using classical methods to calculate sample size. For a flock of 10,000 animals or more, this comes to 97 animals to be sampled per flock (Win episcope 2.0) for an estimated prevalence of 50% .

One cloacal swab was taken from all chickens. For one farm, five *E.coli* colonies per sample were isolated to explore the within animal variability. In the four other flocks, only a single isolate was tested. For the between animal variability all five herds were taken along.

E.coli was isolated and tested for antimicrobial susceptibility according to a standardized method as described in Catry et al. (2007). The 14 antimicrobials that were tested are given in Table 1.

Results were entered into a Microsoft Excel spreadsheet. Zone diameters were converted to their respective susceptibilities (S; I; R). For further analysis, all intermediate resistances were considered resistant. Data were converted to Matlab® for further analysis. First, bootstrap samples are generated by resampling n_1 animals and n_2 isolates with replacement from the data on farm A with (n_1, n_2) being all possible combinations out of N1 = {1,2,…80} and N2 = {1,2,3,4,5}. Second, since antimicrobial resistance profiles are heterogeneous across farms, bootstrap samples are generated by resampling n_1 animals from all five farms, with only the first isolate taken from farm A. To assess the effect of sample size on the global antimicrobial resistance profile, thus for all 14 antimicrobials simultaneously, the proportion of prevalences estimated with sufficient precision, thus within the required confidence interval, was determined. This proportion was determined for sample sizes varying from 1 up to 100 samples per flock, and for 95% confidence interval widths of 0.1, 0.2 and 0.3.

Economic efficiency was determined by a cost effectiveness analysis of the sample size. Both the fixed and variable costs connected to the different sample sizes were taken into account.

3. RESULTS

Figure 1 shows the joint effect of varying numbers of colonies and number of animals sampled, clearly showing that gain in precision is negligible when increasing the number of isolates per animal. Table 1 shows the resistance prevalence and bootstrap confidence intervals for the original dataset as well as for smaller subsets for all 5 farms (only 1 isolate per animal).

Table 1. Prevalence of antimicrobial resistance and corresponding bootstrap confidence intervals for different herd level sample sizes (1 colony per sample).

antimicrobial agent (disk load, µg)	resistance prevalence (%)	Width of the 95% CI for n samples					
		Original dataset	75	50	30	20	10
Florfenicol (30)	0,76	0,01	0,01	0,02	0,03	0,04	0,06
Gentamicin (40)	3,06	0,03	0,03	0,05	0,08	0,11	0,16
Apramycin (40)	5,78	0,04	0,06	0,09	0,14	0,18	0,25
amoxicillin-clav. acid (30+15)	14,35	0,07	0,07	0,12	0,19	0,24	0,36
Enrofloxacin (10)	16,31	0,07	0,07	0,14	0,20	0,25	0,36
Chloramphenicol (60)	16,73	0,07	0,09	0,14	0,22	0,29	0,41
Neomycin (120)	25,04	0,08	0,10	0,18	0,27	0,37	0,50
Flumequine (30)	27,73	0,08	0,09	0,16	0,25	0,33	0,44
Ceftiofur (30)	35,95	0,08	0,09	0,17	0,27	0,32	0,45
trimethoprim-sulpha (5.2+240)	52,79	0,09	0,11	0,20	0,32	0,41	0,61
Streptomycin (100)	61,77	0,08	0,10	0,19	0,29	0,38	0,59
Tetracycline (80)	62,64	0,09	0,11	0,20	0,30	0,40	0,59
Nalidixic acid (130)	70,25	0,08	0,10	0,18	0,27	0,37	0,54
Ampicillin (33)	78,10	0,08	0,07	0,14	0,23	0,30	0,45

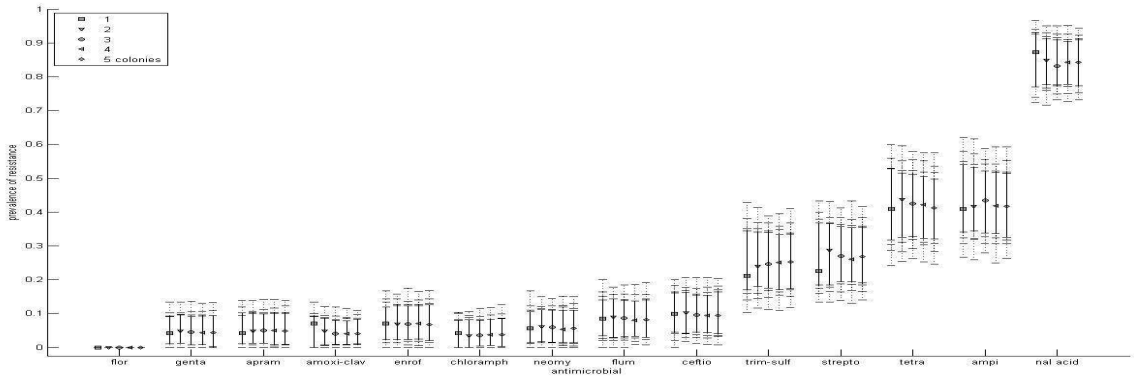

Figure 1. Effect of varying numbers of isolates per animal for different number of animals sampled (original dataset, 75, 50 and 30 animals, represented by the error bars).

To get an overview of the influence of reducing sample size on all 14 antimicrobials tested simultaneously, the proportion of prevalences that can be estimated within a predefined width of confidence interval is calculated for varying sample sizes. Figure 2 shows the results for three widths of 95% confidence intervals: 0.1, 0.2 and 0.3. The results for the cost-effectiveness analysis are presented in Figure 3.

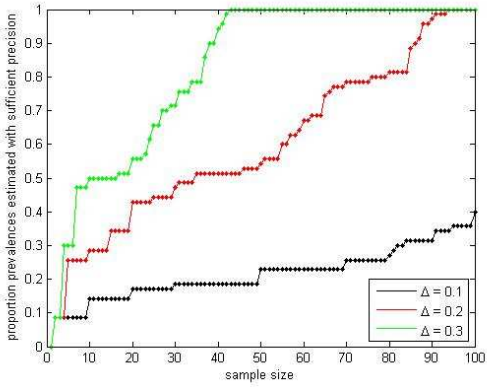

Figure 2. Fraction of prevalences estimated with sufficient precision for different widths of the 95% CI as function of sample size.

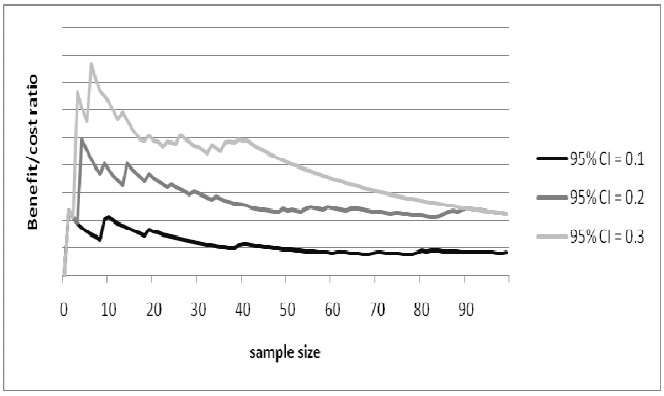

Figure 3. Cost-effectiveness.

4. DISCUSSION

To our knowledge, this study is one of a kind in exploring and analyzing sample size for herd level antimicrobial resistance profile determination in broiler chickens. As a new approach to the problem, a bootstrapping based method was used to assess both within and between animal variability. Results show that focus should be on the number of animals per flock rather than on the number of isolates being tested within one animal. The proportion of prevalences within a herd profile that can be estimated with sufficient precision was determined as a way to globalize the results for the whole range of antimicrobials tested. This proportion allows us to predict or check the accuracy of a given sample size. Because of the financial constraints research often has to deal with, sample size was submitted to a cost-effectiveness analysis. An optimal sample size should provide a good compromise between the epidemiological and economical concerns. A good sample size in this case would be 27. This is the most cost-effective sample size that will allow estimating 70% of the prevalences within a 0.3 confidence interval.

5. AKNOWLEDGEMENTS

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HEIFER AND QUARTER CHARACTERISTICS ASSOCIATED WITH PERIPARTURIENT BLOOD AND MILK NEUTROPHIL VIABILITY

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Increased blood and milk polymorphonuclear neutrophilic leukocyte (PMN) apoptosis around parturition can partly explain the high prevalence of intramammary infections (IMI) in fresh dairy heifers. Neutrophil apoptosis in blood approximately one week before expected calving date and in blood and milk at one to four days in milk was determined using flow cytometry. Information on heifer and quarter characteristics was collected before calving and in early lactation. Data were analyzed using multivariable, multilevel regression analysis.
Supplementation of minerals/vitamins prior to calving was associated with less blood and milk PMN apoptosis around calving. Both blood and milk PMN apoptosis showed a seasonal variation with the highest proportion of apoptotic cells observed between April and June. Heifers losing body condition around calving had higher proportions of apoptotic blood PMN in early lactation. Milk PMN apoptosis was less pronounced in quarters having teat orifices colonized with non-*aureus* staphylococci. The variation in PMN viability mainly resided at the heifer and quarter level.

1. INTRODUCTION

A high proportion of heifers freshens with one or more subclinically infected quarters whereas clinical mastitis is not uncommon either (5). The impact of subclinical intramammary infections (IMI) on the heifers' future performances is most likely related to the persistence of the infection when milk production has started (11). Some heifers eliminate IMI at calving within a short time span whereas others do not. This difference is due to variation in the pathogen virulence and its capability of persisting, but also due to the immune status of the host (15). The available number of polymorphonuclear neutrophilic leukocytes (PMNs) and their functionality contribute to a considerable extent to the first line of immune defence of the mammary gland (13). Differences in the blood and milk PMN quality could therefore, at least partly, explain why an IMI occurs and why it persists or not in a specific heifer/quarter rather than in others. After all, apoptosis of bovine PMNs is closely related to their activity (8).

The aims of the present study were (1) to identify heifer and quarter characteristics associated with periparturient blood and milk PMN viability, and (2) to determine the contribution of herd, heifer and quarter to the total variance of blood and milk PMN viability.

2. MATERIALS AND METHODS

2.1. Herds, animals and data

Nineteen dairy herds were randomly selected from a database of the Flemish Cattle Breeding Association

(Oosterzele, Belgium) including all dairy herds (n = 241) within the vicinity (radius of 30 km) of the Faculty of Veterinary Medicine. Herds treating heifers with antibiotics before calving were excluded. Eighty-two clinically healthy dairy heifers (on average 4 heifers per herd, ranging from 1 to 7) were included in the study.

As cell apoptosis equals a lower viability and as apoptosis is closely related to PMN activity (8), blood and milk PMN quality was expressed by the proportion of apoptotic PMNs. Blood PMN apoptosis was determined at approximately one week before expected calving date and at 1 to 4 days in milk (DIM), whereas milk PMN apoptosis was determined on quarter foremilk samples at 1 to 4 days DIM. Blood and milk PMN apoptosis were quantified flow cytometrically as described (12).

Body condition and cleanliness of the heifer were scored visually based on published scoring systems (4, 7) at approximately one week before expected calving date. At the same time, the presence of udder oedema, teat end lesions and teat skin lesions were recorded. Also, teat orifices of each heifer were sampled to determine whether colonization with non-*aureus* staphylococci was present[2]. Body condition was scored again at 5 to 8 DIM. Quarter milk samples taken at 1 to 4 DIM were cultured as previously described (10). Information on the age at calving, season of calving, supplementation with minerals/vitamins, admittance to pasture before calving, and contact with lactating cows before calving was also collected.

2.2. Statistical analysis

A square-root transformation was used to normalize the data. The regression-model building process involved several steps as described by De Vliegher et al. (2004) (3) using MlwiN 2.02 (Centre for Multilevel Modelling, Bristol, UK). The proportion of variance in the dependent variables occurring at the different levels was calculated by fitting a two-level (blood PMN) and three-level null model (milk PMN), respectively.

3. RESULTS

The proportion of apoptotic blood and milk PMN at the herd, heifer and quarter level is presented in Table 1.
Overall, blood and milk PMN apoptosis around calving showed a seasonal variation with the highest number of apoptotic PMNs occurring in April to June. Apoptosis of blood PMNs in the first days after calving was more pronounced in heifers losing condition (threshold of 0.25 points on a 5-points scale) around calving compared to heifers not losing condition during that period. Supplementation of minerals/vitamins before calving was significantly related with a lower proportion of apoptotic blood PMNs before calving and a lower proportion of apoptotic milk PMNs after calving. Overall, 71.4% and 98.4% of the variation in the proportion of apoptotic blood PMNs before and after calving, respectively, occurred at the heifer level. For the proportion of apoptotic milk PMNs, 8.8%, 45.7% and 45.5% of the variation resided at the herd, heifer and quarter level, respectively.

4. DISCUSSION AND CONCLUSIONS

Some practical heifer characteristics associated with the blood and milk PMN apoptosis around calving were revealed. Heifers on Belgian dairy herds should freshen as much as possible in autumn and winter time as then their blood and milk PMN quality around calving are expected to be at highest level. In Flanders, heifers calving during these months were indeed at a higher risk to suffer from an elevated somatic cell count in early lactation (3). Supplementation with minerals and vitamins has a beneficial impact on the immune cell activity and the udder health in multiparous cows, but the effect has hardly ever been studied for heifers (9). In the present study, blood PMN apoptosis before calving and milk PMN apoptosis after calving were less pronounced in supplemented heifers, substantiating the role of e.g. vitamin E and selenium. Body condition loss around calving can cause an increase in the non-esterified fatty acids and ketone bodies as from calving to approximately 10 days in milk (1) which may negatively affect the bovine blood PMN viability (6, 14). The variation in PMN viability mainly resides at the heifer and quarter level indicating that measures to optimize the innate immunity around calving or new studies should focus at these levels rather than at the herd level.

Table 1: Descriptive statistics of the proportion of apoptotic blood and milk PMN around calving of 82 dairy heifers from 19 dairy herds in Flanders (Belgium).

Data	N	Mean	Median	IQR[2]
Apoptotic blood PMN[1] before calving (%)				
Herd	19	20.3	18.2	11.4 - 26.7
Heifer	82	20.7	16.7	7.7 - 30.3
Quarter	328	…	…	…
Apoptotic blood PMN after calving (%)				
Herd	19	19.7	19.8	13.4 - 24.6
Heifer	82	19.8	14.8	8.3 - 26.0
Quarter	328	…	…	…
Apoptotic milk PMN (%)				
Herd	19	22.6	20.9	18.2 - 25.0
Heifer	82	23.4	21.0	13.9 - 31.6
Quarter	328	24.9	21.9	14.1 - 34.4

[1]Polymorphonuclear neutrophilic leukocytes
[2]Interquartile range

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COAGULASE-NEGATIVE STAPHYLOCOCCI IN BOVINE MILK – A MATTER OF LESSER CONCERN?
- PRESENTATION OF ONGOING STUDY -

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1. INTRODUCTION

In many European dairy farms that have adopted the 10-point mastitis prevention program, coagulase-negative staphylococci (CNS) have become the predominant pathogens found in milk samples from cows with or without elevated somatic cell counts (SCC). Although historically considered to be minor pathogens, CNS are present in milk samples from clinical mastitis and can cause a raise in SCC. On the other hand, CNS appear to be protective commensals on teat apices, and CNS infected cows seem to produce slightly more than culture negative cows. These facts suggest that 'CNS' is a heterogeneous group of bacteria, of which some are presumably clinically important and others are not. Epidemiological studies to elucidate their importance have already been conducted using phenotypic identification methods which lack accuracy, and this could add to the confusion about the relative importance of CNS.
In a first study, we have updated and evaluated tRNA-intergenic spacer PCR (tDNA-PCR) in combination with capillary electrophoresis for species identification of CNS from bovine milk and teat apices (3). This molecular technique is low-cost and user-friendly, and is therefore a useful tool in large field studies. The aim of the present work was to conduct a longitudinal and a cross-sectional field study, in which the importance and epidemiology of CNS species could be elucidated using molecular species identification and strain typing.

2. MATERIALS AND METHODS

2.1. Study design
2.1.1. Longitudinal study
Three well-managed Flemish dairy herds were selected and within each farm 25 cows were randomly selected (blocked for parity). Milk samples are collected monthly during a twelve month period. Bacteriological culture and SCC determination at the quarter level is performed. Additional milk samples are taken by the farmer at special events, i.e. when the cow has clinical mastitis, at drying-off, at calving, and at culling. Relevant information, e.g. body condition score, milk production, parity, teat skin and apex condition, presence of teat lesions … is gathered monthly in order to determine risk factors at the cow and quarter level for infection with the biologically most relevant CNS species.
2.1.2. Cross-sectional study
Once on each of the aforementioned herds, swabs are taken from the teat apices of six of the selected cows, from the milking machine unit liners, and from the milkers' hands. This is done in order to find out the relative contribution of the milking process in the epidemiology of CNS intramammary infection, and which niche (milk or teat apices) every CNS species (and strain of the most relevant species) prefers.

2.2. Sample analysis
2.2.1. Milk samples
Standard bacteriological culture as described by the National Mastitis Council (2) is performed on the milk samples. In addition, CNS are selected for species identification when there are 5 or more morphologically identical colony forming units per ml milk in a specific quarter.
2.2.2. Swabs
Swabs are plated on Colombia blood agar directly after sampling and interpreted after 24h of aerobic incubation (37°C). If 5 or more morphologically identical colony forming units per ml are present, a CNS isolate is selected for species identification.

2.2.3. CNS isolates

Of all selected CNS isolates, tDNA-PCR is performed as described (3) for species level identification. Based on the results of the species level analysis, a decision of which CNS species is biologically most relevant (i.e. cause of clinical mastitis, raise of SCC, protective against infection with major mastitis pathogen), will be made. Of this species, strain typing using random amplification of polymorphic DNA (RAPD) (1) will be carried out.

3. CONCLUSIONS TO BE DRAWN

Epidemiological features of intramammary infections with CNS on species and strain level (for the biologically most relevant species), and the share of the milker and milking machine in the transmission of CNS will be revealed. There will be an indication of possible risk factors on cow and quarter level of the biologically most relevant CNS species. This will help to formulate preventive strategies or therapeutic approaches against CNS infections, if at all necessary.

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FAECAL SAMPLING UNDERESTIMATES THE PREVALENCE OF *SALMONELLA* IN BELGIAN LAYING HENS

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Abstract

In all EU member states, *Salmonella* monitoring in poultry flocks is obligatory. In these monitoring programs a limited number of pooled faeces and / or dust samples are collected to determine whether *Salmonella* is present in the flocks or not. In this paper, a comparison is made between different sampling procedures for the assessment of the between and within-flock prevalence of *Salmonella* in laying hens. In total, 30 farms were sampled. Using a comparable sampling methodology as in the official surveillance programs, *Salmonella* could not be detected in any of the flocks. After transportation of the hens to the laboratory and subsequent analysis of cloacal swabs and caecal contents, *Salmonella* was detected in laying hens from 6 out of 30 farms. Based on the results of this study, it can be expected that, depending on the sampling procedure, different estimates of the prevalence of *Salmonella* can be obtained.

1. Introduction

In the European Union (EU), *Salmonella* is currently the second most important food-borne pathogen (European Food Safety Authority, 2007). Outbreaks of human salmonellosis in the EU are predominantly caused by *Salmonella* Enteritidis and Typhimurium (European Food Safety Authority, 2007). Since many years, *Salmonella* Enteritidis is the main cause of human salmonellosis, both in Europe and in North-America, and this is mainly due to consumption of contaminated eggs (Angulo and Swerdlow, 1998; Delmas et al., 2006; EFSA, 2006).
The EU baseline study on the prevalence of *Salmonella* in laying hens, carried out in 2004-2005, has demonstrated a variable level of infection of laying hen holdings in the different EU member states. The European Regulation No. 2160/2003 makes strict sampling schemes mandatory in the EU member states in order to provide follow-up data on the level of flock contamination. It is unclear however whether these sampling schemes are able to detect low *Salmonella* contamination levels and therefore provides accurate follow-up data on the level of laying hen holding contamination in the EU. The aim of the study described below is to evaluate whether a more intensive sampling methodology results in different estimates of the within and between flock prevalence in commercial layer flocks.

2. Material and Methods

2.1 Selection of the sampled farms

Flocks were selected based upon a list of contact addresses of registered laying hen farms provided by the official Belgian Identification & Registration authorities. The only inclusion criterion used was the flock size (>1000 hens).

The farms which were in the last month of the production cycle were contacted by telephone. Participation was voluntary. All of the contacted farms volunteered to participate. In total 30 flocks from 30 different farms were sampled, comprising conventional battery cage flocks and flocks housed in alternative housing systems. The size of the selected flocks varied between 3500 and 29000 hens. Twenty-nine of the sampled flocks were vaccinated against *Salmonella* and all of them were screened negative for *Salmonella* by the official monitoring program. Participating laying hen farms were sampled one week prior to depopulation.

2.2 Number and sample type taken

On-farm sampling consisted of 5 pooled faeces samples (each weighing 250 gr), 1 mixed dust sample and 40 cloaca swabs of 40 randomly selected laying hens. Gloves were changed in between collection of each pooled faeces sample and the mixed dust sample. The cloaca swabs were taken from hens that could be caught without causing too much agitation. Care was taken that hens were selected evenly throughout the house. Bacteriological analysis started within 12 hours post-sampling. From each flock, 100 hens were caught and placed in cleaned and disinfected transport boxes. The boxes containing the hens were transported to the Faculty of Veterinary Medicine of Ghent University in a cleaned and disinfected van. Transportation time ranged from 30 to 90 minutes. All possible hygienic measures were taken during transportation of the hens to avoid contamination of the hens by the environment. Immediately after arrival, all hens were labeled and a cloaca swab of each hen was taken in the same way as described above. After sampling, each hen was euthanized. Subsequently, the hens were necropsied and both caeca were aseptically removed. Both caeca of each hen were homogenized and pooled for further processing. Bacteriological analysis of all samples started on the day of sampling.

Bacteriological analysis of samples

All samples were analyzed using a modification of ISO 6579:2002, as recommended by the Community Reference Laboratory for *Salmonella* in Bilthoven, The Netherlands. Serotyping of *Salmonella*-isolates according to the Kauffmann-White scheme was performed at the Scientific Institute of Public Health (Brussels, Belgium).

3. Results

A detailed overview of the bacteriological analysis of samples is presented in Table 1. *Salmonella* could not be detected in any of the pooled faeces samples, 40 cloacal swabs and the mixed dust samples. After transportation of the animals, *Salmonella* was detected in laying hens of 6 out of 30 farms, both in cloaca swabs and in the caeca. In positive flocks, estimations of the within-flock prevalence differed depending on the sample type. The within-flock prevalence determined using cloacal swabs was always below 4%, whereas the within-flock prevalence based on the bacteriological examination of the caeca varied between 5 and 14%. In all hens that were positive for *Salmonella* after bacteriological analysis of cloaca swabs, the bacterium was also found in the caecal samples.

Table 1: Overview of the bacteriological analysis of samples taken in 6 positive laying hen flocks (% of positive samples per sample type taken)

Farm	Bacteriological analysis					
	Pooled faeces	Mixed dust	Cloacal swabs	Cloacal swabs after transport	Caeca	Phage types of *S.* Enteritidis
Number of samples/flock	5	1	40	100	100	
1	0	0	0	**3 (0 - 6.33)**	**6 (1.37 - 10.63)**	**PT 1**
2	0	0	0	**3 (0 - 6.19)**	**10 (4.39 - 15.61)**	**PT 21**
3	0	0	0	**1 (0 - 2.94)**	**14 (7.24 - 20.76)**	**PT 12**
4	0	0	0	**4 (0.18 - 7.82)**	**7 (2.02 - 11.98)**	**PT 1, PT 35**
5	0	0	0	**2 (0 - 4.74)**	**5 (0.74 - 9.26)**	**PT 11**
6	0	0	0	**2 (0 -4.70)**	**8 (2.76 – 13.24)**	***S.* Typh.**

4. Discussion

Bacteriological analyses of faecal samples as requested in European Regulation No. 2160/2003 most likely under-estimate the actual prevalence of *Salmonella* in laying hen flocks. Indeed, all 30 flocks were screened negative for

Salmonella by the official monitoring program, using analysis of faecal samples. Even the increased on-farm sampling, combining bacteriological analysis of 40 cloaca swabs, 1 mixed dust sample and 5 pooled faeces samples, did not result in the detection of *Salmonella.* After transportation, *Salmonella* Enteritidis was found in laying hens of 6 farms. Both cloacal swabs and caecal homogenates were found positive in a low number of sampled animals. Based on analysis of these samples the between-flock prevalence can be estimated at 20% (6.7 – 33.3). This is little below the between-holding prevalence estimate of *Salmonella* Enteritidis for Belgium (27.7%) determined during the EU-wide baseline study performed in 2004-2005 at a time when the vast majority of laying hens in Belgium were not vaccinated (EFSA, 2006). In the EU-wide baseline study a flock was identified as being *Salmonella* positive if at least one out of the 5 pooled faeces or 2 dust samples was positive. When using the same decision criterion in this study we did not find any flock positive which would result in an estimated between-flock prevalence of 0%. Also the estimates of the within-flock prevalence in the positive flocks were largely depending upon the sample types considered. The results of the analysis of individual cloaca swabs taken at the farm were all negative, whereas in the infected flocks a limited number of cloacal swabs taken after transport were positive (within-flock prevalence estimate never exceeded 4 %). In the flocks where some cloaca swabs were found positive, analysis of caecal homogenates yielded more *Salmonella* positive animals (estimated within-flock prevalence between 5 and 14%). These differences may be explained by the intermittent excretion of *Salmonella* by infected animals (Van Immerseel et al., 2004) and the fact that stress, caused by the transport, may make hens go from a 'carrier' state to a 'shedding' state. Our data suggest that on farms which are 'apparently *Salmonella*-free', a relatively large proportion of the hens may still carry the pathogen without shedding. Therefore, the numbers of infected flocks based on the official monitoring programs are most likely an underestimation of the true number of flocks in which *Salmonella* is still present. The actual public health risk of the flocks, carrying low levels of *Salmonella* that are only detectable using intensified sampling procedures is unclear. Defining a flock as positive based on the finding of at least one positive sample in whatever bacteriological sampling methodology used is a very crude way of categorizing flocks and does not take into account any estimation of the infection pressure in the flock. The results of this study do suggest that, even in those flocks where *Salmonella* was found, the infection pressure at the time of sampling was probably low. This low infection pressure can probably be attributed to the combination of vaccination and hygienic measures. This raises the question whether the sampling methodology as it is currently used in the *Salmonella* monitoring programs of several EU member states is accurate enough to detect *Salmonella* in low prevalence flocks. In conclusion, it is clear that depending on the sampling procedure different estimates of the between- and within-flock prevalence of *Salmonella* can be obtained. Analysis of faecal samples clearly under-estimates the actual prevalence of *Salmonella* in laying hen flocks.

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BACKYARD POULTRY HOLDINGS IN BELGIUM: AN APPROACH OF A BIOSECURITY SURVEY, IN ORDER TO EVALUATE THE RISK OF SPREADING HPAI

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1. INTRODUCTION

Outbreaks of the newly emerging H5N1 strain of the Highly Pathogenic Avian Influenza (HPAI) have occurred at irregular intervals on all continents (OIE 2008). Measures put up to prevent further spread of the disease levy a heavy toll. Due to its potentially devastating economic impact, there is an urgent need for each country to scientifically evaluate several possible control strategies to fight potential epidemics. Yet, there has been little analysis on which type of poultry holdings are at higher risk of spreading HPAI. Therefore, this study gathers data on the biosecurity of Belgian poultry holdings, to assess the relative risk of HPAI spread between poultry flocks.

To this end, the wide spread practice of smallholder backyard poultry keeping poses a unique challenge as such residences are not required to register their birds. Although government's attention has already been directed at the potential for backyard poultry flocks to be a risk factor for AI outbreaks and further spread, features of their environments that could affect risks of spreading the virus have not yet been described. Therefore, this study is more specifically conducted to better describe Belgian backyard poultry holdings, regarding population density, characteristics and dynamics. The information generated by this study design will be useful to assess the potential AI-related risk that backyard flocks pose to the poultry industry and possible control operations.

2. METHODOLOGY

2.1 Selection

This study will estimate the density and gather information on biosecurity from backyard poultry holdings in close proximity to commercial operations, based on the hypotheses that greater number of horizontal contacts between backyard poultry holdings and commercial poultry operations exist with nearer distances.

A complete list of Belgian commercial poultry operations exists, from an identification and registration database of animals (SANITEL-Poultry, 2005). This database defines a poultry holding as commercial, when more than 200 birds are kept (other than running birds) or a minimum of tree running birds (ostrich, emu, nandu). For the purpose of this study, backyard poultry holdings are defined as residences with fewer than 200 birds or les then tree running birds, kept outside the family house.

80 commercial poultry operations are selected from the Sanitel database, corresponding to a sampling fraction of 4.4%. Selection was done according to a stratified random-sampling technique, using proportional sampling to ensure: (a) appropriate representation of operations across different animal species (chicken, duck, pigeon, pheasant, turkey, quail, guinea fowl and partridge), (b) appropriate representation of operations of different types (rearing multiplier hens, multiplier hens, rearing layer hens, layer hens, broilers/turkeys/ducks – meat-bird production,

selection and show), and (c) a good geographic spread of operations across the Belgian provinces. In addition, all recognized hatcheries, 32 in total, are selected.

After the H7N7 epidemic (2003) in the Netherlands and Belgium, the Federal Agency for the Safety of the Food Chain (FASFC) requested the communities of Belgium (early 2006) to list up all inhabitants owning poultry (by address, kind of birds and number), as a precautionary measure. We contacted 208 different communities which surround the 80 selected commercial poultry operations and 32 hatcheries, and asked whether they want to share their inventory, made in 2006. To date, 60 communities out of the 208 (29%) were able and willing to share their inventory.

Subsequently, all received addresses are geo-coded, as to select all backyard poultry operations within a 3 km radius circle around each selected commercial operation. For those communities from which we do not receive an inventory, equally a 3km radius will be drawn around the selected operations and the coordinates of all residences located within this radius will be generated. Sample size for each circle will then be based on the residence density and the average prevalence of backyard poultry holdings within a 3km radius of commercial poultry operations from those communities with an inventory (considering 95% confidence level and desired accuracy of 5%). According to this sample size, residences will then be selected at random.

2.2 Questionnaire

The selected backyard poultry holdings will receive a letter, introducing the purpose of this research and will be kindly invited to fill in the questionnaire online by giving the web address to the online survey. A preliminary draft was pre-tested on 8 hobby poultry farmers. The revised questionnaire is a standardised semi-closed questionnaire, consisting of 9 pages. To overcome a limited response, an incentive will be given: each participant will receive a voucher worth €2 when buying a bag of 25kg AVEVE poultry feed. In addition, 50 AVEVE gift vouchers worth €10 will be put up for raffle.

2.3 Data processing

Collected data will be coded into a database (Microsoft Excel, 2007). Preliminary analysis and descriptive results will be made in SPSS 16 software. Validation checks will be performed to identify numeric extremes, improper categorical responses, skip patterns not followed, and relational checks.

3. EXPECTED OUTCOMES

Since Backyard poultry holdings are not subjected to specific measures to enhance biosecurity, this study may identify a lack of biosecurity practices. However, since the risk of infection for an individual farm is the combined result of three major components: (1) isolation, (2) traffic control and (3) sanitation, this study will pay attention at each specific component and make an overall balance. One of these components may outweigh the other, thereby changing the risk of introducing or spreading Avian Influenza.

RISK FACTORS FOR STILLBORN PIGLETS

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Abstract
The aim of this study was to identify risk factors for stillborn piglets at herd and sow level in commercial swine herds. Data were collected by means of a written questionnaire and a longitudinal field study. Significant risk factors described are: (a) type of breed, (b) high temperature (≥ 22°C) in the farrowing unit, (c) method of washing the sows before parturition, (d) method of performing supervision of farrowing, (e) herds with clinical PRRSV problems, (f) increasing litter size and parity, (g) poor body condition at parturition. Also, significant interactions between breed and washing or supervision of sows at parturition and between temperature in the farrowing unit at parturition and washing sows were found.

1. INTRODUCTION

In the past many efforts were made by pig producers and breeders to improve sow productivity through genetic selection for increased litter size (5, 11). This evolution has encouraged swine producers to introduce commercial crossbred sows into their sow population. However, concurrently with the selection for litter size, also the number of stillborn piglets has increased, limiting the overall effectiveness of selection for increased litter size (2, 3). Several factors have been associated with the occurrence of stillbirths. Most of these risk factors were related to sow characteristics (1, 2, 6, 9) and/or piglet characteristics (2, 8). Only a few studies reported management related factors influencing the occurrence of stillbirth. In these studies supervision of farrowing in particular was identified as an influencing factor (6, 9). To our knowledge, research on possible risk factors for stillbirths at herd level, including a large range of management factors, is lacking. Therefore, the aim of this study was to identify risk factors for stillborn piglets in commercial swine herds in Belgium 1) at herd level based on a detailed written questionnaire and 2) at herd and sow level by means of a longitudinal field study.

2. MATERIAL AND METHODS

2.1 Survey

In February 2007, a questionnaire was sent by conventional mail to 250 randomly selected swine herds (>150 sows). A reminder was sent after two months to the herds that had not yet responded. Finally, non-responding herds were contacted by phone and asked whether they where yet willing to cooperate or for the reasons of non-response. The survey was closed in September 2007. The questionnaire consisted of 14 pages and contained semi-open questions directly and indirectly related to stillborn piglets. The relationship between risk factors and stillbirths was evaluated with a generalized linear effects model with the percentage of stillborn piglets as outcome variable. All factors with a $P < 0.05$ were retained in the final model. Finally, all two-way interactions were evaluated.

2.2 Longitudinal field study

A longitudinal study was conducted in 21 commercial swine herds (> 150 sows). In total, 545 sows were included in the study. The study was conducted between March and September 2007. During the first herd visit, a questionnaire was filled in to collect general information regarding potential risk factors for stillborn piglets at herd level. The herd level data pertained to herd size, a number of management practices and PRRSV infection status of the herd. The sow level data pertained to type of breed, parity, litter size, number of piglets born alive and stillborn piglets, occurrence of interventions such as farrowing induction, vaginal palpation and oxytocin administration. Body

condition was assessed using back fat measurements with a 5 MHz linear probe ultrasound (Tringa 50 S, Esaote Pie Medical Tringa Linear, Maastricht, The Netherlands). The back fat at the last rib of all sows was measured at different times during the reproductive cycle: 3 weeks before farrowing, at farrowing and at weaning. Statistical analyses were performed with a logistic regression model. After univariable analysis, a multivariable model was build accounting for herd as random effect and the clustering of sow in the herd (MLWin). The percentage of stillborn piglets defined as a categorical variable (≤ 7% and > 7%) was used as dependent variable.

3. RESULTS

2.1 Survey

In total, 111 questionnaire forms were successfully returned by post. The response rate was 44.4 %. From these responders, four used another definition of stillborn piglets, leaving 107 farms for analysis. The reported frequency of stillbirth was on average 7.45% (S.D. 2.83%). Table 1 shows the final result of the general linear mixed model.

Table 1: Results of a multivariable analysis of risk factors for stillborn piglets based on 107 swine herds (Adjusted R squared = 0.62).

Risk factors and their interactions	P-value
Type of breed (1)	0.000
Washing sows before entering the farrowing unit (2)	0.010
Supervision of farrowing (3)	0.006
Temperature of farrowing unit at parturition (4)	0.004
(1)*(2)	0.002
(1)*(3)	0.026
(2)*(4)	0.009

Type of breed used on a farm determined to a great extent the risk for stillborn piglets. A high temperature in the farrowing unit (≥ 22°C compared to < 22°C) caused significant more stillbirths (8.54% vs. 7.28%, respectively). Washing the sows with warm water before farrowing resulted in less stillbirths (5.8%) than no washing at all (7.7%) ($p<0.01$) or washing with cold water (7.0%) ($p<0.26$). When the supervision of farrowing was performed occasionally, significantly more stillbirths (8.1%) were observed in comparison with no attending to farrowing (6.5%) ($p<0.01$) or frequent supervision of farrowing (6.94%) ($p<0.01$). Significant interactions between breed and washing or supervision of sows at parturition and between temperature in the farrowing unit at parturition and way of washing sows were found.

2.2 Longitudinal field study

The mean % of stillborn piglets in the field study was 8.1 ± 12.8. Fifty nine percent of the sows had less than 7% stillborn piglets, whereas 41% had more than 7% stillborn piglets. Breed of the sows was also a significant risk factor for stillborn piglets ($p<0.01$). When compared with Crossbred landrace sows (n= 98), sows of breed A (n= 28) and breed B (n=53) had 3.5 and 2.6 times higher odds for stillbirth, respectively. Sows in herds with clinical PRRSV problems (n=2) had 3.2 times higher odds of stillbirth occurrence compared to sows from herds without clinical PRRSV problems ($p<0.01$). Both for increasing parity (min 1; max 18) ($p=0.02$) and litter size (min 2; max 22) ($p<0.01$) the odds of stillborn piglets was 1.1-times higher for each unity increase. Sows with a poor condition at parturition (≤ 16 mm) had 2.7 times higher odds of stillbirth compared to sows in good condition (16-22.9 mm) ($p<0.01$). The odds for stillborn piglets of sows with ≥ 23 mm back fat at parturition did not differ significantly from sows in good condition (16-22.9 mm). No significant interactions between the risk factors were observed.

3. DISCUSSION

The difference in % of stillbirths between breed can be explained by inherent breed properties. Different studies provided evidence for a genetic influence on stillbirth and reported an heritability between 0.02-0.05 for number of stillborn piglets (3, 4). It was mentioned that purebred lines have significantly more stillbirths per litter than crossbred lines (7). In the present study, all sows were crossbred lines and still there was a significant difference in % of stillbirths between these crossbred lines. Also, the fact that both breed and litter size remained significant in the multivariable regression model indicates that differences in average litter size of the different breeds can't solitarily explain the effect.

The ambient temperature and the supervision of farrowing are significant risk factors for stillbirths. Elevated temperature around farrowing can induce stress (10). Although it has been shown that supervision at farrowing can

reduce stillbirths (6, 9), it appeared the supervision as performed in present study may have disturbed and possibly stressed the sow during farrowing. It has been demonstrated that acute stress during the process of delivery reduces the plasma oxytocin level, leading to prolonged farrowing duration and increased number of stillbirths (12). When the sows were washed with warm water before parturition, significantly less stillbirths were observed and the % was even lowest when the temperature at parturition wasn't elevated. Further studies are necessary to elucidate the possible mechanism behind these associations.

In the present study there were also significant interactions between some management factors like washing or supervision of farrowing and type of breed. These significant interactions indicate that the effect of washing or supervision is not homogenous across the breeds. This unequal effect of specific differences of the environment, in this case management-factors like washing or supervision of sows, on different genotypes might be explained by a varying degree of sensitivity of the different breeds to the environment. It means that practices with a negative effect on the reproductive performance of certain breeds don't have the same effect on other breeds. This implicates that each breed requires specific management practices to obtain optimal productive performance.

The observation that the risk for stillbirth increases with litter size and parity is in agreement with literature (2). Sows originating from herds that were experiencing a clinical PRRSV outbreak were at significant higher risk for stillborn piglets which is logical since an increase in stillbirth was described as one the clinical signs of infected sow with PRRSV (13). The measurement of the back fat of the sows indicated that sows with a poor condition at parturition had significant higher odds of stillbirth compared to sows in good condition. Whereas sows that were too fat did not have an increased risk. This finding confirms once again the importance of sufficient condition of the sows at parturition.

In conclusion, this study clearly demonstrated that breed is a major factor involved in stillborn piglets. Some management practices before or at parturition may further reduce the number of stillborn piglets.

4. ACKNOWLEDGEMENTS

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ECONOMISCHE BETEKENIS VAN DE DIERLIJKE PRODUCTIE IN BELGIË

Piet Vanthemsche

Boerenbond

België kent gezien zijn relatief beperkte oppervlakte een intensieve landbouwproductie. De productieomstandigheden verklaren grote regionale verschillen. In Vlaanderen bestaat een grote variëteit aan teelten en veeteeltsectoren, terwijl de productie in Wallonië homogener is en zich voornamelijk toelegt op akkerbouw en rundvee. De meeste landbouwbedrijven zijn familiebedrijven die in verhouding weinig betaalde werknemers in dienst hebben. De hoge kapitaalsinbreng voor de opstart van nieuwe bedrijven is een belemmering voor vele jonge landbouwers. Daarentegen hebben veel landbouwbedrijven een modern karakter en vereist de hedendaagse landbouw een grondige kennis en opleiding. Dit resulteert in een bijkomende graad van specialisatie van de bedrijven. Maatschappelijke en andere beperkende factoren leiden echter ook tot een noodzakelijke diversificatie van de activiteiten (hoevetoerisme, zorgboerderijen, beheerscontracten, …).

Alhoewel minder dan 2 % van de beroepsbevolking actief is in de landbouw, blijft de sector enorm belangrijk voor de Belgische economie. De totale omzet van de dierlijke productiekolom werd in 2007 geraamd op ruim 19 miljard euro. De dierlijke productiekolom is op te splitsen in de sector toelevering, de veeteelt en de verwerking. Wat de veeteeltsector betreft, gaat het over de rundveehouderij, de varkenshouderij, de pluimveehouderij en de kleine herkauwers en overige hoefdierenhouderij. Voor de toelevering beschouwen we de landbouwmachines (vervaardiging, reparatie en verhuur), de veehandel, de veterinaire diensten, diensten verwant aan de veeteelt en de veevoederindustrie. De overheidsdiensten worden door gebrek aan cijfermateriaal niet opgenomen. In principe zouden hier de lonen van de volgende diensten of verenigingen ook mee opgenomen moeten worden: het Ministerie van Landbouw, de VLAM, de Mestbank, het FAVV, het DGZ-ARSIA, het CDV, en de Melkcontrolecentra. De noemer verwerking bestrijkt de slachthuizen en uitsnijderijen, de vleeswaren- en vleesconservennijverheid en de zuivel. De vermelde bedragen zijn verkregen bij de nationale bank aan de hand van de NACE-BEL codes.
Indien we stellen dat de bruto toegevoegde waarde 30% is van de omzet, krijgen we voor de hele dierlijke productiekolom van België in 2007 een bruto toegevoegde waarde van 5,8 miljard euro. Dit is 1,7% van het bruto nationaal inkomen, wetende dat dit in 2007 gelijk was aan 337 miljard.euro.

De dierlijke sector van de Vlaamse landbouw kende in 2008 een totale omzetstijging van 4 % of 190 miljoen euro. De boer van vandaag kampt echter vooral met gestegen kosten. In 2008 (voorlopige raming) betaalt een boer , in vergelijking met 2007, gemiddeld 57 % meer voor meststoffen, 24 % meer voor energie en 23 % meer voor veevoeders. Verder stegen de onderhoudskosten, het loonwerk, de gewasbeschermingsproducten en de dierenartsenkosten gemiddeld 7 à 10 % meer. Wanneer alle kosten in rekening worden gebracht schat de Boerenbond dat de kosten in 2008 gemiddeld 17,5 % hoger zullen liggen dan in 2007. De enorme stijging van de kosten weegt veel zwaarder door dan de gestegen omzet. Een voorzichtige raming stelt dat het arbeidsinkomen in Vlaanderen in 2008 49 % lager zal liggen dan in 2008. Probleem dat zich stelt is dat de landbouwers deze gestegen kosten niet kunnen doorrekenen naar de schakels verderop in de keten.

De dierlijke productie in de landbouw is een belangrijke schakel in de voedselketen en bijgevolg ook gevoelig voor allerhande problemen die zich in de diverse schakels van de keten kunnen voordoen: veevoederindustrie, zuivelindustrie, vleesnijverheid, distributie, voedselverwerkende industrie. In het recente verleden zijn er talrijke voorbeelden te noemen: dioxinecrisis, industriële contaminaties, vervuilde geneesmiddelen (IBR Nederland), melamine in melk, vleesfraude, … Alhoewel de veehouders vaak geen direct betrokken partij zijn, zijn de gevolgen op de prijsvorming van hun producten vaak enorm en langdurig.

Veehouders zijn bovendien sterk afhankelijk van het opduiken van nieuwe en epidemische dierziekten. In het verleden hebben diverse uitbraken (mond-en klauwzeer, varkenspest, aviaire influenza, …) in West-Europa aangetoond welke de desastreuze gevolgen kunnen zijn op de diergezondheid en het inkomen van de veehouder. Zo bedroeg de directe economische schade door blauwtong in 2007 naar schatting ongeveer 45 miljoen euro voor de Vlaamse schapen- en rundveehouderij en ondervonden veehouders tot ver in 2008 de gevolgen voor de diergezondheid en afzet van hun producten.

Op het individuele vlak investeren veehouders preventief en curatief in de gezondheid van hun veestapel. Aan kostenzijde zijn er de uitgaven aan dierenarts en geneesmiddelen, zowel voor curatief als preventief gebruik.
Vanuit collectief oogpunt bestaat het Sanitair Fonds. Het Fonds steunt op de principes van medefinanciering, medeverantwoordelijkheid en medebeheer door de producenten en financiert met name de tussenkomsten in het kader van de officiële dierenziektebestrijding, geregeld door de Dierengezondheidswet van 24 maart 1987. De werking van dit Fonds berust op de solidariteit tussen producenten en sectoren onderling. De solidariteit laat toe om op te treden bij uitbraken van besmettelijke dierenziekten (bv. mond-en klauwzeer of bij dreiging van aviaire influenza, varkenspest …)
Die solidariteit wordt gerealiseerd via verplichte bijdragen van alle natuurlijke of rechtspersonen die dieren houden of verhandelen. Het geld van het Fonds dient tot schadeloosstelling van de veehouders die hun dieren moeten laten slachten in het kader van aangifteplichtige ziekten zoals BSE, maar ook ter betaling van de prestaties die de erkende dierenartsen uitvoeren in het kader van de dierenziektebewaking: bloedafname voor onderzoek van brucellose en leucose of voor de tuberculinaties.
Het Fonds bouwde de voorbije jaren een totale strategische reserve op van +/- 60 miljoen euro. Jaarlijks dragen de veehouders méér dan 12 miljoen euro bij aan dit Fonds.

Tabel: Omzet dierlijke productiekolom in België (in euro)

Toelevering	**6.428.151.088**	**33%**
- Landbouwmachines	1.387.394.459	22%
- Veehandel	402.043.176	6%
- Veterinaire kosten	477.020.183	7%
- Andere dienstverlening	120.437.331	2%
- Veevoederindustrie	4.041.255.939	63%
Veeteelt	**3.184.673.018**	**17%**
- Gemengd bedrijf	1.440.202.834	45%
- Fokvarkenshouderij	391.855.902	12%
- Varkensvetmesterijen	392.409.037	13%
- Kippenkwekerijen	230.308.153	7%
- Productie van eieren	42.596.033	2%
- Overige pluimveehouderijen	34.429.577	1%
- Rundveehouderij (incl. melk)	517.376.801	16%
- Geiten-, schapen, en andere hoefdierenhouderij	134.894681	4%
Verwerking	**9.730.548.732**	**51%**
- Zuivelfabrieken en kaasmakerijen	4.125.792.217	42%
- Slachterijen	3.021.734.757	31%
- Vervaardiging van verse vleeswaren en vleesconserven (m.u.v. de vervaardiging van verse kant en klaarmaaltijden die vlees bevatten)	2.583.021.758	27%
Totaal	**19.343.372.838**	

THE ECONOMICS OF REDUCING *CAMPYLOBACTER* IN THE BELGIAN POULTRY MEAT CHAIN**,

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**Original Scientific Paper

ABSTRACT

Campylobacter infections are a serious public health problem in Belgium. Poultry meat is most likely responsible for 40% of human campylobacteriosis cases in Belgium. On a yearly basis, consumption of poultry meat causes at least 22,000 campylobacteriosis cases with a costs-of-illness of €11 million. This study aims to evaluate the efficiency, i.e. the ratio of reduced costs-of-illness on intervention costs, of various intervention measures. These measures were selected by representatives from the poultry meat sector and experts in the field. The selection comprised measures at farm level (administration of bacteriocins to feed of broilers shortly before slaughter), at the processing plant (spraying of carcasses with lactic acid or electrolyzed oxidizing (EO) water, crust-freezing or irradiation of carcasses and chicken filets at the end of the slaughterline) and at consumer level (improving kitchen hygiene and application of home-freezing). Among these measures, the decontamination of carcasses with EO water is the most efficient. Administration of bacteriocins to feed of broilers is also efficient. Irradiation is the most effective intervention, however, one of the least efficient. There seems to be less gain by trying to improve food handling in the kitchen. The cost to reach consumers is large while only a very limited fraction of the consumers is willing to change their behavior.

Key words: *Campylobacter*, chicken meat, interventions, electrolyzed oxidizing water, cost-benefit analysis

Introduction

Campylobacter is the leading cause of zoonotic enteric human infections in most developed countries *(43)*. In Belgium, *Campylobacter* enteritis is mainly caused by *Campylobacter jejuni* (80% of the isolates) and *Campylobacter coli* (12%) *(12)*. The most common clinical symptoms of campylobacteriosis are fever, abdominal pain and diarrhea occurring within 2 to 5 days after ingestion of food or water contaminated with *C. jejuni (6, 34)*. Symptoms are usually self-limiting and are resolved within a period of 3 to 10 days. The infection may lead to serious ongoing sequelae and may even be fatal. The most common complications are reactive arthritis (ReA), Guillain-Barré syndrome (GBS) and inflammatory bowel disease (IBD) *(33)*.
Handling and consumption of poultry meat have been identified as 40% of human campylobacteriosis. Several case-control studies have shown that consumption of poultry meat is one of the principal sources of infection *(10, 13, 14, 40, 43)*. Reducing the contamination of *Campylobacter* in the poultry production chain may be obtained by interventions i. at the farm and during transport, ii. at the processing plant and iii. during storage and meat preparation *(21)*. Several Quantitative Microbial Risk Assessment (QMRA) studies have been undertaken to assess the risk of human infection with *Campylobacter* upon the consumption of poultry meat and used to investigate the effect of interventions *(20, 29, 35)*.
The aim of this study is to estimate the efficiency of various intervention measures in Belgium to control *Campylobacter* in the poultry meat chain. The efficiency is defined as the ratio of reduced costs-of-illness on intervention costs. Available models for risk assessment and cost-of-illness of *Campylobacter* infections and sequelae served as input for the current calculations.

Materials and Methods

Figure 1 describes the framework for calculating the efficiency of various selected intervention measures. The year 2004 is used as reference in this economic evaluation.

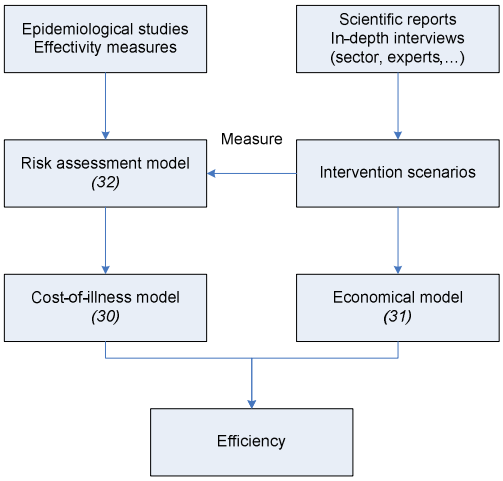

Figure 1 The framework for calculating the efficiency of various selected intervention measures

Reduced cost-of-illness. The QMRA model is based on the model developed by Hartnett *(20)* and describes the chain from farm-to-fork in a modular fashion. The modules considered are 1) rearing and transport, 2) slaughter and processing, 3) preparation and consumption and finally 4) health consequences. At each stage, the model estimates the probability that a bird/carcass/product is colonized/contaminated with campylobacters and the associated microbial levels. Both fresh and frozen whole carcasses and filets, consumed at home, were included. The original model was adapted to the Belgian situation and updated to incorporate results of recent studies *(27)*. In Belgium, only air cooling of the carcasses is used, which is assumed to have no effect on the contamination levels on the carcass. For portioning, the model described in the Campylobacter Risk Management and Assessment (CARMA) project *(29)* was used. For duration of refrigerated and frozen storage at home, own Belgian data involving 471 respondents was used *(18)*. At the preparation and consumption stage, only poor hygiene during cooking was considered, as inadequate cooking poses a much lower risk as shown by Hartnett *(20)*. Cross contamination to raw vegetables was modeled as described in the CARMA project *(29)*. This can either be from the raw meat through hand-salad contact or from raw meat via the cutting board to the salad. Consumer data was used to make the model applicable to the Belgian situation *(4)*.

Estimating the intervention costs (according to Mangen et al., 2005a). The annual total cost (*TC*) for an intervention *m* comprises the estimated annuity (*A*) of the non-recurrent costs (*NRC*) for intervention *m* and the recurrent costs (*RC*) of intervention *m*:

$$TC_m = A_m + RC_m \quad (1)$$

The NRC includes purchase costs and installation and reorganization costs and are mostly long-lasting investments (lifetime of $\geq$ 8 years). These investment costs will be depreciated over the lifetime (n) of the equipment at an interest rate (i) of 4%.

Results and Discussion

Reduced cost-of-illness. Based on epidemiological studies, the annual incidence of *Campylobacter* associated gastro-enteritis (GE) and sequelae in Belgium are calculated. It is estimated that 30 to 40% of GBS is attributable to *Campylobacter* infections and that about one out of a thousand patients with campylobacteriosis develops GBS *(28)*. The annual incidence of GBS in Belgium is 1.5 cases per 100,000 inhabitants *(2)*. Consequently, the annual incidence of *Campylobacter* associated GE cases in Belgium with a population of 10.5 million, is about 55,000 cases. Among these cases about 19,300 patients visit a general physician (GP), 345 are hospitalized and 20 cases are fatal. The latter distribution is obtained from Mangen et al. *(25)*. Only the parameter "proportion people visiting the GP" is multiplied by 1.5, since Belgian people visit the GP more frequent than Dutch people *(5)*. The annual incidence of *Campylobacter* associated GBS is estimated to be 56 cases.

Based on a Belgian 3-years prospective study, the annual incidence of Crohn's disease and ulcerative colitis is estimated at 4.5 respectively 3.6 cases per 100,000 inhabitants, giving a total of 8.1 cases of IBD per 100,000 inhabitants *(29)*. *Campylobacter* is involved in up to 6.2% of Crohn's disease and up to 3.7% of ulcerative colitis *(7)*. Accordingly, the estimated annual incidence of *Campylobacter* associated IBD in Belgium is 43 cases. The rate of ReA associated with *Campylobacter* is fairly low, ranging from 0 to 2.6% *(19, 36)*, resulting in an average of about 700 cases of *Campylobacter* associated ReA in Belgium.

When assessing the intervention measures, many factors should be taken into account such as effectivity, levels of microbial contamination, potential for introducing other food safety hazards, impact on the environment, effect on sensory properties and quality of the product, feasibility, and consumer perception *(3)*. Information and

data for the selection of the various intervention measures and estimates of effectivity are obtained from scientific reports, in depth interviews with representatives from the Belgian poultry sector and from experts in the field. A summary of the intervention measures studied together with their effectivity (pessimistic, most likely and optimistic scenario) is presented in Table 1.

Table 1. Effectivity (pessimistic *(P)*, most likely *(ML)* and optimistic *(O)* scenario) of the various selected intervention measures

Stage	Intervention	Effectivity			References	reduction in campylobacteriosis cases (%)		
		P	ML	O		P	ML	O
Farm	Bacteriocins	5.3[a]	6[a]	6.9[a]	*(38, 39)*	100	100	100
Processing plant	Lactic acid	0.3[a]	1.3[a]	2[a]	*(37, 41)*	0	38	72
	EO water	1.1[a]	2.3[a]	3[a]	*(23, 30)*	28	80	91
	Crust-freezing	0.4[a]	1.1[a]	1.7[a]	*(9)*	32	61	82
	Irradiation	4.7[a]	10.5[a]	20.8[a]	*(9, 15, 16, 22)*	99.8	100	100
Consumer	Kitchen hygiene	0%[b]	3%[b]	7%[b]	*(11)*	0	3	9
	Home-freezing	0%[b] (0.13[a])	3%[b] (0.24[a])	7%[b] (0.46[a])	*(11, 37)*	0	6	9

[a] *log reduction*
[b] *change in behavior after one communication campaign*

At consumer level two interventions are analyzed, i.e. mass-media campaigns to improve kitchen hygiene and home-freezing of poultry meat. As only a very limited fraction of the consumers is willing to change their behavior, these have a limited reduction in number of campylobacteriosis cases.
The cost-of-illness associated with *Campylobacter* infections and sequelae in Belgium is shown in Table 2.

Table 2. Estimated cost-of-illness associated with gastro-enteritis (GE), Guillain-Barré syndrome (GBS), inflammatory bowel disease (IBD) and reactive arthritis (ReA)

Description	Cases/year	Cost/case (€)	Total costs (€ thousand)	% of the costs-of-illness
GE case not visiting GP	35,445	126	4,466	16,3
GE case visiting GP only	19,314	485	9,367	34,3
Hospitalized GE case	345	2,661	918	3,4
Fatal GE case	21	1,774	37	0,135
GBS	56	67,852	3,799	13,9
IBD	43	202,385	8,712	31,9
ReA	714	23	16	0,059
Total costs			27,317	100

As mentioned before 40% of these costs is attributable to the consumption of poultry meat, resulting in a yearly cost-of-illness of €10.9 million. The reduced cost-of-illness of the different intervention measures is obtained by multiplying this value with the percentage risk reduction of the intervention measure. The results are presented in Table 3.

Table 3. Estimated reduced cost-of-illness and efficiency for the different interventions

Stage	Intervention	Reduced cost-of-illness: X (million €/year)		Treatment costs: Y (million €/year)			Efficiency[1]	
		Belgian cons: X_1	All cons: X_2	Farmer	Industry	Government	X_1/Y	X_2/Y
Farm	Bacteriocins	10.9	15.8	5.2	-	-	**2.1**	**3.1**
Processing plant	Lactic acid	4.2	6.0	-	1.0	-	**4.1**	**5.9**
	EO water	8.7	12.7	-	0.5	-	**17.7**	**25.6**
	Crust-freezing	6.7	9.7	-	18.0	-	0.4	0.5
	Irradiation	10.9	15.8	-	35.1	-	0.3	0.5
Consumer	Kitchen hygiene	0.3	-	-	-	1.9	0.2	-
	Home-freezing	0.7	-	-	-	1.9	0.4	-

[1] *The intervention measure is efficient if the ratio > 1 (in bold)*

Intervention costs. One potential approach to control *Campylobacter* colonization at the farm level is the administration of bacteriocins to the feed at a dose of 250 mg/kg, 1 to 3 days before slaughter *(8, 38, 39)*. With a daily feed utilization of 100 g per broiler *(42)*, the cost ranges between €2.6 million (1 day administration) and €7.8 million (3 day administration).

At the processing plant, several interventions were studied: acid decontamination of carcasses, decontamination of carcasses with EO water, crust-freezing and irradiation**.** For acid decontamination of carcasses a spraying device is installed immediately at the end of the slaughter line, before entering the chilling tunnel. According to the suppliers prescription, each carcass needs to be sprayed with 50 ml lactic acid solution *(32)*, but practices in the slaughterhouse show a surplus of 20%, i.e. 60 ml/carcass. With a total of 38 slaughterlines, the sector cost is about € 1.02 million (range € 0.98 - 1.06 million).

The EO-water generator and spraying device would also be installed before entry to the chilling tunnel. The sector cost is about € 0.49 million (range € 0.45 - 0.54 million).

In the slaughterhouse, crust-freezing would be applied after cooling the carcasses and possible portioning. The purchase and installation costs are estimated between € 1.5 and € 3.0 million per piece of equipment (Airproducts, UK). The sector cost is about € 18 million (range € 13.7 – 22.3 million).

Since the purchase cost per piece of equipment used for irradiation is rather high (several millions), it is assumed that gamma irradiation would not be applied in the processing plant itself built instead by a specialized company. Isotron is Europe's leading provider of contract sterilization services. The closest site is in Etten-Leur (Breda, The Netherlands). The sector cost is about € 35.1 million (range € 20.6 - 49.6 million).

At the consumer level, communication campaigns can be applied. According to Allos *(1)* and Peterson *(31)*, poultry meat should be adequately cooked, and cutting boards and utensils used in handling uncooked poultry or other meats should be washed with hot soapy water before being used for the preparation of salads or other raw foods. Another possible intervention measure is the freezing of fresh meat at home by the consumer. Freezing has a damaging effect on *Campylobacte*r, resulting in fewer organisms in broiler carcasses *(17)*. Both intervention measures presume a change in consumer behavior. Such a change might be realized through communication campaigns. The annual cost of such a national communication campaign is €1.85 million (Interministerieel Commissariaat Influenza, Belgium, 2006).

Efficiency and sensitivity analysis. Dividing reduced cost-of-illness by intervention costs indicates the efficiency. With a self-sufficiency degree of 145%, about one third of the Belgian poultry meat is exported. This implies that measures taken to reduce the contamination with *Campylobacter* of poultry flocks or meat will not only have a positive effect on the health risk of consumers in Belgium, but also in countries importing Belgian products. In the analysis, the benefits realized on the Belgian market and export markets are integrated. Results of the various interventions are presented in Table 3, assuming most likely values for effectivity and intervention costs. Three interventions are considered efficient, i.e. having a ratio > 1. The most efficient intervention measure is the decontamination of carcasses with EO-water with an efficiency near 18. The decontamination of carcasses with lactic acid and the addition of bacteriocins to the feed have an efficiency of 4.06 and 2.10 respectively. Considering all consumers, the efficiency is even higher.

The social perspective is traditionally the perspective chosen in economic evaluation. It is assumed that investments are worth doing, when society as a whole is better off than under a status quo situation. However, in our study the “benefactors” and the “losers” are not identical. Costs are made in the food supply chain, while the

benefits are realized at consumer level. The distribution of the intervention costs over the different stakeholders, namely farmers, industry and government are summarized in Table 3.

Conclusions

Campylobacter infections pose a serious public health problem in Belgium with poultry meat being most likely responsible for 40% of human cases of campylobacteriosis. On a yearly basis, this results in at least 22,000 campylobacteriosis cases with a costs-of-illness of € 11 million/year.
Of all analyzed intervention measures, the decontamination of carcasses with EO water is the most efficient, assuming that all other levels in the poultry meat chain continue with good practice behavior. The administration of bacteriocins to the feed of broilers 1 to 3 days before slaughter might be another efficient intervention measure. However, care should be taken considering these results. Up-to-day, the application of bacteriocins has only been investigated in 10-day-old chickens. Further research is necessary to investigate whether these effects remain valid when the bacteriocins are administrated shortly before slaughter. Irradiation is the most effective intervention, however, one of the least efficient.

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EVALUATION OF THE BELGIAN ACTIVE SURVEILLANCE PROGRAMME FOR LOW PATHOGENIC AVIAN IINFLUENZA IN PROFESSIONAL POULTRY HOLDINGS

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ABSTRACT

In accordance to the Commission decision 2007/268 (2), Belgium has set up an active and passive surveillance in wild and domestic birds. The aim of this study was to evaluate the effectiveness of the Belgian active surveillance programme for domestic birds in professional poultry holdings. A scenario tree identifying each step in the detection or infection process was developed and implemented in @RISK 5.0. The uncertainty and variability around certain parameters was taken in account in the different possible scenarios, leading to a final sensitivity accounting for these differences. The results enabled the reallocation of the samples foreseen for 2009 according to the differential risk in each risk group. This study has proven that the use of such models provides an efficient tool in evaluating surveillance programmes in substantiating disease freedom with a statistical confidence level as required by the international standards.

1. INTRODUCTION

The severe ethical and economical repercussions left by the passed avian influenza epidemics led to the implementation of different surveillance programmes in the different EU member states. The Belgian surveillance programme for AI is carried out in accordance with these Commission Decisions 2005/734 (1) and 2007/268 (2). It concerns both domestic poultry (e.g. chickens, turkeys, geese, ducks, ratites, other poultry (guinea fowl, partridges, pheasants, meet pigeons)) and wild birds and consists in both passive and active surveillance. The aim of this study was to evaluate the sensitivity of Belgian serological active surveillance programme for detecting LPAI in professional poultry holdings, in order to provide a certain level of confidence in substantiating freedom of disease, and also to determine subgroups where target sampling would enhance the total sensitivity of the system.

2. MATERIAL AND METHODS

A scenario tree as proposed by Martin et al. (2007) was implemented in @risk software (Palisade, 2007). Different category risk nodes partitioned the population in different proportions following their differential risk of infection or detection. Relative risk (obtained through historical data or passed experience, as well as expert opinion) were attributed to each of these category nodes together with the population proportions and set design prevalence. This enabled the calculation of respective effective probabilities of infection, for each category node. These effective probabilities of infection were combined to the lab detection nodes together with the sampled populations in order to obtain respective sensitivities for each risk group identified. The different riskgroups were characterised based on regions (five different regions were considered according to the relative risk zone surface within each region), risk zones (defined as zones where in between farm distance and likelihood of disease introduction was higher), holdings with or without outdoor facilities and species (chickens, turkeys,

geese and ducks, ratites, other poultry). Through a set number of iterations, sensitivities were obtained for each risk group. The allocation of probability distributions in each relative risk and lab sensitivity took in account the uncertainty and variability around those parameters, in the final sensitivity calculations. Following the obtained sensitivities an optimal sample size was obtained through Freecalc software (Survey Tool box (Ausvet, 2008)). An optimised sample scheme, redistributing the samples foreseen to be taken in 2009, was proposed following the optimal results obtained.

3. RESULTS

The sensitivities and the initial sample sizes, the optimal sample sizes obtained following our model and the risk-based redistribution of the 10,000 samples foreseen for 2009 in Belgium (optimised sample size) are stated in Table I. We notice that the effective probability of infection is higher in risk zones with outdoor facilities, and the specie more at risk is geese and ducks.

Table I **Summary for Belgium of the sensitivities, previous sample sizes, as well as optimal and optimised sample sizes estimation obtained in each risk group in Belgium**

RG	Risk zone				Non risk zone			
	Se	Initial SS	Optimal SS	Optimised SS	Se	Initial SS	Optimal SS	Optimised SS
Outdoor Chicken	0.99	120	70	450	0.99	140	90	580
Outdoor Turkey	0.93	40	30	200	0.61	180	60	380
Outdoor GD	0	0	0	0	0.57	800	300	1930
Outdoor Ratites	0.93	40	50*	250*	0.86	380	90	580
Outdoor OtherP	0	0	0	0	0.57	160	80	500
Indoor Chicken	0.99	920	90	580	0.99	5640	90	580
Indoor Turkey	0.89	80	30	200	0.84	720	40	260
Indoor GD	0	0	0	0	0.74	2600	200	1300
Indoor Ratites	0	0	0	0	0.39	200	200	1300
Indoor OtherP	0.88	80	40	260	0.54	300	90	580

* Unable to achieve desired accuracy by sampling every unit, therefore we estimated the ideal number samples to be taken. It could be considered to spread over the year, this number divided by the number of holdings.

4. DISCUSSION

This study has enabled the identification of risk groups where disease tends to cluster according to biological, ecological or housing systems differences. Even though data used for the quantification of these differential risk was not based on empirical data, the fact of accounting for this inaccuracy by fitting appropriate distributions and taking in account this uncertainty, actually enhances the value of the risk estimate in a first stage and sensitivity in a later stage.

The Commission Decision 2007/268 (2) insists on having an active surveillance system in domesticated birds to substantiate freedom of disease, as well as an early detection system and prevalence estimation of circulating LPAI. The sampling design imposed by this Decision is only able to substantiate freedom of disease with a certain confidence level. Therefore, this study aimed at evaluating the efficacy of the surveillance system in substantiating freedom of disease, with a statistical confidence level based on risk quantification. The sampling design disregards sampling in time, and the likelihood of disease introduction between different sampling periods. The actual surveillance system substantiates freedom from H5 or H7 LPAI strains with yearly intervals.

Using Bayes theorem, the results of the surveillance programme of previous years can be used to enhance its confidence during a next year (3). Also accounting for disease introduction from year to year would be interesting to consider in a future study.

For an early detection system of circulating LPAI, a different sampling design is required. The results of this study provide insight on risk groups where intensifying the sampling would be appropriate. The proposed methodology can be extended to evaluate the efficiency of the early detection system by taking the repeating sampling and the repeating probability of introduction into account. For early detection of HPAI passive surveillance based on clinical findings is more relevant as stipulated in the Commission Decision 2005/734 (1).

The objective of Commission Decision 2005/734 (1) is also to estimate the design prevalence in order to estimate a target prevalence to reach in each Member State. When estimating prevalence in a population, diagnostic test sensitivity and specificity are two crucial parameters. The set guidelines of the commission is mainly aimed at detecting the infection if were present, thus focalises on the minimum sample required for detecting the disease if it was present. In order to estimate prevalence of disease the sample size would be different.

Extending the model to additional parameters influencing the introduction and spread of the virus would be interesting to consider in future studies, such as wild birds, biosecurity measures, and seasonal effects.

Being able to quantify this sensitivity accounting for the complexity of the surveillance system provides great advantage to such models. Furthermore the more accurate sensitivity obtained through this model enables us then to conduct a more efficient target sampling. For assessing surveillance system in a country, this model is a valuable tool as it enables not only to provide a sensitivity but also a distribution around this sensitivity, thus substantiating freedom from disease with set statistical confidence level as required by the international standard guidelines (3).

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