"HOST-PATHOGEN-ENVIRONMENT INTERACTIONS"

28 oktober 2016
KU LEUVEN
TECHNOLOGIECAMPUS GEEL

studiedag ingericht door

PROCEEDINGS

OF THE

23th ANNUAL MEETING OF THE FLEMISH SOCIETY FOR VETERINARY EPIDEMIOLOGY AND ECONOMICS

HOST-PATHOGEN-ENVIRONMENT INTERACTIONS

Editor: S. Ribbens

28 October 2016

GEEL, BELGIUM

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"Host-Pathogen-Environment Interactions"

| 8h30-9h15 | Onthaal met koffie en koffiekoeken |
| 9h15-9h30 | Verwelkoming door KULeuven Technologiecampus Geel en voorzitter VEE |

OCHTEND PROGRAMMA

Plenaire sessie: Johannes Charlier

9h30-9h40	Inleiding van de keynote speakers
	Dr. Johannes Charlier
9h40-10h30	'Mycobacteria: interactions between humans, animals and environment'
	Dr. Vanessa Matthijs (WIV)
10h30-11h20	'The impact of global changes *sensu latu* on vector-borne diseases'
	Dr. Guy Hendrickx (Avia-GIS)
11h20-11h50	koffiepauze
11h50-12h40	'Predicting effects of climate change on parasite epidemiology in grazing systems: the need for integration'
	Prof. Dr. Eric Morgan (University of Bristol)
12h40-14h00	Lunch met postervoorstelling

NAMIDDAG PROGRAMMA

Open session: sessievoorzitter: Stefaan Ribbens

14h00-14h10	Inleiding van de namiddagvoordrachten
14h10-14h30	X. Simons: ''Modelling the spread of African swine fever in the Belgian pig livestock industry: Validation of a simulation model for policy assessment'
14h30-14h50	C. Rojo Gimeno: 'The economic value of milk biomarkers: ex-ante analysis of fat to protein ratio and fatty acid profile for the early detection of subacute ruminal acidosis in dairy cows'
14h50-15h10	B. Damiaans: 'Assessing motivators and obstacles for implementation of biosecurity measures in animal production in Europe'
15h10-15h45	Koffiepauze en postersessie

15h50-16h10   M. Postma: 'Reducing antimicrobial usage in pig production without jeopardizing production parameters'

16h10-16h30   F. Vangroenweghe: 'Improved weight gain and reduced mortality and antibiotic use following oral live non-pathogenic Escherichia coli F4 strain vaccination in piglets against post-weaning diarrhea'

16h30-16h50   S. Sarrazin: 'The influence of the introduction of antimicrobial use guidelines on prescription habits in first-line, small animal veterinarians'

16h50-17h-00   Poster Prijs AVIA-GIS en afscheidswoord voorzitter VEE

17h00-18h00   **Receptie aangeboden door KUL Technologiecampus Geel**

Oral presentations

EVOLUTION OF THE MYCOBACTERIAL SPECIES IDENTIFIED IN THE NATIONAL REFERENCE LABORATORY, 2007-2014

Karine Soetaert[1], Brigitte Vanfleteren[1], Vanessa Mathys[1]

[1] *Bacterial Diseases Service, Operational Direction Communicable and Infectious Diseases, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium*

Correspondence: Karine Soetaert. Bacterial Diseases Service, Operational Direction Communicable and Infectious Diseases, Scientific Institute of Public Health (WIV-ISP), 642 rue Engeland, 1180 Brussels, Belgium. Tel: 0032 2 373 32 10. Fax: 0032 2 373 32 81. Email: ksoetaert@wiv-isp.be

ABSTRACT

As mycobacteria can be responsible for different types of pathology, a correct species identification is important to quickly adapt the treatment and the management of the patient. In order to better characterize the mycobacterial strains infecting the Belgian population, this study was initiated with the objective to determine the relative frequency of the different mycobacterial species and to evaluate their clinical significance.

All species identifications performed on cultures received by the National Reference Laboratory (NRL) during the period 2007-2014 were included in the study. Here, we report the large and increasing presence of nontuberculous mycobacteria (NTM) among the mycobacterial cultures analyzed by the NRL; with M. avium, M. intracellulare and M. xenopi being the most frequently clinically relevant species identified. The evolution of the species over the years suggests an increasing number of infections provoked by M. intracellulare. This study also reveals a significant and increasing reception of false-positive cultures, generating unnecessary laboratory work.

INTRODUCTION

The gender Mycobacterium contains a large variety of species regrouped into three different categories: Mycobacterium tuberculosis complex, nontuberculous mycobacteria (NTM) and non-cultivable mycobacteria. As these species can be responsible for different kind of pathologies in human (tuberculosis, mycobacteriosis or leprosy) requiring different types of treatment, the realization of species identification on positive mycobacterial cultures is primordial in order to start the adequate treatment and management of the patient (Falkinham, 1996). In Belgium, mycobacterial species identification is routinely performed in 12 clinical laboratories and in the National Reference Laboratory (NRL).

In order to better characterize the mycobacterial strains circulating in Belgium and infecting the Belgian population, we performed an analysis of the species identified on positive cultures received by the Belgian Reference Laboratory "Mycobacterium" (WIV-ISP) during the period 2007-2014. This allowed to determine the relative frequency of the different mycobacterial species isolated and their eventual differences in regard to the clinical significance.

MATERIAL AND METHODS

2.1. Clinical isolates

This study comprises all positive cultures received from the 1st January 2007 to the 31st December 2014, by the Belgian Reference Laboratory "Mycobacterium" (WIV-ISP) for species identification and/or drug susceptibility testing. These cultures were sent from 107 different Belgian clinical laboratories.

2.2. Patients' information

All data recorded in the context of the present study had not been collected for research purposes but as part of the routine data collection for diagnosis. Anonymity of data was ensured prior to analysis.

2.3. Species identification

After reception of positive cultures, a research of M. tuberculosis complex was first performed by PCR detection of the Insertion Sequence IS6110, only present in the genome of the M. tuberculosis complex members. In case of negativity, a research of NTM was initiated by sequencing of the gene coding for the 16SrRNA (Kirschner, 93). Briefly, PCR products were analysed by electrophoresis on a 2% agarose gel and visualized by staining with ethidium bromide and UV illumination. The remaining PCR products were purified using a QIAquick 96 PCR Purification kit (Qiagen) and sequenced with primer 285 (Kirschner, 93). Sequencing was performed by using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and analysed on an ABI 3130xl Sequencer (Applied Biosystems). The obtained sequences were submitted to BLAST (Basic Local Alignment Search Tool) analysis against the NCBI (National Centre for Biotechnology Information) and RDP (Ribosomal Database Project) databases. In case of non-concluant/imprecise result, other molecular assays were used like the commercial GenoType CM/AS Line Probe Assay (Hain Lifescience, Germany) based on the analysis of the 23SrRNA gene or home-made species specific PCRs.

2.4. Evaluation of the clinical relevance of NTM

The NRL performs a drug susceptibility testing for all clinically-relevant NTM complying with the American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) criteria (Griffith, 2007). These criteria are based on clinical, radiological and microbiological evaluation. In this study, the number of NTM for which susceptibility testing was asked by the medical doctor was used as a criteria to evaluate the number of pathogenic isolates (distinction between clinically relevant NTM and environmental contaminant of the specimens).

RESULTS

3.1. Frequency and distribution of mycobacteria isolated in 2014

In 2014, a total of 1929 cultures were characterised at the Belgian NRL "Mycobacterium". Among these, 460 (23.8%) were identified as Mycobacterium tuberculosis complex, 857 as NTM (44.4%) and 612 (31.7%) were reported as negative for mycobacteria (false positive cultures).

Among the 857 NTM strains, 229 (26.7%) were identified as M. gordonae, a species which is commonly considered as clinically irrelevant, followed in frequency by M. intracellulare (n=173, 20.2%), M. avium (n=165, 19.2%) and M. xenopi (n=102, 11.9%) that are clinically relevant. The remaining NTM species were less frequent and represented altogether 188 strains (21.9%). See Figure 2 for more details.

3.2. Evaluation of the clinical relevance

A drug susceptibility testing (DST) was performed on all strains identified as M. tuberculosis complex as infection by M. tuberculosis complex is always clinically relevant.

A NTM DST was asked and performed for 249 patients infected by the following mycobacteria: 79 M. avium, 58 M. intracellulare, 23 M. xenopi, 16 M. kansasii, 22 M. chelonae-abcsessus complex, 6 M. malmoense, 5 M. fortuitum, 11 M. marinum and 29 other NTM. This selection (29%) is considered as of the fraction of pathogenic isolates among the NTM analysed in the NRL.

3.3. Evolution of the NTM frequency over the time

The evolution of the mycobacterial identifications over the years is presented in Figure 1 and 2. From 2007 to today, we can clearly observed an increase of the NTM and false-positive cultures received by the NRL for analysis, while the number of M. tuberculosis complex cultures remains stable. The evolution of the species identified among the NTM isolated from Belgian patients over the years (Figure 2) reveals an increase of the relative frequency of M. intracellulare. Indeed, M. intracellulare currently represents about 20,2% (173/857 NTM analysed in 2014) of all NTM isolated from patients while in 2007 this percentage was only of 12,3% (74/598 NTM analysed). Conversely, a decrease from 21% to 12% of the NTM is observed during the same period for the species M. xenopi.
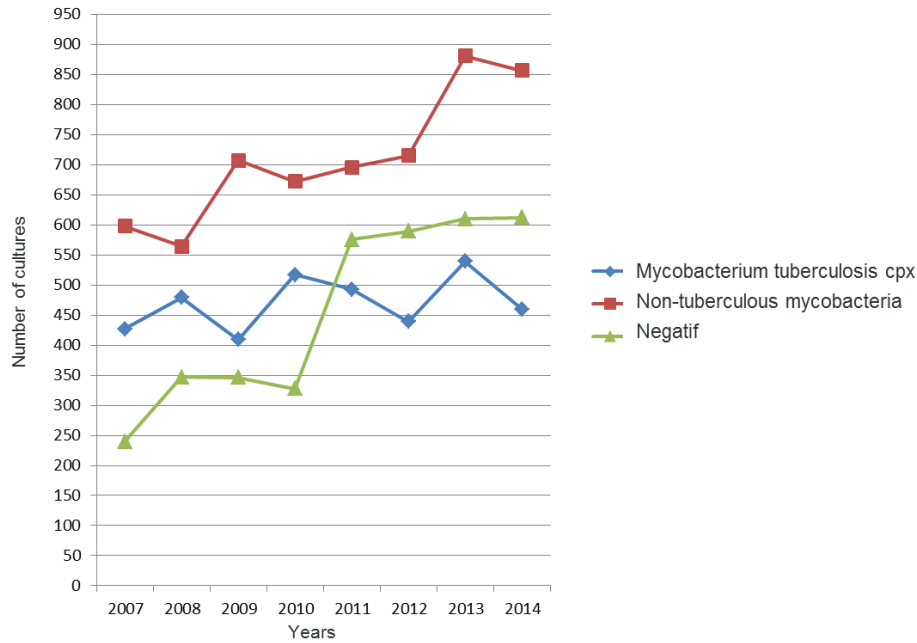
Figure 1: Evolution of the identification results obtained on the cultures received in the National Reference Laboratory, 2007-2014
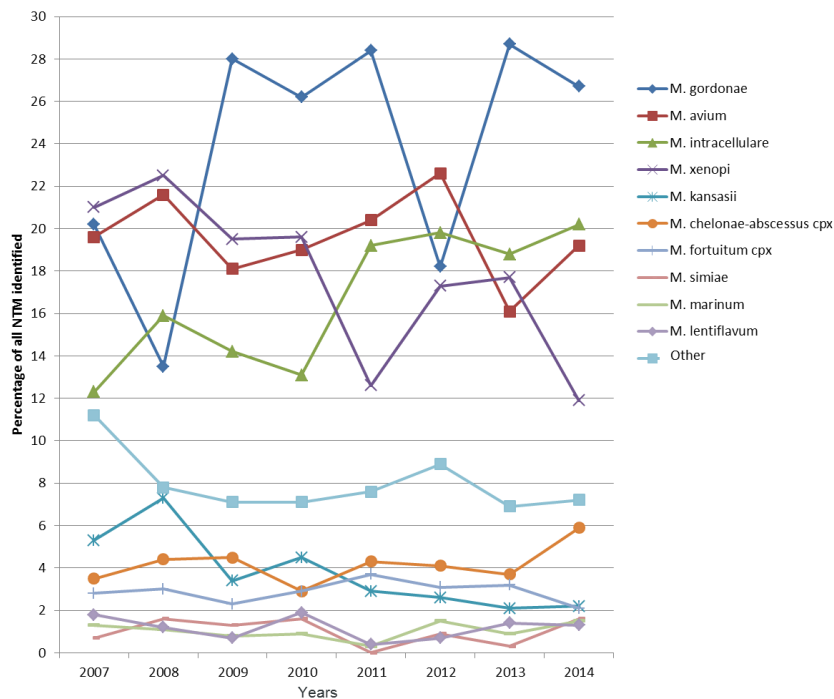

Figure 2: Evolution of the species identified among the nontuberculous mycobacterial cultures received in the National Reference Laboratory, 2007-2014

DISCUSSION

As mycobacteria can be responsible for different types of pathology, a correct species identification is important to quickly adapt the treatment and the management of the patient. Also among the infections caused by NTM, there are increasing evidences that different NTM species and sub-species harbour different clinical and epidemiological presentations. Based on

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this observation, the present study was initiated in order to better characterize the species of the mycobacterial strains isolated from Belgian patients.

This study shows the increasing reception at the NRL of cultures containing NTM; M. gordonae, M. avium, M. intracellulare and M. xenopi being the most frequently species identified. This study also reveals that a significant number of cultures sent to the NRL as "positive" don't actually contain mycobacteria and this number is increasing over the time. A setup of the automated growth system and/or revision of the sample decontamination procedure should be evaluated in clinical laboratories, as these "false-positive cultures" generate unnecessary laboratory work.

The evolution of the species identified among the NTM isolated from Belgian patients over the years suggests an increasing number of infections caused by M. intracellulare. Although a true relative increase in these infections is suspected, this observation could also be partially attributed to the increased awareness of NTM disease these last years and the increased availability and sensitivity of laboratory techniques.

For practical reasons, the close species M. intracellulare and M. chimaera have been grouped under the name "M. intracellulare" in Figure 2 but since two years the NRL performs the distinction between these two species as M. chimaera has been recently reported as implicated in severe cardiovascular and systemic infections following cardiac surgery through the use of contaminated "Heater Cooler" devices (Haller S, 2016; Sommerstein, 2016).

One of the main limitations of this study is linked to the fact that notification of NTM infections is not mandatory in Belgium, although these pathogens have become an increasing public health concern (Hoefsloot, 2013; Winthrop, 2010; Cassidy, 2009). Therefore, the frequency of myobacterial infections reported in this study is probably under-estimated.

Species identification among the nontuberculous mycobacteria was mainly performed by sequencing of the 16SrRNA sequencing. Although sequencing and analysis of other genomic regions are also efficient (like rpoB or hsp65 genes, …) we consider that 16SrRNA sequencing is a valuable technique for the differentiation between members of this gender. However, for the differentiation between genetically close species, conducting further molecular analysis may be worthwhile.

While considerable attempts were made to determine the number of pathogenic NTM (and avoid environmental contaminant of the specimens) by selecting isolates of patients for which DST was asked by the medical doctor, we consider it likely that the selection of pathogenic isolates studied may be underrepresented as some medical doctors probably treat their patients after species identification without asking for a DST.

In conclusion, we report the large presence of nontuberculous mycobacteria (NTM) among the mycobacterial cultures sent to the Belgian Reference Laboratory "Mycobacterium". Among these NTM, the most common clinically relevant species isolated were M. avium, M. intracellulare and M. xenopi. The analysis of the evolution of the species over the years suggests an increase of M. intracellulare infections. This study also reveals that a significant and increasing number of cultures sent to the NRL are "false-positive". A setup of the automated systems for mycobacteria growth or a revision of the decontamination procedures by clinical laboratories would be suitable to avoid unnecessary laboratory work.

ACKNOWLEDGMENTS

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PREDICTING CLIMATE CHANGE IMPACTS ON PARASITE TRANSMISSION IN GRAZING LIVESTOCK: THE NEED FOR INTEGRATION

Eric Morgan, Hannah Rose and Richard Wall

University of Bristol, School of Veterinary Sciences, c/o Bristol Life Sciences Building, 24 Tyndall Avenue, Bristol, BS8 1TQ. Eric.morgan@bristol.ac.uk

INTRODUCTION

Climate change appears to be altering parasite infection patterns worldwide. For gastrointestinal nematodes of grazing ruminants, the level and seasonality of infection have changed in the UK over recent decades, as evidenced by collated veterinary laboratory diagnoses (van Dijk et al, 2008). Such standardised longitudinal data are rare, and this limits the ability of statistical analyses to detect changes in infection patterns. Moreover, statistical approaches are poorly suited to predicting future infection patterns under climate change, since the relationships between climate and infection – and not just their outcome – are likely to be non-stationary. Here, we summarise our progress in developing mechanistic models of parasite transmission in ruminants, and applying them to predict effects of climate and management change on infection patterns. Case studies illustrate how such models can be used by farmers to support decisions on treatment and other management interventions. Our experience mainly concerns small ruminants, but principles apply more widely to cattle and other systems. Finally, we assess future research needs in this area of study, especially regarding better integration of parasite biology with dynamic changes in farm management and the forces that drive them.

EFFECTS OF TEMPERATURE ON PARASITE POPULATIONS

Parasites very often have free-living stages or stage in poikilothermic intermediate hosts. Generally, development rate increases at warmer temperatures (Fig.1). Therefore, it is commonly assumed that global climate warming will tend to favour the development of infectious parasite stages and consequently infection pressure, and worsen disease challenges in livestock.

Fig. 1: Temperature-development relationship for Toxocara canis *larvae within eggs. From Azam et al. (2012).*
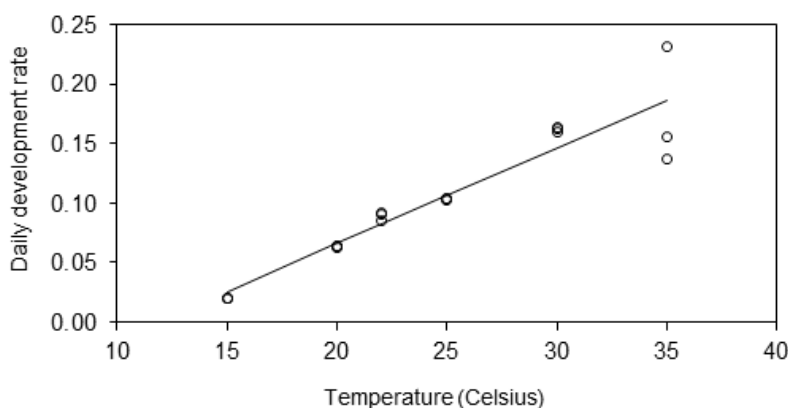

A net increase in infective stage availability, however, is not inevitable. Thus, for example, the survival of infective third stage larvae (L3) of gastrointestinal nematodes (GIN) of ruminants decreases not only at very low (sub-zero) temperatures but also at high temperatures, such that there is an optimum zone for maximum L3 yield (Fig. 2). Climate warming, therefore, could increase or decrease L3 yield, depending on the ambient temperatures in relation to this zone. The exact outcome will be highly species-specific and is not always precisely known, so the effect of warming on parasite communities is somewhat unpredictable and compounded by seasonal temperature fluctuations. For GIN, the 'sweet spot' is expanded by spring and autumn warming, more than in summer.

Fig. 2. Cartoon representing increase in parasite infective stage development with increasing temperature, and a convex relationship of survival with temperature, forming a zone of optimum larval yield.
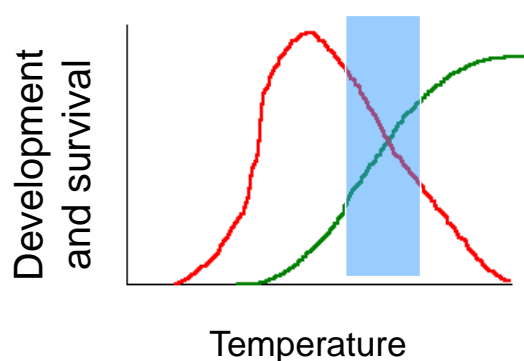
Apart from net larval yield, the effect of climate on the time-course of larval appearance on pasture is important to infection risk. Thus, faster development and mortality can result in a larval peak that is earlier and higher, but abates more quickly, such that pastures become safe for grazing (Fig. 3). Therefore, net effects on infection pressure depend fundamentally not only on direct effects of climate on parasite biology, but also on how the pastures are grazed, i.e. on farm management.

Fig. 3. Effect of increasing temperature (red line, left) on L3 availability over time, relative to baseline (green line, right), assuming simultaneous increases in development and survival.
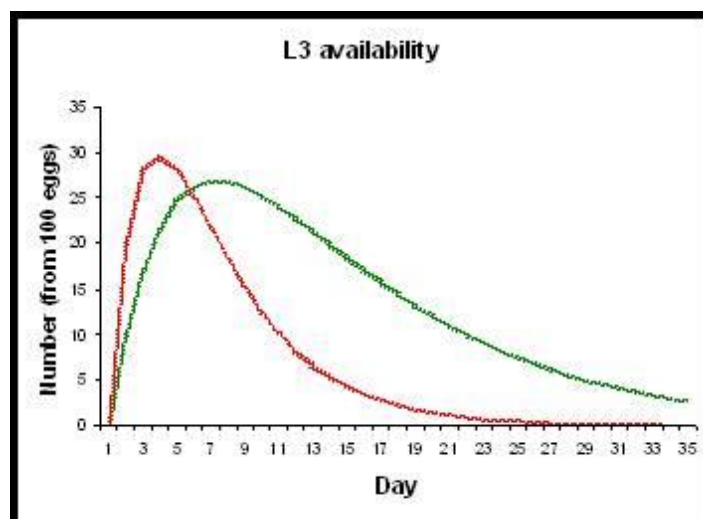
PRECIPITATION AND PARASITE TRANSMISSION

Rainfall modifies the effects of temperature on GIN larval availability, being necessary for larval development and migration from dung to grass (Wang et al. 2014). Other parasites also benefit from humid conditions. Increases in temperature are sometimes associated with low rainfall, e.g. in warm summer weather in Europe. The projections for rainfall under climate change are less precise and confident than those for temperature, and include increasing rainfall variability. This is not only a major source of uncertainty for predicted epidemiology under climate change, but also challenges current control strategies, that often rely on fairly predictable seasonal patterns. More flexible approaches are needed, supported by decision support tools cognisant of the effects of climatic variation on parasite challenge.

HOST FACTORS

Effects of climate on parasite biology are modified by the host reaction – for example immunity under situations of higher infection pressure could result in earlier age of peak infection and lower average infection levels in older age classes. Assessments of production impacts resulting from altered parasite infection patterns should therefore take account of the full production cycle. This is currently rarely the case. Farm management can also be a strong modulator of climate change impacts on parasite populations. Predictive mechanistic models can be helpful in elucidating likely changes in complex systems, to inform control strategies and to develop hypotheses.

Often, management factors are more important than biological processes in determining net changes in risk under climate warming. For example, early shearing can negate increased risk of strike in warm springs (Fig. 4). Other examples include decreased spring scour in lambs caused by early hatching of Nematodirus larvae, when few lambs are ingesting grass.

Fig.4. Effect of early shearing on risk of blowfly strike in sheep. Warming spring leads to early emergence of flies (top graph) and an earlier predicted peak of strike cases, but only in ewes (bottom line, circled). Early shearing can attenuate that peak.
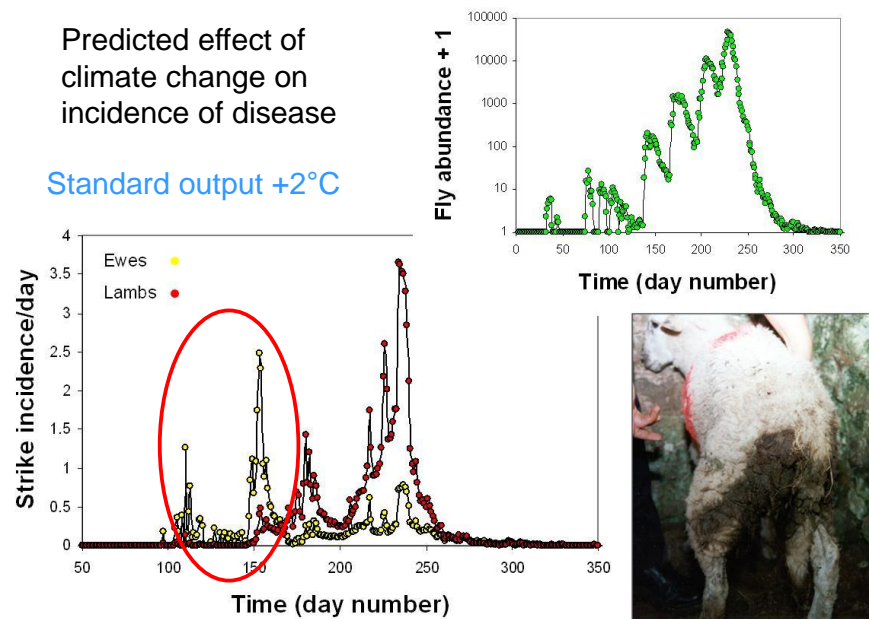
THE NEED TO INTEGRATE NON-STATIONARY MANAGEMENT

Early attempts to include management into models of parasite dynamics have been made, but these usually consider different management scenarios as static. An emerging challenge in the field is to include evolution of management strategy as dynamic, alongside epidemiological and evolutionary change in parasites. New work on farmer behaviour as well as farm economics offers hope for such integrated models.

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MODELING THE SPREAD OF AFRICAN SWINE FEVER IN BELGIUM: VALIDATION OF A SIMULATION MODEL

Simons, X.1, Roelandt, S. 1, Halasa, T.2, Boklund, A. 2, Koenen, F. 1, Welby, S. 1
[1] *CODA-CERVA, Groeselenberg 99, 1180 Brussels Belgium*
Unit of Epidemiology, Risk Analysis and Surveillance (ERA-Surv)
[2]*Technical University of Denmark, Section of Epidemiology,*
The National Veterinary Institutes, Copenhagen, Denmark

ABSTRACT

African swine fever (ASF) is a notifiable infectious pig disease. Current uncontrolled outbreaks of ASF in Eastern Europe represent a non-negligible risk of ASF spread towards Western Europe. The present study aims to validate the use of a Danish model to simulate the spread of ASF in Belgium following a hypothetical introduction of the virus. The results show that the model predicts relatively small spread of the ASF virus in Belgium, with only 3 infected herds as median. Sensitivity analysis shows that the within-herd transmission rate (beta) is the most sensitive parameter; for higher betas, the disease is detected more quickly and the epidemic consequences are lower. Networks analysis of animals movements suggest that the simulation of movement between herds can be improved to better fit historic data. This simulation model could be a valuable tool for contingency planning.

1. INTRODUCTION

African swine fever (ASF) is a notifiable highly contagious viral disease that affects pigs and wild boars (Costard et al., 2013). Since 1997 most of the European countries are ASF free. In 2007, an outbreak was declared in Georgia, since then the disease has spread in numerous eastern countries (Guinat et al., 2014), so that currently, in 2016, uncontrolled outbreaks are reported in Poland and Lithuania (OIE, 2016). Consequently, there is a non-negligible risk of ASF spread towards Western Europe (EFSA panel on Animal health and Welfare, 2014).
Recently the Technical University of Denmark (DTU) published a stochastic model that simulates the spread of ASF in Denmark and predicts its consequences (Halasa et al., 2016a). The DTU-DADS-ASF model uses a huge amount of input parameters, some of them specific to the disease (incubation period, transmission rate,…), other specific to the country (herds positions, types, data on animal movements,…). Starting with a hypothetical introduction of the virus, the model simulates the spread (within and between herds), the possible detection and control of the disease. Moreover an additional economical module computes the costs related to the simulated epidemics.

The main objective of the present study is to investigate if the DTU-DADS-ASF model can be adapted to the Belgian context and to simulate the spread of a potential introduction of the virus in Belgium. The model was studied and its strengths and weaknesses were explored. Such model can provide valuable information to policy makers while build contingency plans.

2. MATERIAL AND METHODS

2.1 Data source
Details of movements of pigs in Belgium for years 2013 and 2014 were extracted from the National Animal Registration and Identification database (Sanitel). Data cleaning was carried out to exclude herds without full information on herd size, location and movements. In turn, this data enabled the computation of the main model inputs. It is important to notice that movements of individual pigs are not stored; only batch movements are registered, and the amount of animals moved per batch is specified. Parameters specific to the disease and those related to the detection and control modules were validated by expert's opinions.

2.2 Model description
The DTU-DADS-ASF model was developed in R software and is freely available on Github (https://github.com/THalasa/DTU-DADS-ASF). The core of the model is composed by 4 modules: within-herd spread, detection, control measures, and between-herd spread. The simulation starts in day 1 with one (or more) infected herd selected by the experimenter. The within-herd spread module is based on a SLSCR model (Susceptible-Latent-Subclinical-Clinical-Removed) (Halasa et al., 2016b). The disease is detected when the

amount of sick and dead animals (SIED) exceeds several thresholds. The control measures include restrictions of movements and clinical visits, which can accelerate the detection of the disease. Spread between herds is based on the simulation of animal movements, which is computed from historical movement data. Movements of sows and weaners are modeled independently. The daily number of outgoing movement is computed by a Poisson distribution which lambda parameter is herd-specific and equals the total amount of movements per year divided by 365. The herd that will receive the movement is selected as a function of the distance between the sending and receiving herds and their types.

2.3 Sensitivity analysis
Sensitivity analysis was carried out on potentially influent model parameters. Three different transmission rates (beta) were proposed: 0.3, 0.6 and 2 that represent respectively low, normal and high transmission rates (Carvalho et al., 2013). Multiple parameters were modified in order to investigate extreme situations regarding disease detection. The first detection occurs if the 3 conditions specified in table 1 are fulfilled.

Table1: Sensitivity analysis parametrization for first detection

Detection conditions	Parameter	Basic scenario	Weak detection	Strong detection
SIED ≥ Expected mortality * x	x	2	2	2
SIED ≥ Herd size * y	y	0.0255	0.04	0.003
SIED ≥ z	z	5	7	5

* SIED stands for 'sick and dead animals'.

The impact of these modifications was assessed for the following outputs: proportion of non-detected epidemics (iterations), time after first detection, epidemic duration and number of infected herds. 2000 iterations were realized for each scenario. In order to reflect a 'worst case' scenario, an herd with more than 10000 animals and more than 1 out-movement per week (high spread potential) was selected as the source of infection in day 1.

2.4 Network analysis
Network analysis was carried out on historical movement data and on data of simulated movements in order to validate the model assumptions on movements between herds. In the networks, each node represents a herd and each edge represents a shipment of a batch of animals. Directed weighted networks were computed so that an edge's weight equals the frequency of that particular movement in the course of the year. The amount of animals moved per batch was not taken into account in the network analysis.
The simulations of the movements between herds were computed using 2 different methods. *Simulation 1* follows the strategy used in the DTU-DADS-ASF model (see 2.2). In *Simulation 2*, the selection of the herd that will receive the movement is based on the distance between the sending and the receiving herds, their types, and an additional parameter, herd-specific, that reflects the probability of receiving a contact (herd yearly ingoing movements/total yearly ingoing movements).
The network analysis was carried out using R software version 3.1.2 and R package 'igraph'. The metrics of the different networks were compared: number of movements, network density, proportion of isolates, centrality, reciprocity, clustering coefficient and diameter.

3.   RESULTS

Following the examination of Sanitel data for the year 2014 and after the data cleaning step, the database consisted of 6012 herds, more than 13 million animals, 33627 batch movements and near 7.5 million animal movements between herds.

Table 2 summarizes the models outputs and the sensitivity analysis. In the basic scenario, the transmission rate is set to 0.6, according to recent study on the current Georgian strain circulating in Eastern Europe (Guinat et al., 2015). The model outputs for the basic scenario predict relatively small outbreaks for Belgium, with a median of 3 herds infected (Max=9) and an epidemic duration of a week, with a maximum duration of 49 days. The disease first detection was after 30 days (median). As ASF is very contagious the detection could be expected earlier; this can be explained given that all the scenarios were 'worst case scenarios', and were started in a herd with more than 10000 animals so the detection only occurs when SIED animals exceed 250 or if another smaller herd is infected. Sensitivity analysis shows that the most influent parameter is the within-herd transmission rate (beta). For higher betas, the disease is detected more quickly and the epidemic consequences are lower. The detection parameters can also have an important impact on model output, nevertheless even with a weak detection strategy, the epidemic consequences are of the same order than the basic scenario.

Table2: Model outputs and sensitivity analysis

Scenarios	Basic scenario	Transmission rate (β)		Detection scenarios	
Output	$\beta = 0.6$	0.3	2	Weak	Strong
Undetected epidemics (%)	3	30	1	3	3
Time after 1st Detection (days)	30	42	23	32	21
Epidemic duration (days)	7 Max=49	17 Max=206	4 Max=10	13 Max=63	1 Max=31
Numer of herd(s) infected	3 Max=9	3 Max=13	2 Max=8	3 Max=11	1 Max=5

* Medians are displayed

Results of the networks analysis show that the historical movements are more specific than the simulated movements. This can be explained by the fact that preference exists in real movements; this means that herd *x* will more often send animals to herd *y* than to herd *z*, even if herd *z* is of the same type and at the same distance than *y*. This specificity is not taken into account in the simulated network 1; consequently the movements are more at random in the *Simulation 1* compared to historical data. The *Simulation 2* meant to correct this bias; indeed networks metrics of *Simulation 2* show less randomness compared to *Simulation 1*, and better fit network metrics of historical data.

4. DISCUSSION

This study shows that the DTU-DADS-ASF model could be used to simulate the spread of ASF in Belgium. Indeed, the data is available via Sanitel database and expert's opinions. First results predict small outbreaks if ASF would be introduced in Belgium, which is consistent with the results obtained by the model for Denmark. Given with high biosecurity and tracing systems, this is reasonable to expect outbreaks will be quickly detected and controlled in our countries. Though only limited data is available regarding historical outbreaks, it is comforting to see that data from 1985 corroborates our findings. Indeed, during the last outbreak of ASF in Belgium, in 1985; 12 herds were infected, 60 herds slaughtered (34041 animals), and the disease was eradicated after 6 month (Biront et al., 1987).

Nevertheless, the between-herd spread module should be improved to better reflect real situation and preference in animal movements between farms. Tools and R packages exist to deal with movement networks that could be used to improve the model. The user must also be aware that the model outputs can be strongly affected by the parametrization, so it is crucial to have reliable information on for instance transmission rate of ASF virus.

The main strength of the DTU-DADS-ASF model is its flexibility; written in R it can be easily adapted to country specific data. The majority of the input parameters are expressed as distributions, thereby reflecting the variability and uncertainty of the situation. The model is also comprehensive, as it takes into account all steps interfering with the surveillance system of ASF (from infection to detection and eradication). Moreover a new wild boar module has been developed very recently and the economic module still adds valuable information for policy makers. The next step is the validation of the economic module for Belgium.

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THE ECONOMIC VALUE OF MILK BIOMARKERS: EX-ANTE ANALYSIS OF FAT TO PROTEIN RATIO AND FATTY ACID PROFILE FOR THE EARLY DETECTION OF SUBACUTE RUMINAL ACIDOSIS IN DAIRY COWS

Cristina Rojo Gimeno[1*], Karen De Ridder[2], Veerle Fievez[3], Erwin Wauters[1]

[1]*Social Sciences Unit, Institute for Agriculture and Fisheries Research (ILVO), Burg. Van Gansberghelaan 115, box 2, Merelbeke, 9820, Belgium;* [3]*Ghent University, Department of Animal Production.* [*]*Email address: Cristina.rojogimeno@ilvo.vlaanderen.be*

ABSTRACT

The key objective of this study was to develop a methodology to estimate the value of precision dairy farming monitoring systems (PDFMS) prior to implementation and, hence, in a situation of limited data availability. The second objective was to investigate which variables have the highest influence on the value of information (VOI) provided by PDFMS. To illustrate these objectives, we used a case-study of two milk biomarkers to monitor subacute ruminal acidosis (SARA) in dairy cows, the fat to protein ratio (FPR) and the fatty acid profile (FAP). A stochastic decision tree was designed to estimate the expected monetary value of: (i) not monitoring, monitoring with (ii) FPR, or (iii) FAP. The results suggest that under the most probable scenario not monitoring was a better decision than monitoring. Using FAP to detect SARA will be of value in herds with a prevalence between 0.24 and 0.80. While an improvement of the sensitivity of the FAP to 1 did not yield a positive VOI, an improvement of the specificity of the FAP to 1 did lead to a positive VOI.

1. INTRODUCTION

When evaluating to what extent PDFMS improve health management, a key element is the economic value of the information (VOI) that they provide. The VOI is the potential improvement in profitability stemming from the adoption of the monitoring system (Pannell and Glenn, 2000). Usually a rational farmer will invest in a PDFMS if its associated benefits exceed its costs, so deriving a high VOI. Despite the fact that numerous PDFMS have been developed in the last two decades, their associated VOI have been rarely investigated.

The primary objective of this study is to propose a methodology to evaluate the economic VOI of PDFMS, prior to their adoption, hence in the presence of data scarcity. The secondary objective is to give insights about the factors influencing the VOI, to steer the focus of PDFMS. A crucial issue regarding future research of PDFMS is whether developers should concentrate on improving the accuracy of the PDFMS or on the identification (or selection) of specific groups of cows at higher risk. We pursue both objectives using a case-study about milk biomarkers for detecting SARA. Measuring the pH of the ruminal fluid by ruminocentesis is the practical alternative to the gold standard which consists of continuous measurement of the ruminal pH (Duffield et al., 2004). In comparison to the former, the use of non-invasive tools such as milk biomarkers presents many advantages (Colman et al., 2012). Presently in Belgium, the fat to protein ratio (FPR) is used as a milk biomarker of SARA (Mertens, 1997). Models based on the milk fatty acid profile (FAP) have been recently developed for early diagnosis of SARA (Fievez et al., 2012; Colman et al., 2012) which showed a similar sensitivity (Se) but a higher specificity (Sp) than the FPR. However, due to the lack of routine method for the analysis of all milk fatty acids required to diagnose SARA, FAP-based models are not commercially available yet (Colman et al., 2015).

When SARA is not monitored, farmers can only take herd-levels decisions. As a consequence monitoring using a milk biomarker could better tailor treatment decisions by enabling cow-level decisions, thereby optimizing the herd-level management. In this study we investigated: (i) the VOI of monitoring either with FPR or FAP versus no monitoring and (ii) the most influential factors affecting the VOI of FAP to detect SARA.

2. MATERIALS AND METHODS

Our procedure consisted of a stochastic decision tree model applied to a typical specialized dairy farm. This model was fed with data of the sensitivity (Se) and specificity (Sp) of both biomarkers, true prevalence (True Prev),

disease costs (DC), and treatment costs (TC). The data of the last three variables were scarce and we used the methodology proposed by Hardaker and Lien (2010) to address the evaluation of different potential decisions when there is data scarcity. In this case, Hardaker and Lien (2010) advocate to use a combination of fitted distributions, data obtained from previous literature and reports combined with expert judgement modeled as subjective probabilities. Data gathered from literature, consultation with experts, dairy cattle veterinarians, and feed advisors allowed to obtain information on the True Prev of SARA, TC of SARA, and DC of SARA. The data used for the test characteristics of FAP and FPR originated from four previously conducted experiments, that aimed at identifying relevant milk fatty acids to diagnose SARA and reported the Se and Sp of both milk biomarkers (Colman et al., 2015). Table 1 contains data on the input variables fed into the decision tree. The expected monetary value (EMV) of the three different strategies was assessed: (i) not monitoring, monitoring with (ii) FPR or (iii) FAP. The VOI of the milk biomarker is estimated by the difference between the EMV of the strategy to manage SARA based on monitoring results from each of the milk biomarkers (FPR and FAP) minus the EMV of the strategy to manage SARA without monitoring.

Table 1. Input variables fed into the stochastic decision tree.

Variable	Distribution	Source
TC (€/cow/year)	Uniform (20, 250)	Literature, reports, triangulation
DC (€/cow/year)	Pert (100, 210, 400)	Literature, reports, triangulation
True Prev	Fitted distribution (Exponential, mean = 0.16, SD = 0.16)	5 previous studies
Se and Sp FPR	Se = 0.72, Sp = 0.31	Colman et al. (2015)
Se and Sp FAP	Se = 0.64, Sp = 0.89	Colman et al. (2015)

Different scenarios were described. First, we simulated the scenario in which the combination of the different variables better described the situation and corresponded to the most probable distributions of DC, TC, True Prev, and the most probable deterministic values of the Se and Sp of FAP and FPR. In the remainder of this text this scenario is referred to as default scenario. An elasticity analysis was conducted to explore the influence of uncertain variables on the VOI of the FAP. The effect of the True Prev on the VOI of FAP was investigated. Furthermore, the effect of improving the Se and Sp of the FAP were investigated.

3. RESULTS AND DISCUSSION

3.1. Default Scenario

Under the most probable scenario, not monitoring was better in 70% of the iterations. Furthermore, in 96% of these iterations, it was better not to treat than treating the herd. This is mainly a consequence of the low True Prev (on average 16%). When using biomarkers, FAP to detect SARA dominated by first degree stochastic dominance the decision to use FPR to monitor SARA which was related to the low test performance of the FPR test (Se=0.72 and Sp=0.3143) as compared with the FAP-based models (Se=0.6421 and Sp=0.8877). The low value of information provided by the FAP coincides with the rather low VOI obtained after using of different precision agriculture (Pannell and Glenn, 2000) and precision livestock monitoring systems (Jørgensen, 1993; Bewley et al., 2010). These studies found that information provided by these systems do not always enable better decisions than when no monitoring was used. Sometimes the minor benefits obtained did not even cover for the cost of the technology, like in the study conducted by Jørgensen (1993). He found that the VOI from most precise weightings of pigs in comparison with batch delivery with no weighing was only €2/pig and did only cover the cost of implementing individual identification tags (Jørgensen, 1993).

3.2. Elasticity and Sensitivity analysis

At average TC and DC, using FAP to detect SARA had a positive value when the True Prev was between 0.24 and 0.80, with its maximum between these two values at 0.60. Within this range of True Prev, the VOI of FAP increased with herd size (Fig. 1). It seems that FAP could be profitable in a herd at higher than average risk for SARA. For instance in 25 out of 78 herds from previous studies used to fit the True Prev distribution had a prevalence between 0.24 and 0.58. Alternatively, FAP could be profitable in subgroups of a herd with a higher risk of developing SARA such as cows which are between 15-30 days in milk with individual compound feeding which is rapidly build up during this period. Yet, this only holds when the use of FAP does not entail fixed investment or implementation costs, and hence only implies a cost when it is actually used.

Figure 1. Surface graph of the effect of the herd size (N, Z-axis) and true prevalence (True Prev, X-axis) on the VOI of the FAP to detect subacute ruminal acidosis versus no monitoring (Y-axis). Arbitrary VOI categories are presented with different color codes as indicated in the legend.
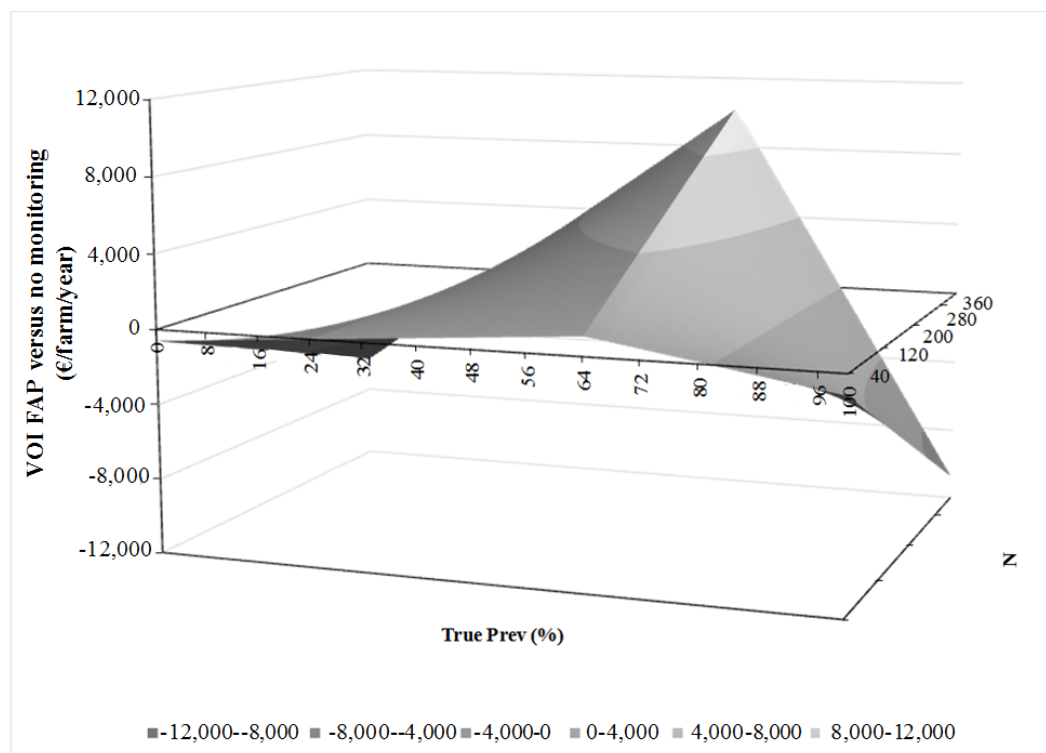
Under the default scenario, even when Se of FAP could be improved to 1 with the same Sp, the VOI of FAP remained always negative. Whereas, if the Sp increased up to 0.95, the VOI of FAP was positive. These results are in agreement with the desires of farmers who prefer to have as few false alerts as possible and therefore wish for a PDFMS with a high Sp (Claycomb et al., 2009). However, given the plausible combinations of Se and Sp of FAP, the VOI of the current FAP-based models remained always negative. As improvement of Se and Sp require large amounts of time, money and resources, ex-ante evaluation of this improvement should be explored first, as done in the current study.

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ASSESSING MOTIVATORS AND OBSTACLES FOR IMPLEMENTATION OF BIOSECURITY MEASURES IN ANIMAL PRODUCTION IN EUROPE

Bert Damiaans[1], Steven Sarrazin [1], Jeroen Dewulf [1]

[1]*Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*

Preventing disease transmission between and within farms by implementing biosecurity in farms is an important step towards a more preventive approach in veterinary medicine. This study aimed to better understand possible motivators and obstacles for its implementation in farms by letting people of different farming sectors (pigs, poultry, cattle) judge biosecurity measures for efficacy, feasibility and return-on-investment. Highest scoring biosecurity measures were those meant to limit infection pressure, such as cleaning and disinfection of vehicles and stables and a carcass storage separated from the stable. Other high scoring measures were those preventing disease entrance on farm: herd specific clothes and boots and a hygiene lock. The biosecurity measures scoring lowest were showering and an animal free period before entering the stable. These measures were considered especially unfeasible. Across species the same trends could be noticed, yet for pigs and poultry the answers were on average substantially higher than for cattle.

Het voorkomen van ziektetransmissie tussen en binnen bedrijven door het implementeren van bioveiligheid op deze bedrijven is een belangrijke stap naar een preventieve aanpak binnen de diergeneeskunde. Deze studie was erop gericht om mogelijke drijfveren en belemmeringen voor implementatie van bioveiligheid beter te begrijpen door bioveiligheidsmaatregelen te laten beoordelen door mensen uit verschillende landbouwsectoren (varkens, pluimvee, rundvee) op efficiëntie, haalbaarheid en rendement. De hoogst scorende waren bioveiligheidsmaatregelen bedoeld om de infectiedruk te verlagen, zoals reiniging en desinfectie van voertuigen en stallen, en een kadaveropslagplaats, afgescheiden van de stallen. Andere hoog scorende maatregelen voorkomen ziekteinsleep op het bedrijf: bedrijfskleren en –laarzen, en een hygiënesluis. Het laagst scorend waren douchen en een diervrije periode voor het binnengaan van de stal. Deze maatregelen werden vooral niet haalbaar bevonden. Over de diersoorten heen konden dezelfde trends worden opgemerkt, hoewel voor varkens en pluimvee de antwoorden gemiddeld duidelijk hoger lagen dan voor rundvee.

1. INTRODUCTION

Farm biosecurity includes the prevention of disease transmission within and between farms and is important to establish the shift from cure to prevention in veterinary medicine (Lin et al., 2003; Villarroel et al., 2007; Derks et al., 2013; Laanen et al., 2013). This concept is well established in pig and poultry production and also in cattle production the potential to implement biosecurity is present (Laanen et al., 2010; Gelaude et al., 2014; Sarrazin et al., 2014). However, there is still room for improvement in all species. To identify key elements of known biosecurity measures and the motivators and obstacles for their implementation, a workshop was organized by European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-AGRI).

2. METHODS

Sixty-one participants involved in animal production including farmers or their representatives, researchers, farmer advisors, practicing veterinarians, animal health services and representatives of trade, industry, government or the European Union from 22 European countries attended the biosecurity workshop in Brussels in January 2015. Respectively 22, 26 and 13 of the attendees represented the cattle, pig and poultry sector. Each of these participants evaluated 21 biosecurity measures for their own species of interest by considering for each measure the assumed application in their country, expected efficacy in disease prevention, expected feasibility to implement in a farm and expected return on investment (ROI) on a 5-level Likert scale. The 5-level Likert scale was set up to assign a score of -2 to +2 to each biosecurity measure. These biosecurity measures were overall the same for the three species, yet some were species-specific. The results were analysed using basic descriptive analysis in Microsoft Excel.

3. RESULTS

Scoring well across the species for the combination of feasibility and efficacy are biosecurity measures meant to limit infection pressure, such as cleaning and disinfection of transport vehicles before entering the farm, cleaning and disinfection of the stables after every production round and a carcass storage separated from the stable (top 3 ranking across the species). Preventing spread between groups by using holding specific equipment scores well, except for poultry where it has one of the lowest scores of all measures (rank 17/21). Scoring among the top in two of the animal species are measures which prevent disease entrance on the farm by use of herd specific clothes and boots and passing a hygiene lock before entering the stable. Also rodent control is considered important. All of these biosecurity measures are expected to have a high ROI. They contain measures of internal and external biosecurity, showing it is considered important to adhere to both. The biosecurity measures scoring lowest (rank 20 and 21 in each species) were showering and an 24 hour animal free period before entering the stable. Both measures scored consistently low on feasibility.

Except for these common ones, some species-specific remarks can be made. In cattle a separate box for parturition is the biosecurity measure scoring highest on the combined score for feasibility and efficacy, as well as for ROI. Also scoring high for the combined score of feasibility and efficacy is the avoidance of contact with animals from another farm. The measures considered to be most feasible for cattle were a rodent control program and a carcass storage separated from the stables. The measures judged most efficient were avoidance of contact with animals from other farms, cleaning and disinfection of transport vehicles before entering the farm and of the stables after every production round. Other low scoring measures were an insect control programme and following strict working lines. Drinking water analyses were considered to be feasible but not efficient. All low scoring measures were considered to have a low return on investment, including separation of the farmyard into a clean and dirty part (scoring low on feasibility).

For pigs multiple biosecurity measures score high on all three criteria and most of these have been mentioned among the measures scoring high across the species: herd specific clothes and footwear, cleaning and disinfection of transport vehicles before entering the farm and of the stables after every production round, followed by holding specific equipment, passing a hygiene lock before entry of the stables. A high scoring measure that did not return in the other species is the prohibition of employees to work on other pig farms. Drinking water analyses, avoid access to pets in the stable and a carcass storage separated from the stables scored high on feasibility, with a lower score on efficacy. Strict separation of different age groups in compartments and a strict all-in, all-out system per batch for each age category had a high score specifically for efficacy and a lower one for feasibility. These last measures also had a high score for ROI. Only a systematic insect control programme could be found as an additional low scoring biosecurity measure for pigs.

For the poultry sector, no additional high scoring biosecurity measures could be found. Showering as a low scoring measure scored especially low on feasibility. The other low scoring measures including a systematic insect control programme scored higher on feasibility, but are considered less efficient. Only one measure had a negative score for ROI, the prohibition of partial depopulation.

It is striking that the answers for pigs and poultry are on average substantially higher than for cattle. Respectively the results for pigs and poultry were 17.5% and 15% higher for efficacy, 15% and 22.5% for feasibility and 20% and 15% higher for ROI, respectively).

4. DISCUSSION

Across species the same trends were noticed though for cattle the scores for efficacy, feasibility and expected ROI were substantially lower than for the other species. People working in cattle seem to have a less positive view on the expected effects of biosecurity in comparison to pig or poultry production. One third of all biosecurity measures judged for cattle obtained on average a negative score for ROI, while this was not the case for any measures for pigs and only for one in poultry.

The fact that using holding specific equipment only scored low in poultry can likely be explained because in a poultry stable little equipment is used during a production round.
As measures that aim to prevent disease entrance on the farm, such as use of herd specific clothes and boots and passing a hygiene lock before entering the stable as well as measures to limit infection pressure, such as cleaning and disinfection of transport vehicles and stables and a carcass storage separated from the stable, and measures to prevent spread between groups by using holding specific equipment scored high, both internal and external biosecurity were considered important.

Biosecurity measures receiving low scores were those considered especially unfeasible. The ROI for the low-scoring biosecurity measures common among the three species, showering and a 24 hour animal free period, is lowest of all measures as well, since high investments may be required to implement them. Moreover, limiting a veterinarian to perform only one visit a day seems unrealistic. Overall, feasibility seems to be deemed more important than efficacy to assess ROI. This is most clear in the answers for pig production, but can be seen in the other species as well. For example a systematic insect and rodent control programme that scored high across the species for feasibility and scored lower for efficacy, also had higher scores for ROI compared to keeping new animals in quarantine for at least 4 weeks, which scored lower for efficacy and higher for feasibility. A remarkable exception is the drinking water analysis which obtained negative scores for both efficacy and ROI in cattle, but this was still considered quite feasible.

As a first step in improving their awareness towards biosecurity, people in the cattle sector should be informed of possible consequences and benefits of raising the biosecurity level. Yet also in the other sectors, benefits should still be further communicated. Analysing the results of this workshop gave us a clear ranking of the judged biosecurity measures. The higher ranked measures can be communicated towards farmers to help them implement biosecurity on their farm, while for the lower ranked measures it should be investigated why they were judged so low. If they are indeed infeasible, solutions might be found to improve them.

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REDUCING ANTIMICROBIAL USAGE IN PIG PRODUCTION
WITHOUT JEOPARDIZING PRODUCTION PARAMETERS

Merel Postma [a] [*], Wannes Vanderhaeghen [b], Steven Sarrazin [a], Dominiek Maes [c], Jeroen Dewulf [a]

[a] *Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820, Merelbeke, Belgium*
[b] *AMCRA (Center of Expertise on Antimicrobial Consumption and Resistance in Animals), FPS Health, Food chain safety and Environment, Eurostation II, Place Victor Horta 40 box 10, 1060 Brussels, Belgium*
[c] *Porcine Health Management Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820, Merelbeke, Belgium*
[*] *Corresponding author: Belgium, Tel: +32 9 264 75 48. E-mail address: Merel.Postma@ugent.be (M. Postma).*

ABSTRACT

Background

Antimicrobial usage (AMU) is high in pig production. Little information is known about interventions to reduce usage.

Objective

Evaluation of extent to which AMU could be reduced in pig production by optimization of herd management, biosecurity status, vaccination strategy and advice on prudent use of antimicrobials.

Materials/Methods

Intervention study in 61 Flemish pig herds, three visits/herd. A herd specific plan of action was evaluated in a period of +/-8 months.

Results

Significant improvement of biosecurity, combined with improved vaccination, anthelmintic therapy and prudent AMU. A significant reduction of 52% in AMU from birth till slaughter and 32% for breeding animals (treatment incidences). Technical results improved; number of weaned piglets/sow/year (+1.1), daily weight gain (+5.9 g/day), finisher mortality (-0.9%). An economic model found a net profit of €2.67/finisher or €42.99/sow/year.

Discussion

Guided interventions as a team effort of farmer and herd veterinarian/advisor are a promising method in reduction of AMU in pig production.

SAMENVATTING

Achtergrond

Antimicrobieel gebruik (AMG) is hoog in varkensproductie. Weinig informatie is gekend over interventies om gebruik te reduceren.

Doelstelling

Evaluatie hoeveel AMG kan reduceren in varkensproductie door optimalisering van bedrijfsmanagement, bioveiligheid, vaccinatie strategie en advies over prudent gebruik van antimicrobiële middelen.

Materialen/Methoden

Interventiestudie in 61 Vlaamse varkensbedrijven, drie bedrijfsbezoeken/bedrijf. Een bedrijfsspecifiek actieplan werd geëvalueerd in een periode van +/-8 maanden.

Resultaten

Significante verbetering bioveiligheid, gecombineerd met verbeterde vaccinatie, anthelmintische therapie en prudent AMG. Een significante reductie van 52% in AMG van geboorte tot slacht en 32% voor fokdieren (behandelingsincidenties). Technische resultaten verbeterden; aantal gespeende biggen/zeug/jaar (+1.1), dagelijkse groei (+5.9g), sterfte vleesvarkens (-0.9%). Een economisch model vond een netto winst van €2.67/vleesvarken of €42.99/zeug/jaar.

1. INTRODUCTION

Reduced and prudent use of antimicrobials in animal production might lead to a halt in the selection of resistant bacteria and could ultimately result in a reversion to susceptibility. Within the animal production sector, pig production is one of the top sectors using antimicrobials (Callens *et al.*, 2012, Filippitzi *et al.*, 2014, Van Boeckel *et al.*, 2015, MARAN *et al.*, 2014) and therefore a substantial decrease in the unnecessary use in pig production is required. To be able to achieve this reduction, it is important to assist and guide farmers in this process, since they are generally aware of the problems and risks, but not sufficiently knowledgeable on the implications on herd level (Visschers *et al.*, 2015). To provide farmers as well as veterinarians and other advisors on farms with good information on these alternatives, it is required that effects of aforementioned alternatives are illustrated in field conditions.

Previous studies have provided information on the association between herd characteristics, disease problems and AMU (Hybschmann *et al.*, 2011, Fertner *et al.*, 2015, Laanen *et al.*, 2013). The EU study of the minapig consortium (Postma *et al.*, 2016, Postma *et al.*, 2016, in press) indicated associations between biosecurity, improved production results (e.g. number of weaned piglets/sow/year) and reduced AMU. Information on national reduction and the related influence on productivity and evolution of diseases is also substantiated (MARAN, 2015, Swedres-Svarm, 2015, DANMAP, 2015). However, herd specific information on the possible reduction in AMU in a longitudinal intervention study over a relatively short period of time at herd level combined with a follow-up of the herds' productivity has been unknown until now. The present study therefore aimed at evaluating the potential reduction of antimicrobial use by promoting prudent usage combined with farm management optimization through the guidance of farmers.

2. MATERIALS AND METHODS

2.1. Herd selection
Motivated farmers with a farrow to finish herd with minimum 150 sows located in Flanders.

2.2. First herd visit
During the first herd visit, information was gathered on herd characteristics, management and technical performances of the herd. In addition, a biosecurity audit was performed by means of the Biocheck.UGent™ (Ghent University, 2010) system. The herd veterinarian was asked to provide information on the purchased antimicrobials.

2.3. Follow up herd visits
Based on the collected information, a herd specific plan of action was prepared focusing on biosecurity interventions and preventive measures including vaccination and the prudent use of antimicrobials. Interventions that were estimated to be feasible by the farmer and herd veterinarian were agreed upon to be implemented in the next coming months. These final agreements were listed and sent to the farmer and veterinarian by email in a summary document highlighting the action points for the forthcoming months.

2.4. Data collection
Data was collected by means of a visual herd inspection during the visit of the herd and a paper and pencil questionnaire about the production results and herd management characteristics. Technical parameters included the following information: the number of weaned piglets per sow per year, growth performance (g/day) for the finisher period, mortality till weaning, mortality in the nursery, mortality in the finisher period and feed conversion rate (FCR) of the year preceding the visit.

2.5. Quantification of antimicrobial usage
Antimicrobial usage was quantified by calculating the "treatment incidence" (TI) per herd and per age category as described by Timmerman *et al.* (2006).
The TI quantifies how many animals from a theoretical group of 1000 animals receive an AM treatment daily. A TI for standard treatment was calculated for the breeding animals (sows, gilts, boars), suckling piglets, weaned piglets and finishers. Furthermore, the AMU from birth till slaughter was quantified over a standardized lifespan period of 205 days (TI_{205}) for those herds where all age categories from birth till slaughter were present.

2.6. Statistical analysis
Descriptive statistics were performed for relevant variables (e.g. TIs, biosecurity scores, production results). Normal probability tests and plots were examined to check whether assumptions of normality and

homoscedasticity were fulfilled. All statistics were performed using IBM SPSS version 23 (IMB Corp., 2014). Stacked-clustered bar charts were prepared using Microsoft Excel following the method described by Blakeston (2013).

3. RESULTS

3.1. Herd characteristics & herd visits
61 herds were partaking in the intervention period. Herd visits were performed between December 2010 and May 2014. On average 8 months passed between the 2nd and 3rd visit ($\bar{x}$= 8.20, SD= 3.8).

3.2. Initial production parameters and preventive measures
The main production characteristics are described in Table I. On average 2.6 anthelmintic therapies were applied per year to sows (range 0 (n= 2) - 7.4). Piglets received anthelmintic therapy (including anti-coccidial treatment) on 24 herds (24/53 herds with piglets, 1 unknown) with a minimum of 1 therapy per nursery group and a maximum of 3. Fatteners received on average 2 anthelmintic therapies per round (range 0 (n=2) - 3.5, 1 unknown). The average number of pathogens vaccinated against was 5.4 (range 3 - 10) for sows (52/52 herds) and 1.3 (range 0 - 3) for piglets (46/54 herds). Only 2 herds vaccinated finishers, both only against one pathogen.
The internal biosecurity status at the initial herd visit was on average 49 (range 25 - 84), external biosecurity was higher with a score of 63.5 (range 43 - 95) (Table 3). The highest score was obtained for "purchasing policy" ($\bar{x}$= 85), the lowest for "supply of fodder, water and equipment" ($\bar{x}$= 34).

Table I Average numbers of animals and production results over all farms for visit 1 and visit 3. Significant p-values (p≤ 0.05) for the paired samples t-test are presented in bold font.

	Visit #	n farms	Mean	SD	Minimum	Maximum	p-value
Number of sows	1	52	320.7	252.2	85	1750	0.15
	3	52	328.3	263.1	85	1750	
Number of finisher places	1	57	1456.6	759.8	500	3915	0.57
	3	57	1469.8	772.4	500	3915	
Number of weaned piglets per sow per year	1	49	26.4	2.4	22.2	33.2	**<0.01**
	3	48	27.5	2.8	22.1	34.6	
Mortality till weaning (%)	1	49	12.8	3.5	6.3	21.1	0.68
	3	48	12.6	3.3	6.7	19.1	
Weaning age (days)	1	50	23.1	2.6	19.0	28.0	**0.02**
	3	47	22.6	2.5	18.9	28.0	
Feed conversion ratio finishers	1	47	2.7	0.2	2.3	3.4	**0.01**
	3	36	2.6	0.3	1.5	3.1	
Daily weight gain (g/day) finishers	1	49	667.5	77.7	456.0	782.0	**0.01**
	3	33	675.2	83.2	487.0	801.8	
Mortality in finisher period (%)	1	54	3.2	2.0	0.8	11.5	**0.04**
	3	39	2.6	1.8	0.8	7.6	

3.3. Initial antimicrobial usage
The average TI_{205} for standard treatments was 121.16 (SD= 120.63, n= 53), implying that on average over these farms 121 out of 1000 finisher pigs received a daily dose of antimicrobials for standard treatment purpose, or an average finisher pig on these farms was treated with antimicrobials in the framework of standard treatments during 12% of their lifespan from birth till slaughter. The usage in the different age categories is described in Table 4.

3.4. Interventions
For biosecurity and herd management a total of 39 different interventions were defined. On average over the herds 12 biosecurity/management interventions were deemed feasible and eventually on average 8 (range 0 – 15) of the interventions were implemented. For vaccination, anthelmintic therapy and preventive measures, on average 6 advices were deemed feasible and 5 were implemented (range 0 – 12), out of 38 interventions given across all farms. Out of 38 herds where the advice was given, 25 altered their vaccination scheme. This included mainly additional vaccination against atrophic rhinitis, *E. coli*, Glässers disease, influenza and porcine circovirus 2 (Table 3), and was often combined with additional diagnostic testing (n= 38 out of 56 where the advice was given). Over

all farms, 15 different advices were given for prudent antimicrobial treatment; on average 4 interventions were perceived to be feasible and 3 (range 0 – 7) were eventually implemented.

3.5. *Visit 1 versus visit 3*

The average number of weaned piglets per sow per year increased significantly with 1.1 piglet. Significant beneficiary changes were also observed in the feed conversion rate (FCR) (-0.1), daily weight gain (+5.9 g/day) and mortality in the finisher period (-0.4%) in paired samples. Furthermore, the weaning age reduced significantly with 0.4 days (Table 1).

Biosecurity levels improved in general. On average, external biosecurity improved significantly with 2.4 points, mainly consisting of significant improvements of its subcategories "purchasing policy" (+3.5), "removing animals, manure and carcasses" (+4.0) and "vermin and bird control" (+4.4) (Table 3). The internal biosecurity improved substantially with an overall average increase of 7.0 points.

AMU reduced on average over the herds in all age categories. For the overall period from birth till slaughter, the reduction was 52.0% (95% confidence interval in reduction in TI = -76.9 (upper bound), -191.3 (lower bound)) (Figure 1). A linear regression model for the association between the reduction in AMU (visit 3 minus visit 1) and the rank of initial AMU showed that high users in piglets, finishers and breeding animals were able to reduce AMU significantly more compared to initially low-users. The use of critically important antimicrobials was largely reduced.

Figure 1 Graphical visualization of the AMU reduction in breeding animals (sows), piglets, finishers or over the total period from birth till slaughter after advices were given to 61 herds to improve herd management, biosecurity and to use AM more prudently. The values in the bars represent the AMU for prophylactic usage (red) or curative usage (green) and lighter colors represent the results of the AMU after implementation of the interventions. The percentage above the bars represent the percent reduction in AMU that was achieved over all participating herds.
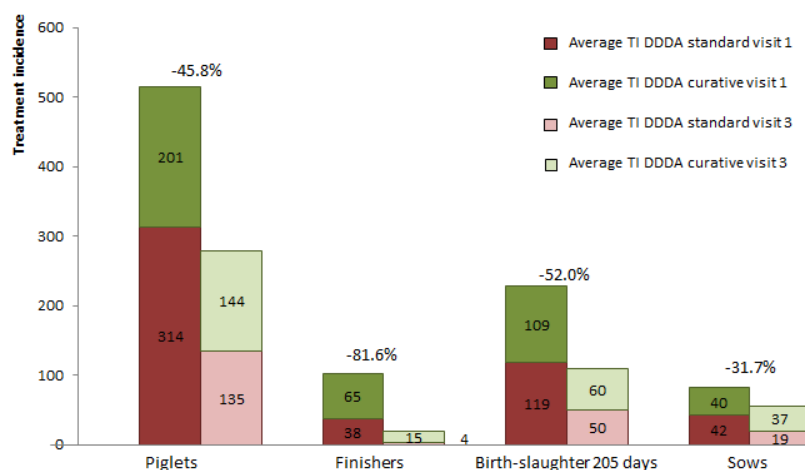
4. DISCUSSION & CONCLUSION

A significant reduction of 52% in AMU from birth till slaughter in the finisher pigs and 32% in the breeding animals was achieved together with improved production results. Breaking routines, through motivation and guidance of farmers on general herd management, improved biosecurity, optimized vaccination and a prudent use of AMU, as a team effort, has shown that the much needed reduction of AMU in pig production is possible. The presented approach could be used by veterinarians and other herd advisors when guiding farmers in the reduction of the AMU in their herds and the subsequent active participation in the fight against AMR.

5. ACKNOWLEDGEMENTS

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IMPROVED DAILY WEIGHT GAIN AND REDUCED MORTALITY AND ANTIBIOTIC USE FOLLOWING ORAL LIVE NON-PATHOGENIC ESCHERICHIA COLI F4 STRAIN VACCINATION IN PIGLETS AGAINST POST-WEANING DIARRHEA

Frédéric Vangroenweghe

Elanco Animal Health Benelux, Plantijn en Moretuslei 1 – 3rd floor, 2018 Antwerpen, Belgium

Post-weaning diarrhea (PWD) remains a major cause of economic losses and excessive antibiotic use to the pig industry. The present study investigated changes in production parameters following the implementation of Coliprotec® F4 vaccination against PWD by F4-ETEC in a commercial pig farm as compared to addition of ZnO or use of a safe dietary formula. Several production parameters were collected: weight (d0-d21-d50), feed consumption, mortality and number of individual treatments. Based on the fecal score and average daily gain (ADG), the addition of ZnO performed better in the first period (d0-21). In the second period (d21-50), however, vaccination against ETEC F4 had higher ADG (+11%) and lower mortality. Treatment incidence was significantly lower in the vaccinated groups (TI = 22) as compared to the safe formulation (TI = 82). In conclusion, vaccination with Coliprotec® F4 results in lower antibiotic use, lower mortality and improved growth.

1. INTRODUCTION

Post-weaning diarrhoea (PWD) remains a major cause of economic losses for the pig industry due to mortality, morbidity, decreased growth rate and cost of medication (Fairbrother *et al*., 2005). PWD typically causes mild to severe diarrhoea after weaning, which can be associated with marked dehydration, loss of performance, and mortality. A number of preventive measures have been recommended in order to prevent and minimize the impact of PWD, including all-in/all-out management of the nurseries, thorough cleaning and disinfection between batches and a draft-free environment, with appropriate constant temperature for the recently weaned pig (Fairbrother *et al*., 2012). Antimicrobials are frequently used on farms to treat PWD. However, emergence of antimicrobial resistance has been reported recently in *E. coli* strains causing PWD (Rosengren *et al*., 2008; Boyen *et al*., 2010; Luppi *et al*., 2013). In addition, feed supplementation with zinc oxide at therapeutic doses may increase *E. coli* antimicrobial resistance (Bednorz *et al*., 2013). The emergence of *E. coli* antimicrobial resistance and the risk of transmission to pathogenic bacteria in humans have increased the need of alternative strategies for preventing and controlling PWD, such as vaccination. Coliprotec® F4 (Prevtec Microbia) is a live non-pathogenic *E. coli* vaccine indicated for active immunization of pigs against enterotoxigenic F4-positive *E. coli* following a single dose oral administration (volume of 2 ml) of piglets from 18 days of age. Coliprotec® F4 reduces the incidence of moderate to severe post-weaning *E. coli* diarrhoea in pigs and decreases the fecal excretion of pathogenic *E. coli* bacteria from infected animals. This study aims to investigate changes in production parameters following the implementation of Coliprotec® F4 vaccination against PWD by F4-ETEC in commercial pig farm.

2. MATERIALS AND METHODS

2.1. Selection of the farm
The farm was managed according to a 4-week batch-management system with weaning at 21 days of age. The farm had a history of PWD in nursery pigs in which the disease is typically observed within 1 week after weaning as evidenced by typical clinical signs (sudden death, diarrhoea, decreased feed consumption, dehydration and depression). Laboratory confirmation of the involvement of F4-ETEC as cause of PWD by PCR identification of virulence factors was obtained in the previous production group. Coliprotec® F4 vaccination was administered at 18 days of age, 7 days before the onset of clinical symptoms of diarrhea.

2.2. Experimental design
Complete litters were randomly allocated to one of the following groups (1 to 5) on the day of vaccination. Sow parities were homogeniously distributed across all treatment groups. At weaning, each group contained 128 piglets,

distributed over 4 feeders and 8 pens with 16 piglets in each pen. All details on different treatment groups are given in Table 1.

Table 1. Description of treatment groups

Group code	Coliprotec® F4 vaccination	ZnO addition (first 14 days)	Safe formulation with barley	# phases	Protein content (%)
A	X			1	16.9
B	X			2	18.5 – 17.0
C	X			3	16.6 – 17.2
D			X	3	16.1 - 17.2
E		X		3	16.5 – 16.3

Pigs in all groups were kept separately during the study. Before weaning, mixing of vaccinated and non-vaccinated litters was avoided. After weaning, treatment groups were housed separately per 2 pens at 1 feeder. However, all piglets of all treatment groups were in the same nursery compartment with identical housing conditions. Same type of feeders, feed composition and drinkers will be used in all groups. Depending on the farm layout, different biosecurity measures will be implemented to avoid the transmission of faeces between groups (i.e. boot covers).

2.3. Clinical examinations.
Pigs will be inspected daily and any unusual clinical findings, including but not limited to altered behaviour, ambulation and appetite will be recorded individually. The presence of diarrhoea will be assessed daily at pen level for the first 14 days after weaning according to the faecal score system in Table 2.

Table 2. Faecal score system.

Fecal score	Definition	Description
0	Normal	Normal faeces, solid.
1	Slight	Pasty, soft faeces.
2	Mild	Presence of liquid but more solid particles than liquid
3	Moderate	More liquid than solid particle.
4	Severe	Liquid, watery, projectile faeces.

2.4. Production parameters
The following production parameters were registered throughout the trial: weight at weaning (d0), weight after the 1st phase (d21) and weight at end of nursery (d50); feed consumption (kg feed per feeder), mortality (day of mortality, weight) and number of individual treatments (injections per pen, reason for treatment).

2.5. Antibiotic treatments
Individual antibiotic treatments for PWD, when necessary due to the critical state of the pigs and according to the clinical criteria of the farm veterinarian, were used in all treatment groups. Same product, active principle, formulation and dose were used in all groups. Based on local legislation (no enrofloxacin on Belpork certified pig farms) and the upcoming ban on some critically important antibiotics (colistin), the antibiotic choice for treatment has been decided to be injectable lincomycin-spectinomycin (Linco-Spectin; Zoetis). Treatment incidence (TI per 1000 piglets at risk d0-30) was calculated as previously described (Timmerman *et al.*, 2006).

2.8. Data processing and statistical analysis.
Data generated in this study were analysed for differences between treatment groups using the Student's t-test with correction for multiple comparison.

3. RESULTS

The results on fecal score, production parameters and antibiotic treatments are summarized in Table 3. The AUC of the fecal score during the 1st period (d0-21) was significantly lower in the group with ZnO addition as compared to both the group on a safe dietary formulation and 2 (A, C) of the Coliprotec® F4 vaccinated groups. Total weight gain over the entire post-weaning period was only numerically different between the group with ZnO addition and the other groups. Average daily gain was significantly lower in the group with ZnO addition as compared to the other groups. Treatment incidence was significantly higher in the group with the safe diet formulation as compared to the other groups.

Table 3. Summary of the fecal score (AUC d0-21), total weight gain (d0-21; d21-50; d0-50), average daily gain (d0-21; d0-50), feed consumption (d0-21; d21-50; d0-50) and treatment incidence (TI per 1000 piglets at risk d0-30). Statistical differences (P<0.05) are shown with different superscripts.

Group		A	B	C	D	E
	Treatment group	Coliprotec® F4	Coliprotec® F4	Coliprotec® F4	Safe diet	ZnO addition
Parameter	*Period*					
Fecal score (AUC)	d0-21	42.88 [a]	41.63 [ac]	43.13 [a]	52.63 [a]	15.75 [c]
Total weight gain (kg)	d0-21	5.29 [a]	5.34 [a]	5.31 [a]	5.33 [a]	5.44 [a]
	d21-50	7.45 [ab]	7.69 [ab]	7.42 [ab]	7.01 [b]	8.74 [a]
	d0-50	18.01 [a]	18.41 [a]	18.16 [a]	17.50 [a]	16.94 [a]
Average daily gain (g/d)	d0-21	106 [a]	110 [a]	97 [a]	71 [a]	157 [a]
	d0-50	248 [a]	258 [a]	254 [a]	230 [a]	226 [b]
Feed consumption (kg)	d0-21	4.23 [a]	4.05 [a]	3.80 [a]	3.29 [b]	5.37 [c]
	d21-50	17.19 [a]	16.53 [a]	16.57 [a]	17.07 [a]	16.25 [c]
	d0-50	21.43 [a]	20.58 [a]	20.28 [a]	20.36 [a]	21.62 [a]
Treatment incidence	d0-30	19.8 [a]	19.0 [a]	27.3 [a]	82.0 [b]	2.9 [c]
Mortality (%)	D0-50	4.7	3.9	3.1	12.5	7.0

3. DISCUSSION

The presented results clearly show that the frequently used safe diet formulations for post-weaned piglets under Belgian conditions do not prevent severe clinical outbreaks of PWD without supplementary additions of antimicrobial agents or ZnO. Based only on clinical evaluation of fecal scores, which is frequently the only evaluation under practical farm conditions, addition of ZnO at 3000 ppm for a maximal period of 14 days results in the best piglet performances. However, after the end of the ZnO supplementation, piglet performances gradually decreased in the 2nd production phase, resulting in the lowest total weight gain (-8%) and overall average daily gain (-12.5%). Vaccination with Coliprotec® F4 clearly resulted in a serious decrease of treatment incidence as compared to the standard used safe diet formulation group. The group with ZnO addition had the lowest treatment incidence, however, the aspect of potentially increased *E. coli* antimicrobial resistance (Bednorz *et al.*, 2013) following feed supplementation with zinc oxide at therapeutic doses should also be considered. Moreover, no significant differences related to the different feeding strategies could be observed among the 3 vaccinated groups, which implies that protection against the pathogenic *E. coli* bacteria is more important in obtaining good performances relative to the specific details of the chosen feeding strategy. In conclusion, vaccination against ETEC F4 using a live non-pathogenic *E. coli* strain results in lower antibiotic use, reduced mortality and improved daily weight gain.

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THE INFLUENCE OF INTRODUCING ANTIMICROBIAL USE GUIDELINES ON PRESCRIPTION HABITS IN FIRST-LINE, SMALL ANIMAL VETERINARIANS

S. Sarrazin[1], M. Schlepers[1], F. Vandael[1], A. Van Cleven[1], E. De Graef[2], H. De Rooster[3], J. Dewulf[1]

[1]*Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.*
[2]*Centre of Expertise on Antimicrobial Consumption and Resistance in Animals, Eurostation II, Victor Hortaplein 40 mailbox 10, 1060 Brussels, Belgium.*
[3]*Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium.*

A prospective study was used to evaluate the impact of introducing antimicrobial use guidelines on the prescription habits of fourteen first-line, small animal veterinarians during first consultations of cats and dogs, one month prior to the implementation of antimicrobial use guidelines and at least 20 days thereafter. The guidelines listed first, second and/or third choice antimicrobials per condition based on scientific literature regarding efficacy. The proportion of consultations where antimicrobials were prescribed decreased in cats and dogs after the introduction of the antimicrobial use guidelines. There was an increase of consultations of cats and dogs where veterinarians handled according to those guidelines: 1) antimicrobials prescribed only when indicated and 2) only a first (or second) choice antimicrobial was prescribed. However, an increase in the prescription of third choice antimicrobials and highest priority critically important antimicrobials both in cats and dogs was noticed.

In deze interventiestudie werd de impact van richtlijnen omtrent verantwoord antibioticumgebruik bij veertien eerstelijns dierenartsen bij consultaties van kat en hond onderzocht door het voorschrijfgedrag voor en na de introductie van deze richtlijnen te vergelijken. Gebaseerd op wetenschappelijke literatuur geven deze richtlijnen per aandoening een overzicht van eerste, tweede en derde keuze antibiotica. Na de introductie van deze richtlijnen daalde zowel bij kat en hond het aantal consultaties waar antibiotica werden voorgeschreven en er was een stijging in het aantal consultaties waarbij gewerkt werd volgens de richtlijnen. Dit hield in dat antibiotica enkel werden voorgeschreven wanneer ze ook geïndiceerd waren en dat enkel een eerste keuze (of tweede keuze) antibioticum werd voorgeschreven. Verder werd bij kat en hond echter ook een stijging in het voorschrijven van derde keuze en kritisch belangrijke antibiotica vastgesteld na de introductie van de richtlijnen.

1. INTRODUCTION

The use of antimicrobials in small animals, as in any other animal species including humans, can promote the selection of less susceptible and resistant bacteria (Dewulf *et al.* 2007, Soares Magalhães *et al.* 2010, Chantziaras *et al.* 2013, Burow *et al.* 2014). Currently there is a lack of accurate and complete data from small animal practices regarding the use of antimicrobials in cats and dogs in Belgium. An expert group in human medicine defined five strategic goals to optimize antimicrobial use among which the development and implementation of antimicrobial use guidelines (Goldmann *et al.* 1996). The Belgian Centre of Expertise on Antimicrobial Consumption and Resistance in Animals (AMCRA) developed guidelines for prudent use of antimicrobials in cats and dogs to support veterinarians in the choice for an antimicrobial agent when treatment is necessary. The aim of this study was to evaluate the impact of introducing these antimicrobial use guidelines on prescription habits of veterinarians in small animal practices.

2. MATERIALS AND METHODS

The antimicrobial use guidelines concern 25 conditions and described what requirements should be met for diagnosis and whether antimicrobials (first, second and/or third choice) are indicated (at www.e-formularium.be). From the participating practices the consultations from at least a month prior to the visit were extracted from the practice management system. Next, the antimicrobial use guidelines were explained and distributed as a pocket-size booklet. The veterinarians were asked to implement the guidelines as much as possible when treating cats and dogs during at least twenty working days. The clinical conditions included in the study were listed in six groups:

skin, respiratory tract, digestive tract, urogenital tract, ear and other (osteomyelitis, sepsis and antimicrobial prophylaxis).

The first outcome of interest was whether a veterinarian had prescribed antimicrobials during a consultation. A second outcome of interest was whether a veterinarian had handled according to the antimicrobial use guidelines during a consultation: 1) a clear indication to use antimicrobials as part of the treatment protocol was present and 2) a first (or second) choice antimicrobial was selected. For each outcome of interest a generalized linear mixed model was fitted to examine the association with the introduction of the antimicrobial guidelines, the clinical condition and the self-reported frequency of working according to the guidelines (sometimes versus most of the times). To examine the strength of the association between the prescription of antimicrobials and whether antimicrobials were indicated according to the guidelines, odds ratios (OR) and Cohen's kappa coefficients were estimated with 95% confidence intervals (CI) (McHugh 2012).

3. RESULTS

3.1. Cats

Antimicrobials were prescribed in 72% (142/198) and in 60% (148/247) of the consultations before and after the introduction of antimicrobial use guidelines, respectively. Although a considerable decrease was noticed, the introduction of antimicrobial use guidelines did not significantly influence the proportion of consultations where antimicrobials are prescribed ($P = 0.71$). The prescription pattern varied significantly with the clinical condition ($P < 0.001$). As expected, antimicrobials were more likely prescribed when indicated by the guidelines, both before (OR 6.0, 95% CI 1.8; 20.4) and after (OR 6.5, 95% CI 2.5; 17.2) the introduction of the guidelines. However, it was also noticed that a very substantial amount of prescriptions were actually not indicated, 54% (106/198) before and 45% (110/247) after the introduction of the guidelines, respectively. The corresponding values for Cohen's kappa (0.13 and 0.17 before and after, respectively) indicate only a slight agreement between the prescription and the actual indication of antimicrobials. The percentage of consultations handling according to the antimicrobial use guidelines increased from 30% to 43% after introduction of antimicrobial use guidelines ($P = 0.24$). Handling according to the guidelines varied significantly with the clinical condition ($P < 0.001$). An overall significant shift in the prescription pattern concerning first, second and third choice antimicrobial as well as off-label products was noticed after the introduction of antimicrobial use guidelines ($P = 0.02$). The relative number of prescriptions of second choice antimicrobials decreased by 16%, while the prescription of first choice (+4%), third choice (+8%) antimicrobials and off-label products (+3%) increased. Furthermore, the relative number of prescriptions of highest priority critically important antimicrobials increased by 12% ($P = 0.02$) after introduction of the antimicrobial use guidelines.

3.2. Dogs

Antimicrobials were prescribed in 80% (277/347) before and in 68% (356/522) of the consultations after the introduction of antimicrobial use guidelines. Similarly as for cats, this considerable decrease was not significantly associated with the introduction of antimicrobial use guidelines ($P = 0.49$). The prescription pattern varied significantly with the clinical condition ($P < 0.001$). Antimicrobials are more likely prescribed when indicated by the guidelines, both before (OR 2.3, 95% CI 1.2; 4.3) and after (OR 4.4, 95% CI 2.6; 7.2) the introduction of the guidelines (Table 4). Again a considerable amount of prescriptions were actually not indicated, 49% (170/347) before and 42% (218/522) after the introduction of the guidelines, respectively. The corresponding values for Cohen's kappa (0.09 and 0.20 before and after, respectively) indicate only a slight agreement between the prescription and the actual indication of antimicrobials. The percentage of consultations handling according to the antimicrobial use guidelines increased from 35% to 45%. This increasing trend was marginally significantly associated with the introduction of antimicrobial use guidelines ($P = 0.13$). Handling according to the guidelines varied significantly with the clinical condition ($P < 0.001$). An overall significant shift in the prescription pattern concerning first, second and third choice antimicrobial as well as off-label products was noticed after the introduction of antimicrobial use guidelines ($P = 0.04$). The relative number of prescriptions of off-label antimicrobials decreased by 10%, while the prescription of first (+5%), second (+1%) and third (+5%) choice antimicrobials increased. Furthermore, the relative number of prescriptions of highest priority critically important antimicrobials increased by 5% ($P = 0.06$) after introduction of the antimicrobial use guidelines.

4. DISCUSSION

The observed improvements in number of consultations without use of antimicrobials and according to the guidelines were not statistically significant when taking into account the clustering of the results within the veterinary practices. This is likely the result of the fact that the effect of the introduction of the guidelines was not

consistent over the different veterinarians and suggests that some veterinarians are more open to advice and follow-up of guidelines whereas others are more reluctant. It warrants further research to find out what the true hurdles were that withheld some of the participants to apply the guidelines.

The results clearly indicate that antimicrobials were prescribed far more often than indicated both before and after the implementation of the antimicrobial use guidelines, showing that there is still a large margin for reduction of antibiotic use in small animal medicine. There is a need for further understanding how veterinarians can be persuaded to adhere more to prudent use guidelines to reduce the use of antibiotics and the risk of resistance selection.

Notable was the unexpected increase of the prescription of third choice antimicrobials in cats and dogs, in particular the prescription of cefovecin increased substantially. Cefovecin is a broad-spectrum antimicrobial registered for the treatment of cats and dogs and is classified among the highest priority critically important antibiotics. In cats, the antimicrobial activity following a single injection lasts up to 14 days (SPC Convenia 2013). Previous studies have indicated that the ease of administration is the key factor explaining the popularity of this antimicrobial (De Briyne *et al.* 2013), as also stated by the participating veterinarians. The preference is likely also influenced by anticipated low owner compliance in administering a short-term oral antimicrobial therapy (Grave and Tanem 1999). However, the benefit of the therapy compliance guaranteed by a single injection should not overrule the more important considerations related to the antimicrobial resistance selection which is of importance both for animals and humans.

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Poster presentations

QUALITATIVE SPATIAL ASSESSMENT OF HUMAN TBE RISK FACTORS IN BELGIUM

Sophie Roelandt, Els Ducheyne, Vanessa Suin, Steven Van Gucht, Stefan Roels, Guy Hendrickx and Yves Van der Stede
sophie.roelandt@coda-cerva.be

Mapping is a risk-based method that has been used particularly in vector-borne diseases to identify the areas and time periods in which surveillance is more likely to successfully detect emerging health threats at an early stage. This greatly benefits governments and public health agencies and should be performed in interdisciplinary collaboration. Mapping cases and risk factors to study TBE(V) tick borne encephalitis (virus) emergence and epidemiology has been used in many endemic countries.

In QGIS vector layers, we first mapped the sampling frames and gold standard seroneutralisation seropositive sample results collected from three previously published TBEV studies in veterinary sentinel: in dogs (Roelandt et al., 2011), in cattle (Roelandt et al., 2014) and in wild boar (Roelandt et al., 2016). Since Belgium currently has no confirmed autochthonous human TBE cases, 9 clinically suspected human patients with inconclusive serological results were also added. The vaccination and exposure history of these patients is unfortunately unknown (Source data: WIV-ISP; 2009-2013). From the very abundant literature on TBE risk factors, Belgian raster data (1 km2 pixel resolution) was acquired on eight frequently mentioned TBE risk factors (RF). This included roe deer and wild boar predicted densities, cattle and small ruminant actual densities, an I. ricinus tick distribution/density layer, Corine Land Cover 2006 forest presence/absence, human proximity indices to forests ("human exposure"), and the monthly cooling rate during Autumn (August to December).

The TBE-risk of each pixel was then defined by defining a cut-off using the distribution of each RF-layer. We selected the most extreme 20% (upper or lower) quantiles for each layer, except for the forests (presence/absence). Each selection of 20% represents the more extreme cases at the higher end of the TBE risk scale (risk present). All other quantiles were considered to be not at risk or at very low risk (risk absent). In a third step, the number of risk factors per 1 km2 was calculated by simply summing the number of risk factors present per pixel. The resulting qualitative TBE risk factor presence map with RF color code (Range: 0-6 RF's present) will be presented, including the serologically positive municipalities ("Cases"). Finally, the average number of TBEV risk factors for tested infected municipality was calculated and compared to the average number in the tested free municipalities by the one-sided Mann-Whitney-U test (significance: $p<0.05$).

The final map showed a subjective overlap of animal sentinel seropositive communities in areas with higher numbers of risk factors, as well as overlaps between species seropositivity in all Belgian sentinel studies (dog-human-roe deer-cattle-wild boar). This association, however, was only statistically significant for the combined data of cattle and wild boar (MWU: $p<0.05$). We will critically discuss our general approach to this first and qualitative TBE risk (factor) mapping exercise for Belgium, the results obtained and the potential improvements that should allow for future predictive modelling and spatial risk mapping. In conclusion, TBEV does seem to be present in Belgium, where it would be expected to emerge based on TBE risk factors, despite previous negative predictions.

PERFORMANCE OF BROILERS AND BROILER BREEDERS ASSOCIATED WITH REOVIRUS VACCINATION PROGRAM

Peter De Herdt, Marlies Broeckx, Filip Van Driessche, Bart Vermeiren, Geert Van Den Abeele and Stefaan Van Gorp
peter.de.herdt@merck.com

A vertically integrated monitoring program was set up for breeders hatched in 2013 and their offspring, to detect differences in performance related to the reovirus vaccination schedule.

All within the same organization in Belgium, 17 breeder flocks were vaccinated with one dose of live and one dose of inactivated reovirus vaccine while 14 flocks received 2 doses of inactivated vaccine without live priming. The hatchability of the eggs produced by these birds was examined. Further, the daily growth, feed conversion, chick mortality, slaughterhouse condemnation, production index and antibiotic use were monitored in 110 broiler flocks derived from the breeders. All gathered data were examined statistically.

In eggs obtained from breeders vaccinated twice with inactivated reovirus vaccine, a significant 2.88% higher hatchability was observed. The progeny broiler flocks of these breeders showed a significant 18.6 % lower chick mortality during the fattening period.

The results may indicate that -under the epidemiological conditions of this study- double administration of inactivated reovirus vaccine in broiler breeders can contribute to higher hatchability of breeder eggs and lower broiler mortality.

A BAYESIAN EVALUATION OF FOUR ANTIBODY ELISA TESTS TO CERTIFY FREEDOM OF BOVINE VIRAL DIARRHOEA INFECTION IN UNVACCINATED CATTLE

S. Roelandt, J.-B. Hanon, M. De Baere, Y. Van Der Stede, C. Faes, A.-B. Caij
sophie.roelandt@coda-cerva.be

Belgium has a Bovine Viral Diarrhea (BVDV) control program in which commercial antibody ELISA kits will be used for the purpose of ascertaining freedom of disease at the herd level, starting July 2017. Therefore, it is important to have an understanding of the field accuracy of these kits. Commercial BVDV Ab ELISA kits were previously evaluated, using Belgian field sera (n=1313) from 19 unvaccinated herds and using VNT as gold standard test. Seroprevalence was 46.10% (npos=603/1308; 95%CI: 43.41-48.81%). However, there are no "perfect" reference tests for in vivo detection of (BVD) infection in adult cattle. Even the VNT has a "grey zone" - 19 samples had VNT borderline-titers and were considered negative - which may favor less sensitive ELISA's. Therefore, four commercial BVDV Ab ELISA tests (Svanova[A] - Idexx[B] - Prionics[C] - Bio-X[D]) were re-evaluated with a no gold standard approach for the purpose of demonstration of freedom of BVDV infection in individual sera from unvaccinated cattle. A total of n=1313 available sera from unvaccinated herds were tested with the four selected ELISA's and in BVDV VNT.

Test results were entered in a "4test1pop" Bayesian model, including 2-way test dependence parameters and uninformative priors for all parameters (UN). As a prior sensitivity analysis, six more prior scenarios were used in the same model: informative priors on test diagnostic accuracy ~ experts and previous work (TEST1-2); informative priors on population prevalence ~ SNT results (PREV1-2); and two scenarios with all informative priors (TP1-2). All models were estimated using MCMC (using two chains, 10,000 iterations used as burn-in, results were based on an additional 50,000 iterations, with thinning of 10); model diagnostic criteria were satisfactory; the Deviance Information Criterion (DIC) indicated little influence of the priors on the posteriors. The accuracy/prevalence parameter estimates were comparable for all scenarios, and are reported from the UN-model (CI: credible interval)

- Diagnostic Sensitivity: Test A: 97.76% (95%CI: 96.08-98.96%) - Test B: 99.86% (95% CI: 99.21-99.99%) - Test C: 99.23% (95%CI: 97.58-99.99%) - Test D: 94.45% (95%CI: 92.13-96.52%);

- Diagnostic Specificity: Test A: 95.24% (95%CI: 92.50-97.47%) - Test B: 91.31% (95%CI: 88.27-93.95%) - Test C: 95.28% (95%CI: 91.26-98.43%) - Test D: 95.18% (95%CI: 92.21-97.33%);

- Population Prevalence: 44.98% (95%CI: 42.17-47.88%)

The four ELISA's together slightly underestimate the population seroprevalence in this model, but the 95% credible interval includes the actual VNT seropositivity (46%). Additional predictive values and Youden's indexes to investigate the performance of the diagnostic tests were calculated within the UN model. These parameter estimates were also high and comparable but some were statistically different (Bayesian p>0.05).

Based on predictive values in the study population and biological interpretation of these accuracy parameters, all tests were sufficiently fit for purpose at the herd level, despite an expected false negative/positive fraction of 1-5% and false positive fraction of 5.5-10% at the individual level.

This no gold standard Bayesian comparative study agreed well with gold standard evaluation and demonstrated that in the Belgian setting all four tests under evaluation possessed high accuracy, though test D could increase the cut-off a little to align DSe/DSp with the other tests. Most observed statistical differences may not be clinically significant at the herd level. Based on predictive values in the study populations, all tests are indeed to a large degree "fit for purpose" for the designated purpose in the Belgian BVD control program. Clinicians and laboratories need to take the predictive test characteristics into account when using them at the individual or herd level diagnostic process, and VNT confirmation testing should be performed for ELISA-seropositive results.

GOOD VACCINATION PRACTICES: IT ALL STARTS WITH GOOD REFRIGERATOR TEMPERATURE

Vangroenweghe F.*, Kelderman K., Vanhooydonck K.
vangroenweghefr@elanco.com

Introduction - Since 2007, stringent measures to reduce antibiotic reduction by 50% in food producing farm animals, including pigs, have been installed in the Netherlands. In Belgium, AMCRA has installed an antibiotic reduction plan with objectives for a strong reduction of antibiotic use by 2020. Due to this antibiotic reduction, a major increase in the use of vaccinations against most currently present swine pathogens, such as M. hyopneumoniae, PRRSv and PCV-2, has been observed. To obtain maximal results from the applied vaccination strategies, the vaccine has to be handled with care from production over storage to application to the target animals under practical field conditions. Based on a survey held in 2014 [1] and on practical on-farm data collection, many farmers and farm workers still do not realize the strong impact of wrong refrigerator temperatures on the quality and efficacy of vaccines. The aim of the current survey was to obtain data on the real in-refrigerator temperatures on farms throughout the Benelux by direct measurement of in-refrigerator temperatures with a digital thermometer during on-farm visits.

Materials and methods - In total, data of in-refrigerator temperatures of over 100 farms have been collected using a digital thermometer. All farms were visited in relation to enteric or respiratory disease problems and were equally distributed throughout the entire Benelux area. The refrigerator sampled was the main refrigerator designated by the farmer to stock the main supply of vaccines on the farm. Data were collected after at least 45 min of temperature measurement using the temperature probe of the digital thermometer.

Results - First analysis shows that only 60-65% of all refrigerators have a well equilibrated temperature range (+2°C to +8°C) suitable for the guaranteed preservation of stocked vaccines. Most extreme situation has been observed with a refrigerator freezing the vaccine vials down to -4°C, resulting in frozen content and subsequent loss of efficacy of the stocked vaccine.

Discussion – Good Vaccination Practices are crucial in obtaining optimal results for vaccine to protect animals from the specified diseases. From the present survey, it is clear that the awareness of refrigerator temperature for vaccine storage is low to unexisting under practical conditions, depending on the farm. With the enormous expansion of farm size during the last decade, it is clear that more hired people are working on several critical task, such as vaccine receipt, storage and administration in swine farms nowadays. The need for regular training on Good Vaccination Practice seems crucial in order to improve the effectiveness of vaccination protocols.

Conclusions - In conclusion, basic knowledge on GVP, and more specifically the continuous monitoring of refrigerator temperatures for vaccine storage, has still room for major improvement in order to maximize efficacy of applied vaccines under Belgian and Dutch field conditions.

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METHODS USED IN A FLEMISH CAMPAIGN TO CREATE AWARENESS ON RESPONSIBLE ANTIMICROBIAL USE IN THE VEAL INDUSTRY

Jozefien Callens, Bart Pardon, Isabelle Degezelle, Stefaan Ribbens, Koen De Bleecker
Jozefien.Callens@dgz.be

Objectives: Antimicrobial consumption in Belgian livestock is among the highest of Europe, and this is translated in high levels of antimicrobial resistance in commensal, zoonotic and pathogenic bacteria. Especially in pigs, poultry and veal calves antimicrobial use is intensive. The Flemish government funded a project (the responsible use of antibiotics in intensive reared livestock) with the objective to create awareness in farmers and veterinarians. The objective of this abstract is to describe the topics communicated, and the ways of communication, used to create awareness about antimicrobial use and resistance in the veal industry.

Materials and Methods: An expert panel consisting of academic staff (n= 3), veterinarians of the animal health service (n=2) and veterinarians active in the veal industry (n=5) was constructed and guided by the Flemish advice organ on veterinary antimicrobial use (AMCRA). Available information on administration ways and indications for antimicrobial use in the veal industry was inspected, and possibilities to reduce were listed. Next, actions to reduce antimicrobial were priority ranked according to their possible contribution to antimicrobial reduction, the likeliness to be implemented at short term and the ratio labor/achieved reduction. Subsequently, the campaign was focused on the top 5 action points. At every stage, outcomes were communicated with the persons involved in the same exercise for pigs and poultry.

Results: Ranking according to the expert panel was as follows: (1) avoiding routine antimicrobial group treatments, (2) avoiding two or more antimicrobials in one treatment, (3) calf quality control at arrival, (4) stable ventilation adjustment, (5) correct dosing of antimicrobials, (6) avoiding oral antimicrobial use, (7) avoiding group treatments and (8) vaccination upon arrival. The first two measures were at the veterinarians discretion and had already been markedly adjusted. The third measure, calf quality control at arrival, could only be influenced by the integrators and not by the farmers directly. Parameters for selection (immunoglobulin levels at arrival, a bodyweight over 50 kg at arrival, no persistent infection with bovine viral diarrhea virus and no diarrhea or respiratory signs) were nevertheless communicated to the farmers. Ventilation audits were performed in four veal farms to collect practical examples including fumigation tests. The main problems on the farms were hypoventilation and draught. To stimulate correct dosing, heart girth measurements were performed on 698 veal calves (6 herds), and the association with body weight was determined by linear regression. In this way, farmers could more accurately estimate body weight by a simple heart girth measurement. Finally, to what considers individual treatments made by the farmer, 'avoiding the use of two or more antimicrobials' was made one of the central one-liners in the communication campaign.

Conclusions: Overall the campaign was focused on a correct stable ventilation, correct dosing of a single antimicrobial with the least importance for human medicine and avoiding group treatments. The methods used and the action points identified in this project might aid other countries with similar industries to create awareness about responsible antimicrobial use.

Comments: Special acknowledgement for the important contribution of Magali Blomme to this project.

CLASSIFICATION OF ADULT CATTLE DISEASES IN BELGIUM BASED ON A VETERINARY AND A LABORATORY SURVEY, YEARS 2013-2015

Véronique Renault, Bert Damiaans, Steven Sarrazin, Marie-France Humblet, Jeroen Dewulf and Claude Saegerman

Bert.Damiaans@UGent.be

Objective

The biosecurity measures and controls in the Belgian cattle industry have been strengthened over the years to improve animal health and the safety and quality of the products. Nevertheless, the level of implementation of those measures remains insufficient due to several perceived constraints (e.g: cost, time and lack of evidence). Biosecurity measures to be applied must be prioritized in accordance with the individual farm conditions and the main diseases affecting cattle. Previous prioritization exercises were neither specific to Belgium nor based on an exhaustive list of diseases. This study aimed at classifying the most important diseases in order to improve the technical guidance of farmers regarding disease control and prevention.

Materials and methods

A list of 72 cattle diseases with occurrences in Europe was developed based on a literature review. Through an online survey, Belgian rural veterinarians were asked to assign a score to each disease according to their frequency, their evolution from 2013-2015 and their perceived importance (list of the top 5 diseases). A total of 107 veterinarians answered the first two questions and 93 the last one. The results of the survey were used to classify the diseases based on their frequency, trends and importance through an additional weighting system.

Additional criteria were taken into account to correct possible underrating (zoonosis of major public health concern or (re)emerging diseases) and overrating (diseases emphasized in recent scientific literature or existing control programs) by the veterinarians. Therefore, regional laboratory databases and previous disease prioritization exercises were also analyzed. A table was developed to synthesize the importance given to each disease based on the outcomes of the veterinary survey, the previous prioritization exercises and the regional laboratories database. Lastly different selection criteria were applied to identify the most important diseases to consider in regard to biosecurity.

Results

From the online survey 28 diseases were selected. Among them eight diseases were listed in the top 5, frequents and with increasing occurrence: mastitis, bovine respiratory disease, diarrhea/enteritis, distomatosis, interdigital dermatitis, neosporosis, paratuberculosis and scabies.

Sixteen additional diseases were identified based on previous prioritization exercises (major zoonosis and/or (re)emerging diseases mainly) and two based on the analysis of the regional laboratory data base.

A total of 46 diseases out of the 72 were therefore classified as important for biosecurity measures implementation.

Conclusion

The list of 46 most important diseases obtained through this study is coherent with the Belgian context as all the communicable diseases in Belgium are included with the exception of lumpy skin disease and yersiniosis. Out of the 46 diseases, 24 are non-communicable diseases of major importance in Belgium due to their economic impact and high occurrence. This additional list could guide the decision makers in terms of future control programs as they do represent a major concern for the cattle owners.

The diseases listed as important will be reviewed to identify their related risk factors and appropriate biosecurity measures in order to improve the technical guidance for farmers.

The table obtained represents a practical tool for other users who could easily adjust the selection criteria to their specific objectives and needs.

EPIDEMIOLOGICAL STUDY OF SALMONELLA IN BELGIAN POULTRY USING MULTILOCUS VARIABLE NUMBER OF TANDEM REPEATS ANALYSIS (MLVA)

Pierré Eva, Brossé Charlotte, Deschuytere Herman, Jasson Vicky, Wattiau Pierre
Eva.pierre@dgz.be

An epidemiological study was performed using multilocus variable number of tandem repeats analysis (MLVA) to (a) define the source of repetitive Salmonella Enteritidis infections in Belgian laying herds, (b) determine if cross-contamination of Salmonella Typhimurium occurred on mixed-species (poultry/pigs) farms and (c) compare Salmonella Typhimurium strains of breeders and broilers.

MLVA was conducted on S. Enteritidis strains isolated from laying herds testing positive at least twice in the past 5 years for this serotype. Mixed-species farms were included in the project if at least one broiler flock was positive for S. Typhimurium at legislative sampling. In that case, six pairs of overshoes were taken spread over all age categories of the pig herd. Salmonella was isolated by standard enrichment method (ISO 6579 annex D - MSRV). Salmonella isolates were typed by MLVA.

Results

Laying hen herds – Salmonella Enteritidis

Farm N°	N° positive samples	N° positive production cycles	N° different MLVA profiles	Same MLVA in different production cycles
1	19	2	3	no
2	7	3	2	yes
3	2	2	1	yes

FARM N°	N° POSITIVE SAMPLES PIGS	N° POSITIVE SAMPLES POULTRY	N° DIFFERENT MLVA PROFILES	SAME MLVA IN DIFFERENT SPECIES
1	1	1	1	yes
2	1	1	2	no
3	1	2	3	no
4	1	2	3	no
5	3	4	2	yes

BREEDING HERDS – SALMONELLA TYPHIMURIUM

N° of breeding farms	N° positive samples	N° different MLVA profiles	N° of MLVA strains detected in both breeders and broilers
6	15	8	1

From this study following aspects may be concluded: (a) S. Enteritidis and S. Typhimurium strains with different MLVA profiles are circulating in Belgian poultry flocks, (b) The same strain can persist on a farm in laying herds or a new strain can be introduced, (c) Indication of spread of S. Typhimurium between animal species, (d) one S. Typhimurium MLVA profile was observed in a breeding as well as in broiler farms. This is no proof of vertical transmission.

This study indicates that MLVA typing can be useful to trace the source of Salmonella on poultry farms and therefore help to take the most appropriate actions on the individual farms.

VEE studiedagen

1993   Epidemiologie en economie in de veehouderij - St. Niklaas

1994   Economische aspecten van dierziektenbestrijding – Klassieke varkenspest: de sector en het beleid – St. Niklaas

1995   Residuen bij antibioticagebruik – Actuele bestrijdingsprogramma's – St. Niklaas

1996   Preventie van overdracht van zoönosen via voedsel door bewaking van de productieketen – Deinze

1997   Biologie en management bij het uitbreken van dierziekten – Boxtel NL

1998   Epidemiologische aspecten van de bedrijfsbegeleiding – Gent

1999   Bedreiging van insleep van ziekten uit de derdewereldlanden, detectiesystemen en economische implicaties – Antwerpen

2000   Preventie van besmetting in de voedselketen – Brussel

2001   Risicoanalyse en kwaliteitsbeheer van de dierlijke producten – Melle

2002   Risk-assessment: dierziekten en bewaking voedselketen – Leuven

2003   Antibioticumresistentie bij landbouwdieren – Torhout

2004   Nieuwe toepassingen van de epidemiologie en de economie in de dierengezondheidszorg – Melle

2005   Survey methodologie – Diepenbeek

2006   De rol van observationele studies bij het identificeren van risicofactoren – Gent

2007   Lustrumviering VEE <> VEEC – Emerging diseases – Wageningen (NL)

2008   Economische gevolgen van infectieziekten bij landbouwhuisdieren: Hoe belangrijk zijn ze? – Leuven

2009   Epidemiologische tools als hoeksteen voor beleid en praktijk – Tervuren

2010   Eradicatie van endemische dierenziekten: hoe ver moeten we gaan? – Roeselare

2011   Is prevention better than surveillance? – Sint-Niklaas

2012   VEE en VEEC organiseren ISVEE 13 in Gent en Maastricht

2013   Impact of globalization on animal health – Drongen

2014   Meeting AESA & VEE - Brussel

2015   Evidence Based Veterinary Medicine - Melle

2016   Host-Pathogen-Environment Interactions - Geel