{ JOINT SYMPOSIUM }

NEW CHALLENGES IN EPIDEMIOLOGY :

HOW DO WE ADAPT ?

FRIDAY, OCTOBER 24TH 2014

PACHECO Center - Brussels

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NEW CHALLENGES IN EPIDEMIOLOGY: HOW DO WE ADAPT ?

Welcome

to the 1st VEE-AESA joint symposium !

It is our pleasure to welcome you in Brussels at the first joint meeting of the Flemish Society for Veterinary Epidemiology & Economics (VEE) and the "Association d'Epidemiologie et de Santé Animale" (AESA). Both societies aim to enhance the interest and education and to stimulate research in the field of veterinary epidemiology and economics of animals, mainly by organizing workshops, conferences and symposia and by providing scientific support in the different areas of veterinary, epidemiology and economics.

Our venue is Brussels and our host is the Federal Agency for Safety of the Food Chain (FASFC). This year's topic of the morning session of this first joint meeting is **"New Epidemiological challenges: how do we adapt?"**
Despite increased interest, research and investment in global animal health and surveillance, the scale of the task is considerable and many challenges remain or new challenges will arise.
During this symposium we will discuss if these epidemiological challenges are as such that our ideas about epidemiological approach should be adapted or not. Recognized Belgian experts with international appeal will try to found answers for questions: What impact have these (new) epidemiological challenges on cost efficiency? What are the major challenges associated with biosecurity & what new intervention methods should be used for this? Together with you, veterinary practitioners, veterinary authorities & stakeholders we will try to find answers.
In the afternoon program, which has a variety of interesting subjects, we will give young scientists the opportunity to present their work during two parallel sessions (oral presentations and posters).
At the end of the presentations, three prizes will be attributed to the best posters by Avia-GIS, the VEE and the AESA.

We thank the sponsors (Zoetis, Life Technologies, Boehringer Ingelheim and IDEXX) for their financial support and the FASFC for its hospitality. A special thanks to the board members of VEE and AESA (for the VEE, Sophie Roelandt, Guy Hendrickx and Jeroen Dewulf and for the AESA, Jean-Yves Houtain, Mathieu Hubaux, Sabine Cardoen and Marc Dispas). We appreciated all the work that had been fulfilled during the organization of this meeting as well during the evaluation of the submitted proceedings, abstracts and posters. We can be proud of the obtained result.

On behalf of the boards of the AESA and VEE we wish you a pleasant and stimulating meeting.

Dr Yves Van der Stede
President of VEE

Prof. Claude Saegerman
President of AESA

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17h00 **Closure drink**

KEYNOTES
LECTURES

Socio-psychological veterinary epidemiology: A new discipline for an old problem ?

Erwin Wauters[1], Cristina Rojo-Gimeno

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Introduction

This paper reviews past evidence on the social dimension of animal health management. Socio-psychological veterinary epidemiology is the study of human behaviour that affects the causes, spread, prevention and control of animal diseases and health problems. Animal health is becoming an increasingly important topic and there is a large body of literature on veterinary and epidemiological approaches to increase our knowledge about animal health and to design animal health interventions. Yet, managing animal health isto a large extent a farm level issue. Ultimately, veterinary and epidemiological solutions and interventions towards better animal health are to be implemented by farmers. As a consequence, researchers have started to study the behaviour of farmers and other stakeholders relating to the implementation of animal health management practices (AHMP). Very recently, the veterinary and epidemiological domain has experienced a rapid increase in behavioural studies investigating the human factor in animal health decisions (Fig. 1).

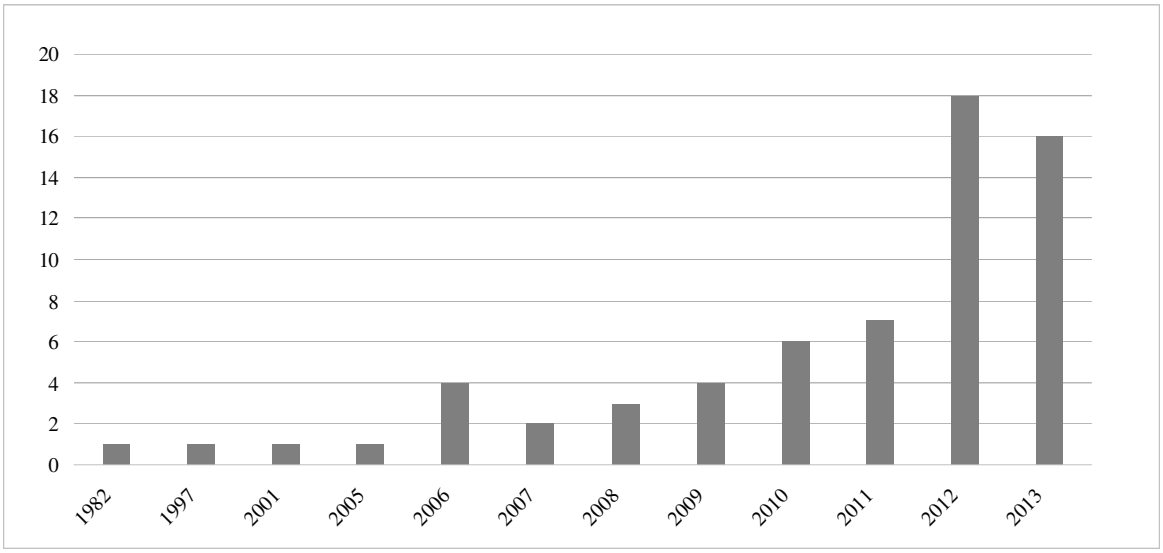

Fig.1 Number of publications related to socio-psychological veterinary epidemiology, per year 1982 - 2013

Inspired by the term social epidemiology, the field that covers research on human attitudes, perceptions and practices influencing human health, we suggest to use the term socio-psychological veterinary epidemiology (SPVE) for this relatively new domain within veterinary epidemiology. As with any developing discipline, it is, from time to time, appropriate to adopt a rear view at both the findings and especially at the assumptions, approaches and methodologies of past studies, in order to provide recommendations for the future development and usefulness of the evolving discipline. This paper provides a literature review of the bulk of past SVPE studies, with two objectives. The first is to summarize and –if possible– draw some universal conclusions on motives, barriers, values and factors that affect how humans manage animal health and welfare in farming systems. The second objective of this literature review is to critically assess the approaches and methods that have been applied and the type of questions that have been studied, in order to provide suggestions for future progress of this discipline, both from a scientific point of view and in terms of the relevance and usefulness of the studies for practical implications.

Summary of the main findings in the existing literature

Knowledge

Knowledge is hypothesized to influence behaviour in several ways. First, knowledge about the disease and its potential consequences is thought to raise awareness and induce a need for action. Second, knowledge of potential intervention strategies and their impact is thought to influence farmers to adopt these strategies.

Problem recognition and responsibility

Problem recognition refers to the degree to which the farmer sees a problem and thus feels a need for action. It also relates to just how serious he perceives this problem to be. Farmers that perceive major problems with the health and welfare status of their herd are more inclined to adopt certain AHMP (Alarcon *et al.*, 2013, Anneberg *et al.*, 2012, Ellis-Iversen *et al.*, 2011). Problem responsibility refers to where farmers put the responsibility to solve a particular problem. There appears to be a difference in this respect between so-called production diseases (endemic diseases with potential negative effect on productivity and profitability, such as mastitis) and zoonotic diseases (diseases that are more a problem in terms of food safety). Hence, different communication strategies and different policy measures will be needed in order to convince farmers to control problems such as salmonellosiscompared to problems such as mastitis.

The social influence of the veterinarian

A major result in many studies is that the role of the veterinarian is of paramount importance. Farmers considered the veterinarian as their main advisor(Ellis-Iversen *et al.*, 2010, Hernandez-Jover *et al.*, 2012b, Alarcon *et al.*, 2013, Derks *et al.*, 2013a,Derks *et al.*, 2013b,Espetvedt *et al.*, 2013) and trusted the information provided by them (Garforth *et al.*, 2006). There is, however, there is evidence that the way veterinarians' deal with farmers and their management of animal health problems leaves scope for improvement.

Social influences other than the veterinarian

Social influences other than the veterinarian relates to the sources of information and their impact. One social influence that has been investigated quite often is other farmers, family and friends. Otherproducers are often identified as important sources of information (Garforth *et al.*,2006, Elliot *et al.*, 2011, Hernandez-Jover *et al.*, 2012, Mafimisebi *et al.*, 2012, Alarcon *et al.*, 2013,Espetvedt *et al.*, 2013). Further, farmers often regard governments as a negative source of information. The information provided by public research institutes or the state were very poorly considered by farmers (Cross *et al.*, 2009, Ellis-Iversen *et al.*, 2010,Hernandez-Jover *et al.*, 2012a, Hernandez-Jover *et al.*, 2012b, Alarcon *et al.*, 2013).

Economic impact of the animal health management practices

Regardless of how farmers think about the necessity to act, regardless of their characteristics, they often consider the costs and benefits of specific proposed interventions separately. In this respect, costs and benefits should not solely be thought of as in monetary terms, but also in economic terms. Non-economists generally think of economic impacts in terms of financial, monetary costs and benefits, whereas economists use a broader definition of economic costs and benefits, encompassing issues such as long term benefits, market effects, the value of foregone opportunities, risk, farming-system interactions and other costs and benefits. The economic impact in economic language bears much resemblance to the attitude concept that is used by social psychologists. In social psychology, attitudes are the overarching concept encompassing all subjective –perceived– advantages and disadvantages of a particular action.

Farm and farmer characteristics

Farm and farmer characteristics refer to observable characteristics of the farm and the farmers that are thought to influence adoption of practices and behaviour. The most studied factors in this category are age, education and experience(Leach *et al.*, 2010, Toma *et al.*, 2013), herd size, land size and household size(Bell *et al.*, 2006, Bhattarai *et al.* 2013, Ellingsen *et al.*,2012, Elliot *et al.*, 2011). In a majority of cases, these variables appear insignificant in explaining the use of certain animal health management strategies, and in those instances where they are significant, their impact is inconclusive: sometimes positive but sometimes negative as well.

Local knowledge and flexibility of practices

Several studies highlight the need to integrate different sources of knowledge and information when implementing a plan to eradicate an emerging disease, something which is in contrast to the strict standards of international organizations (Mather, 2012), who strive to find the best solution and impose this solution to a whole region.

Discussion

Implications for policy, research and extension

There is an absence of clear, universally significant factors that influence animal health management behaviour. The OIE, the WHO and other national and international bodies have a task to develop policies to promote global adoption of sound practices to improve animal health and welfare, and prevent foodborne diseases. The sometimes contradictory results observed across analyses make this task particularly challenging. Indeed, the results above tend to suggest a 'targeted policy approach', whereby policy mechanisms are geared to the particulars of a locale or, preferably, to individual farmers and their farm operations. Yet, this may be too pessimistic altogether. Based on the summary of the literature above, some general recommendations can be

suggested. Firstly, the role and potential of the veterinarian should be exploited better. Secondly, the economic costs and benefits of the proposed practices should be investigated. Thirdly, targeted communication schemes can significantly improve animal health management behaviour. Targeted communication schemes should consider farmers' goals and objectives and tackle farmers' key perceptions about the economic costs and benefits. Fourthly, epidemic and zoonotic diseases pose a specific challenge, since farmers do not always feel responsible for the management of such health problems. Hence, the role of government instruments will be particularly important.

Areas of progress in the research discipline

A few potential areas of progress have been identified. The first deals with the place of SPVE in broader veterinary and epidemiological research projects. Up until now, it has too often been the case that the social research about farmers behaviour is just another work package that needs to be included in order to get project proposals approved, just as the economic work package was a few years ago. In a truly interdisciplinary or even trans-disciplinary research approach knowledge generated in each discipline would be further used by the other discipline in order to make it more according to farmers' perceptions and suitable for practical application.
Second, a larger number of intervention studies is advocated for. Save for a few exceptions, most studies are adoption studies, that investigate farmers' and/or veterinarians beliefs, perception, intended behaviour and actual behaviour related to a specific or more broad animal health problem. Whereas the results of such studies are very useful to suggest intervention strategies that could actually change animal health management behaviour of the relevant stakeholders, there is a lack of studies that investigate what actually works and what not. A few exceptions are worth mentioning (e.g., Huttner *et al.*, 2001, Stringet *et al.*, 2011).
Lastly, in order for SPVE studies to produce results that can successfully inform policy makers, veterinary researchers and extension agents, we advocate for a continuation of the mixed-method approach as the dominant research paradigm combining qualitative and quantitative research methods.

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Biosecurity in livestock ruminants, a pillar of the strategy of disease prevention

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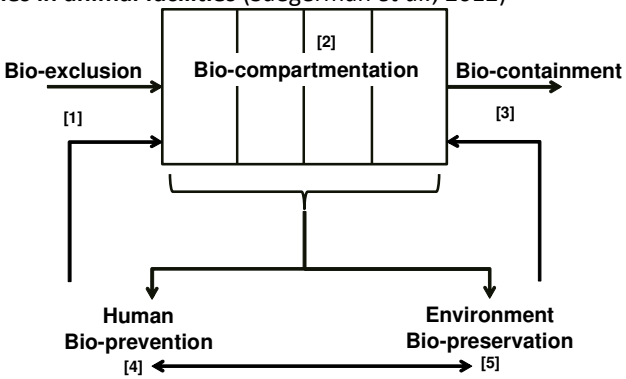
The concept of biosecurity

Biosecurity is recognized internationally as an important tool to help controlling infectious disease both at country and at farm levels (e.g., Negrón *et al.*, 2011; Laanen *et al.*, 2013). In veterinary medicine, biosecurity can be defined as the implementation of measures that reduce the risk of introduction and spread of diseases agents including zoonoses; it requires the adoption of a set of attitudes and behaviours by people to reduce the risks in all activities involving domestic, captive exotic and wild birds and their products (World Organisation for Animal Health, 2008). It greatly relies on the respect of the 5 B's operational concepts: bio-exclusion (to limit the risk of introduction into a facility), bio-compartmentation (to limit the spread of the pathogen within the same facility), bio-containment (to limit the spread of the disease agent outside the facility), bio-prevention (to prevent the risk of human bio-contamination) and bio-preservation (to prevent any environmental bio-contamination and persistence of the pathogen) (Saegerman *et al* , 2012) **(Figure 1).** In terms of epidemic animal diseases, bio-exclusion is the most efficient action.

Figure 1. Biosecurity principles in animal facilities (Saegerman *et al.*, 2012)



Legend: All the stages specified in the figure above are part of a biosecurity approach and contribute to the reduction of the risk of introduction and spread of infectious agents:
1) to limit the risk of introduction (bio-exclusion);
2) to limit the spread of the pathogen within the same facility, e.g. by isolating excreting animals (bio-compartmentation);
3) to limit the spread of the disease agent outside the facility (inter-herd transmission) (bio-containment);
4) to prevent the risk of human bio-contamination (bio-prevention);
5) to prevent any environmental bio-contamination and persistence of the pathogen (bio-preservation). Human can contaminate animals as well (e.g., *Mycobacterium bovis* (Fritsche *et al.*, 2004)). Animals can be re-infected by the contaminated environment, which is especially true for pathogens presenting a long environmental persistence such as *Bacillus anthracis* (Hugh-Jones and Bleckburn, 2009) or *Mycobacterium bovis* (Kelly and Collins, 1978) when ecological conditions are optimal.

Towards preventive rather than curative medicine

It is becoming increasingly evident that there is a need to reorientate farmers and veterinarians towards preventive rather than curative medicine (Saegerman *et al.*, 2009; Sayers *et al.*, 2014). The implementation of biosecurity measures is an important way to contribute towards such reorientation. The putative benefits of implementing biosecurity for disease prevention and/or control include improved production efficiency resulting in greater profits, better animal welfare, improved immune responses to vaccines, less antibiotic use and enhanced job satisfaction for producers, herds health professionals and other agricultural workers (e.g., Brennan and Christley, 2012; Laanen *et al.*, 2013). However, poor communication amongst stakeholders and the provision

of conflicting information from multiple sources result in confusion and apathy amongst farmers with regard to the implementation of biosecurity (Kleen *et al.*, 2011; Gunn *et al.*, 2008).

Current Belgian situation regarding implementation of biosecurity in livestock

Currently, in Belgium, biosecurity recommendations exist for the pig and poultry sectors but are lacking for the cattle sector (Ribbens *et al.*, 2008; Laanen *et al.*, 2010; Van Steenwinkel *et al.*, 2011; Gelaude *et al.*, 2014).Structural biosecurity measures in the pig industry were implemented progressively since 1993as a contribution to deal, in first instance, with classical swine fever that occurred in 1990 and 1993 (Vanthemsche and Saegerman, 1994) and more generally, to all other diseases. Indeed, structural biosecurity measures were mandatory in this species (Royal Decree at 14 June 1993 determining equipment requirements for the housing of pigs). Five years later, structural biosecurity measures were also mandatory in the poultry sector (Royal Decree at 10 August 1998 establishing certain conditions for health qualification poultry).In addition, for thesetwo species, a website application was developed by the University of Ghent, in collaboration with the Flemish Animal Health Organization (DGZ),permitting a free and independent evaluation of the quality of the whole biosecurity in pig or poultry farms through a scientific-based questionnaire and accompanying scoring system (www.biocheck.ugent.be) (Gelaude *et al.*, 2014).

It would be of great benefit for the Belgian cattle sector to have access to biosecurity evaluation and advisory tools that are well adapted to their specific situation and that provide practical suggestions and advise for improvement. To develop such a system, it is important to understand, first, if and how biosecurity measures are being used and what drivers and constrains are influencing the implementation of such measures (population radioscopy). This can help identifying areas for further exploration, such as evidence-based research on the efficacy and cost-effectiveness of biosecurity measures, as well as factors affecting producer decision-making related to biosecurity.

Some preliminary surveys on biosecurity measures in Belgian cattle farms (Sarrazin *et al.*, 2014; Humblet *et al.*, in preparation) indicated that few biosecurity measures are currently implemented by Belgian cattle farmers, thereby exposing themselves to the risk of disease transmission within and between farms. Especially in regions with a high cattle density, where distances to neighbouring farms are short and professional visits frequent, a farm-specific preventive strategy should be developed, thereby using the facilities often already present on the farm. Other preliminary surveys on biosecurity measures in veterinarians (Saegerman *et al.*, in preparation) and other professional herd visitors (Sarrazin *et al.*, 2014; Humblet *et al.*, in preparation) showed that biosecurity measures should also be improved. Currently and based on limited data, the compliance with biosecurity rules (if existing)is intermediate, which should pave the way to a series of measures such as increasing awareness through education of all actors involved. As an example, in terms of education of veterinary students, biosecurity must be accurately addressed in teaching but also in clinical practices and some guidelines should be known, such as the Code of good veterinary practice published by the Federation of Veterinarians of Europe (FVE) and the WOAH[OIE]-FAO Guide to good farming practices for animal production food safety, aiming to control the dangers that could threat animal health and food sanitary security at the farm level. For this purpose, a dedicated teaching website was developed by the Faculty of Veterinary Medicine of the University of Liege and is available at the following address: http://www.fmv-biosecurite.ulg.ac.be/. This website is visited more than 9,000 folds per year by people from more than 100 different countries.

The next part of this communication will target the cattle sector.

General interest to implement biosecurity measures with emphasis on the cattle sector

Preventing and controlling the introduction of new diseases is an agricultural challenge which is attracting a growing public interest. This interest is, in part, driven by an impression that the threat is increasing, partially related to changes in production structures such as herd sizes, but there has been little analysis of the changing rates of biosecurity threat, and existing evidence is equivocal (Waage and Mumford, 2008). In a recent Belgian survey (Laanen *et al.*, 2014), approximately half of the respondents in the cattle sector were convinced of the positive effect of biosecurity on the reduction of diseases in their farms. However, farmers estimated their own level of knowledge on biosecurity as being rather low and their motivation for implementing additional measures as limited. This preliminary information justifies more applied research on biosecurity in the cattle sector.

The overarching goal of biosecurity is to prevent, control and/or manage the risks to life and health as specific to the particular veal and cattle sectors. In doing so, biosecurity is an essential element of a sustainable agricultural development (FAO, 2007). The non-implementation of biosecurity measures in farms make other preventive and control measures less efficient on animal diseases. In a recent study on bovine viral diarrhoea virus, biosecurity combined with vaccination were the main measures decreasing the risk of high-cost outbreaks in herds (Smith *et al*, 2014).

In addition, the agricultural sector is interested in prevention measures towards diseases that have an impact on production and animal health and welfare since they positively influence the sustainability of production. Recently, several international organisations promoted the biosecurity approach, including in the cattle sector, as

a response to the introduction and spread of diseases in animal facilities (e.g., OIE: http://www.oie.int/en/for-the-media/editorials/detail/article/biosafety-biosecurity-and-prevention-of-diseases/ ;
OIE-FAO: http://www.oie.int/fileadmin/Home/eng/Food_Safety/docs/pdf/3_Lang_Good_farming_practices.pdf ;
EFSA: http://www.efsa.europa.eu/fr/efsajournal/doc/3276.pdf ;
FEV: http://www.fve.org/news/publications/pdf/gvp.pdf ;
DG-Sanco: http://ec.europa.eu/food/animal/diseases/strategy/archives/nov2006/conclusion_panel3.pdf).

Specific economic interest of applied biosecurity

Investment in biosecurity is more of a long-term investment in income protection rather than short-term profitability, it is an insurance cost rather than a production input cost, as it is a mean to prevent economic losses for cattle holders. More applied researches are needed in term of economic impacts and cost-effectiveness of implementing biosecurity measures, both at the farm and veterinarian levels, for the veal and cattle sectors. The results of these future researches are of main importance to increase and maintain the motivation of actors involved in the process.

Towards more applied research in biosecurity in Belgium

The future applied research would allow improving the situation in Belgian veal and cattle sectors regarding biosecurity, both at the farm and professional herd visitor levels. Indeed, it is in the producer's best interest to adopt a biosecurity plan designed to prevent the introduction and spread of infectious diseases. With the intensive production systems in Europe in combination with the small profit margins, disease prevention, rather than disease treatment, is becoming the only realistic possibility for the future. Studying the biosecurity situation in the cattle and veal production sector in Belgium will allow identifying and prioritizing the measures to be implemented. Moreover a risk-based biosecurity scoring system will provide the sector with a self-assessment tool that can give a specific feedback to cattle holders on possible improvements. Implementing these effective biosecurity measures will lead to an increased efficiency of production, resulting in greater profits for cattle holders. Reducing the risk of disease introduction and spread of diseases within the holding leads to less reliance on animal therapeutics and improved animal welfare. In turn, this reduction translates into increased profit. Furthermore, less diseases will lead to a reduced risk of transmitting zoonotic diseases to workers and consumers.

Conclusion and perspectives

It is evident that there is a need to re-orientate farmers and veterinarians towards preventive rather than curative medicine and the implementation of biosecurity measures is an important way to contribute towards such reorientation. However, poor communication amongst stakeholders and the provision of conflicting information from multiple sources result in confusion and apathy amongst farmers with regard to the implementation of biosecurity. Indeed, biosecurity measures should be based on evidence to ensure a correct implementation. This important step entails more research. It is a need to understand the field practices, in terms of biosecurity, in the veal and cattle sectors (farmers and professional herd visitors), to understand the determinants of attitudes and behaviours affecting producer decision-making related to biosecurity (sociological factors), and to evaluate the cost-effectiveness of biosecurity measures in farms; it is essential to elaborate a risk-based quantitative biosecurity scoring system per herd typology to ensure the possibility of an ongoing (self) assessment of biosecurity practices over time. These future developments are of great interest directly for farmers (self-evaluation, benchmarking) and professional herd visitors. Such a system could assist producers and herd health advisors in deciding the most effective areas to invest in and could highlight areas requiring further producer/veterinary education and training. Finally, the results of implementing such a system will be of interest for the government since they will provide an overview of the current situation and a prioritised list of measures that would improve the situation. Some of these may be included in policy measures like in the pig and poultry sectors. In addition, some of these results are of first importance for the modelling of the spread of several diseases in the specific Belgian conditions.

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New developments in vaccines and vaccination programs to mitigate the emergence of foot-and-mouth disease and bluetongue

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Introduction

Infectious livestock diseases have a significant negative impact on food security, particularly for rural communities and contribute to malnutrition and poverty in the third world. Highly contagious diseases such as foot-and-mouth disease (FMD) and vector-borne diseases such as bluetongue (BT) decrease the efficiency of the conversion of feed into nutritional food and, together with the mass slaughter of diseased and disease-exposed animals, contribute to the unfavourable environmental footprint of the livestock sector and the non-acceptance of disease control strategies. Diseases such as FMD and BT cause high production losses and impose local and global movement and trade restrictions. This results in denying farmers access to the globalized markets, endangering their income and food security, and negatively impacts the employment in the agri-food sector.

FMD and BT are widespread around the world and cause severe socio-economic problems with an annual global cost of ca. 10 and 3 billion €, respectively. FMD has been eradicated in Europe through vaccination, movement control, slaughter and efficient veterinary services. Occasional incursions have devastating effects in ruminants and swine. In 2001 FMD affected over 2000 premises in the UK, resulting in the slaughter of several million animals and a cost of ca. 10 billion € including losses in trade and tourism. Since 1998 BT virus (BTV) annually causes substantial losses in ruminants in Europe. The peak of BTV-8 in 2006-2008 in France, Germany, the Benelux and Switzerland had a cost of more than 2 billion €.

In 2003, the EU changed its FMD non-vaccination policy in favour of emergency vaccination and vaccination has also aided to control the BTV-8 outbreak. Although inactivated vaccines are available for both diseases, the serotype diversity of FMDV (7 different serotypes) and BTV (26 serotypes) presents a serious challenge for an efficient multivalent vaccination strategy. Also other issues such as the slow onset of immunity, the short duration of protection, the need for high technological purification to ensure the possibility of differentiating infected from vaccinated animals (DIVA) and the obligation of producing in high containment facilities, emphasize the need for new, more efficient and flexible vaccine systems.

To accommodate this, immunological research and vaccine development should go hand in hand. A deeper knowledge must be acquired on the interplay between the virus and the adaptive immune system to induce long-term and heterotypic immunity. A better understanding of the early interactions between host-cells and viruses is essential to support strategies for blocking virus entry and replication and to increase virus clearance. Therefore immunological research mainly focus on how the chemokine signaling cascades initiated during vaccination can be modulated and on how conserved multi-serotypic antigens can be modulated to persist in the host's immune system. The adapted use of immune modulators such as stabilizers, novel adjuvants, non-coding RNAs or viral interferon inducers will enhance the capability to provide early protection and increase the duration of the protective immunity.

Antigen characterisation of relevant proteins/peptides and their interaction with the host immune system will generate knowledge to enhance the multivalent or multi-serotypic nature of vaccines. The broad-spectrum capacity will be obtained by formulating strain cocktails, incorporating specific neutralizing and non-neutralizing B-cell or T-cell epitopes in existing (recombinant) vaccines, dendrimerization of multiple epitopes, producing chimeric subunits or combining expression of different (conserved) viral antigens in next generation vaccines. The technology used for next generation vaccines should allow rapid modification of the constructs and production systems so that fully tailored multivalent vaccines can be produced. This is primarily sought through the use of non-infectious vaccine particles [such as virus like particles (VLPs), viral peptides or subunits, vectored vaccines] or replication deficient viruses (DISC and DISA vaccines) incorporating specifically conserved multi-serotypic epitopes. New developments are also focusing on improved antigen stability and extended shelf lives of vaccines at ambient temperatures which will reduce the costs and cold chain requirements for storage and transportation of vaccines.

An overview is given of some new developments in:

1) immunological research related to vaccine production
2) vaccine production
3) control of vaccination programs

1. Analyses of pathogen-host interactions: induction and regulation of immune responses.

The generation of vaccine-mediated protection is a complex challenge. Although several components of the immune system complement each other, almost all current vaccines confer protection by the induction of specific neutralizing antibodies. However sometimes animals with low levels of such neutralizing antibodies are also protected, suggesting that other mechanisms than neutralization are involved in protection. In addition it is known that long-term protection requires the persistence of antigens (Ag) and/or generation of immune memory cells.

1.1. Better understanding of early interactions blocking virus replication and increasing virus clearance.

The purpose of investigating the early interactions between pathogens and their host is to gain knowledge on the early events triggered upon infection that affect viral entry and can lead to viral clearance or to the presence of residual virus with or without disease. In this process, the mechanisms operating in the host cells aimed at sensing viral motifs as non-self and downstream routes leading to type-I interferon (IFN) and cytokine induction are crucial but are still poorly understood. Therefore current research is addressing different related issues:

- ⇨ the IFN induction pathway in animal cells and the role of interleukin on virus replication in terms of viral clearance;
- ⇨ the interaction of virus-epitopes with dendritic cells and the following events of uptake and antigen presentation;
- ⇨ the analysis of the early immunological profile after infection compared to vaccination.

The results derived from these studies will significantly contribute to improve our understanding of the innate responses elicited by host cells in response to infection as a first line antiviral defence and will therefore significantly advance the design of effective vaccine development.

1.2. Adaptive immune system interaction: towards induction of long-term and heterotypic immunity.

1.2.1. Identification of mimic conformational epitopes and their immunological relevance during viral infection.

Peptide libraries and phage-displayed random peptide libraries (PDPL) are being explored to discover amino acids sequences that mimic conformational epitopes (mimotopes) and subsequently to study their immunological relevance during infection. Peptide libraries and PDPLs are screened with panels of monoclonal antibodies (MAb) i) to enable profiling the immune response against specific epitopes after infection or vaccination (potential identification of antibodies conferring heterotypic and/or long-term immunity) and ii) to design antigens for more effective vaccines.

1.2.2. The role of T helper cell response and persisting viral Ag for induction of a long-term immunity.

Without participation of CD4+ T-cells in the immune response, B-cells cannot undergo isotype switching to generate high-affinity antibodies, the microbicidal activity of macrophages is reduced, the efficiency of CD8+ T-cell responses and CD8+ T-cell memory are compromised, and down-regulation of effector responses is impaired. Therefore, CD4+ T-cells are likely to fulfill an important facilitator role in the maintenance and control of protective immune responses against FMDV/BTV. The importance of specific T-cell responses to promote long term antibody responses to FMDV vaccines is being tested in mice, making use of gene knockout technology to clearly define the role of T-cell subsets. These studies are complementary to those performed to examine antigen persistence in T-cell dependent germinal centres. All this information on the role of T-cell responses in driving long term antibody responses will feed the design of new vaccine formulations and will also substantiate the understanding of how to increase the duration of immunity of killed vaccines.

2. Development of innovative and multivalent vaccines.

2.1. Development of subunit vaccines

A subunit vaccine presents an antigen to the immune system without introducing viral particles. The production involves the synthesis of peptides or the isolation of a specific protein from a virus and administering this by itself. A weakness of this technique is that that those peptides or proteins often don't have the correct conformational (3-dimential) structure or that isolated proteins can be denatured and will then elicit antibodies which differ from the desired antibodies. A second method of making a subunit vaccine involves putting an antigen's gene from the targeted virus into another virus (virus vector such canine adenovirus) to make a recombinant virus expressing the antigen. The antigen (one or more subunits of protein) is then extracted from the vector. In poxvirus-based systems (canarypox vector and capripox vector) the pox virus is used as a vehicle for the transfer of BTV genes into the sheep cells where BTV proteins are then synthesized inside the host cells. Some new generation vaccines are produced in insect cell culture using a baculovirus based protein expression system. This eukaryotic expression system has the advantages that it produces large amounts of protein very efficiently compared to mammalian cell expression systems and it can fold and assemble proteins and large complexes with a 3-dimentional structure that resemble the original form much better. A plant based expression system can also be used to express proteins such as VP2 from BTV strains in large quantities. Combinations of different expressed VP2 proteins and chimeric VP2s to generate a more multivalent immune response are being explored.

Vaccines based on dendrimeric peptides offer several advantages such as more potent immunity with DIVA assurance. Dendrimeric FMD peptides incorporating 3 to 4 copies of an immunodominant B-cell epitope of FMDV linked to a heterotypically conserved T-cell peptide. These special peptides already provided proof of solid protection in pigs against homologous FMDV challenge. Current developments using structural modifications (epitope composition, multiplicity and connectivity) can further improve the immunogenicity and protective response elicited by these B2T dendrimers. Incorporation of determinants relevant for induction of broader, earlier and long-term immunity, identified by the analyses of pathogen host interaction, will allow further improvement of this kind of vaccines. A B2T construction with a B-cell epitope comprising antigenic site A (G-H loop) of VP1 FMDV O/UK/2001 (Pan Asia) and a T-cell epitope corresponding to part of the non-structural protein A3 of FMDV proved to achieve full protection not only in a murine model but also in swine.

Virus-like particles (VLPs) consist of viral structural proteins that, when overexpressed, spontaneously self-assemble into particles that are antigenically indistinguishable from infectious virus or subviral particles. Virus-like particles resemble viruses, but are non-infectious because they do not contain any viral genetic material and thus are a safe alternative to attenuated viruses. VLPs can be produced in a variety of cell culture systems including mammalian cell lines, insect cell lines, yeast, and plant cells. VLPs are a useful tool for the development of vaccines. VLPs contain repetitive high density displays of viral surface proteins which present conformational viral epitopes. Immunisation trials with VLPs for BTV or FMDV and other viruses have shown that they elicit stronger and more long lasting immune responses than unassembled subunit vaccines, and are also efficient at stimulating both B and T cell responses. In challenge experiments, doses of BTV VLPs as low as 10 µg (containing no more than 2.39 µg VP2) were sufficient to afford protection in contrast to immunisation with baculovirus expressed VP2 alone where 2 doses of 100 µg were necessary to achieve complete protection. Vaccine developers are generating chimeric Rabbit Haemorrhagic Disease Virus (RHDV), Parvovirus and baculovirus VLPs incorporating immunogenic FMDV specific B- and T-cell epitopes complemented or not with immunostimulatory molecules such as Toll Like Receptors (TLR) ligands. A recombinant Semliki Forest Virus (SFV) vector that express the FMDV P1-2A capsid precursor (from both serotype O and A) with a low level of the 3C protease is in development. This modified SFV vector is only capable of a single-cycle of infection and will express the FMDV proteins within cells, equivalent to FMDV infection.

2.2. Reverse genetics to develop designed attenuated vaccines or Disabled Infectious Single Cycle (DISC) vaccines.

Traditional live vaccines rely on attenuation of virus by passage in cell cultures, eggs or animals. The mutations that define these attenuations are mostly not documented. The recent development of a reverse genetics system makes the rational design of attenuated vaccines possible. Infectious attenuated BTV is produced entirely from DNA clones by generating one transcript in vitro for each genome segment, and using these to transfect permissive cells. This system allows the introduction of any mutation into the genome of BTV, providing the resulting virus is viable. The risk of reversion to virulence can be reduced greatly by introducing more than one attenuating mutation into the genome of the engineered strain. It will thus be possible to use a defined attenuated genetic background and introduce the antigenically important outer capsid proteins from the serotype(s) of interest. This will be relevant in regions where protection from several co-circulating strains is needed.

Using the new reverse genetics system made it possible to develop disabled infectious single cycle (DISC) vaccines. In these vaccines, one or multiple essential viral genes are inactivated (knockout) in the virus and supplied during vaccine production using a complementing cell line. In unmodified cells and in the vaccinated animal, the virus is unable to replicate because the complementing proteins are missing. The resulting aborted infection allows the expression of viral proteins at natural sites of infection without the production of infectious virus or disease in the animal, and can be considered an extreme form of attenuation. Since the DISC strain would be missing one or more viral proteins, it will be possible to distinguish between vaccinated and infected animals. Such vaccines are compatible with current vaccine-production facilities used to make attenuated and inactivated vaccines.

The reverse genetics technology for bluetongue virus (BTV) has been used in combination with complementing cell lines to recover defective a BTV-1 mutant with large deletions in an essential BTV gene that encodes the VP6 protein of the internal core of the virus. A complementing cell line provided the VP6 protein in trans. The protective capabilities of BTV-1 DISC virus was assessed in sheep by challenge with specific virulent strains and demonstrated that it was highly protective. This DISC vaccine could offer a promising alternative to the currently available vaccines. Further, a defective BTV-8 strain was made by reassorting the two RNA segments that encode the two outer capsid proteins (VP2 and VP5) of a highly pathogenic BTV-8 with the remaining eight RNA segments of the BTV-1 DISC virus. A NS3-based DISC vaccine against BTV 8 has also been developed and evaluated in sheep. A serological DIVA test based on NS3 should be developed. Notwithstanding the promising results, further studies

are needed if these DISC vaccines are to be used for up scaling to an industrial level. The possibility of recombination between the defective gene of the DISC vaccine and the helper cell-expressed VP6 mRNA or with adventitious virus segments should be addressed in depth (genetic stability). Additives (stabilizers) must be optimised in order to maintain vaccine virus titres and ensure the protective efficacy of the vaccine. The insertion of a foreign immunogenic tag onto a capsid protein of the DISC vaccines strains must be evaluated as a potential DIVA and an ELISA must be developed. The duration of immunity and multivalent capacity must still be analysed.

2.3. Improvement of conventional FMD vaccines: Increased antigen stability

FMDV is notoriously unstable. Vaccines formulated from unstable viruses are less immunogenic due to virus degradation. Therefore, during production the manufacturer has to compensate for this instability, which increases production costs and reduces vaccine yield. In addition, to be effective, current FMD vaccine programs require frequent booster vaccinations, a property that may also be linked to vaccine antigen instability. It is shown that single amino acid replacements in the capsid of FMDV increased its resistance to acidic pH and enhanced its thermal stability without impacting on growth properties or antigenic structure, making these viruses candidates for improved, more stable vaccines.

3. Control of vaccination programs

3.1. Development and improvement of accompanied DIVA diagnostics

The control of diseases in livestock requires the use of efficient vaccines which allow a clear differentiation between infected and vaccinated animals (DIVA vaccines). The current DIVA concept in FMD/BTV is based on detection of antibodies that are elicited in infected but not in vaccinated animal: antibodies to non-structural proteins NSP-3ABC / NS3, respectively).
Since novel subunit vaccines contain only a selection of viral epitopes, immunoassays capable to detect subsets of antibodies against those other epitopes which are not represented in these innovative vaccines represent a further DIVA approach that is investigated.

3.2. Post Vaccination monitoring.

Vaccination is one of the most important tools to combat FMD. Various approaches to vaccination have been used based on the local situations and objectives, e.g. mass vaccination, vaccination applied to target animal populations, zones or high risk areas, ring vaccination around outbreaks and vaccination at buffer or protection zones around free areas. Since many factors can affect the effectiveness of vaccination against FMD, the regimens and programs used must be monitored continuously to identify any failings and to ensure sustained control of the disease. Post-vaccination Monitoring (PVM) is also used to substantiate freedom of infection sometime after the last FMD outbreak. However, it is hardly possible to substantiate the absence of occult virus infections in a vaccinated population as that might be masked but not eliminated by incomplete vaccine-induced immunity. Therefore uncertainty remains over the levels of confidence that are required and can be achieved using current tools and strategies, over the extent of surveillance required, and finally over what should be the appropriate responses to and consequences of different serological surveillance findings. It is recommended that the rationale for serosurveillance after emergency vaccination should be complemented. If both vaccination and clinical surveillance are highly effective, only small numbers of herds and animals will become infected. With tests of imperfect sensitivity and specificity it is not possible to detect and eliminate all of these cases without culling many uninfected animals as well. In other words, the cost-benefit will be low. Therefore, investigations post-outbreaks to substantiate freedom of virus infection/transmission should also focus on demonstrating the effectiveness of the control measures and the vaccination program.

ORAL COMMUNICATIONS

Farm economic analysis of improving biosecurity status and good management practices in farrow-to-finish pig farms

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Introduction

Biosecurity implies all the measures that prevent pathogens from entering a farm (external biosecurity) and reducing the spread of pathogens within a farm (internal biosecurity). In densely populated areas like Belgium (it has an average of 6,500,000 pigs, NIS, 2012); biosecurity strategies accompanied by good management practices play a major role to control diseases.

Recently it has been shown that biosecurity combinedwith a reduction of use of antimicrobials in pig farmsleads to improved technical parameters (Laanen *et al.*, 2013). Despite the existence of a positive link between the enhanced biosecurity status, reduced antimicrobial usage and improved technical parameters farmers sometimes remain reluctant to implement biosecuritymeasures (Gunn *et al.*, 2008).

The most important motivators and holdback factors to implement biosecurity strategies are, apparently, of economic nature. The main motivator for farmers is to improve profit due to higher productivity.The major holdback is its costs. Biosecurity measures areperceived as too expensive to implement. The farmers clearly specify that knowledge on the costs of biosecurity strategies will incur and the benefits acquired will motivate them to implement biosecurity strategies and good management practices(Laanen *et al.*, 2014). However, a link between the costs entailed and benefits obtained is lacking. In this study the biosecurity status of farrow-to-finish herds was improved and the herd management optimized by providing tailored advice. The aim of this study was to estimate the benefits of implementing biosecurity measures and good management strategies.

Materials and methods

In total fifty-one Flemish farrow-to-finish pig farms participatedin a study conducted by the Epidemiology Unit of the Faculty of Veterinary epidemiology. Herds were visited in three occasions between the end of 2010 and mid of 2014. In the first visit an evaluation of the situation regardingthe production parameters, disease problems, diagnostic results, vaccination and anthelmintic strategy,biosecurity status and antimicrobial use was conducted. The herds' biosecurity status wassurveyed using the questionnaire Biocheck.UGent (www.biocheck.ugent.be) which uses a scientifically risk based scoring system to quantify biosecurity status from 0 to 100. The status is subdivided into two categories: 1) internal and 2) external biosecurity. Both are disaggregated in 6 subcategories comprising several questions per subcategory.

The information gathered in visit 1 was evaluated by the researcher and herd-specific advice on biosecurity, management and antimicrobial usage was supplied in the second visit (average 356 days after 1st visit). During the third visit the farms' (average 610 days after 1st visit) biosecurity strategies implemented, management, antimicrobial and key technical parameters were recorded.

The costs incurred by implementing biosecurity measures and good management practices were gathered by Animal Health Care Flanders for prices on veterinary costs, Agrologic (Agrologic, 2014) for prices of commodities and prices of vaccinations were obtained by the Faculty Veterinary Practice of Ghent University. We could not include the costs of antimicrobials because, at the time of preparation of this extended abstract, the change in treatment incidence was not available.

Control farms were obtained from AMS data (the acronym in Flemish for 'Department of monitoring and study', Flemish Government). To calculate the difference-in-difference of the key technical parameters (average daily weight gain (g/day), feed conversion, mortality till weaning (%), average weaned piglets per sow per year, mortality of the finishers (%)) we used a propensity score matching. Propensity score matching attempts to estimate the effect of an intervention accounting for the variables that predict receiving the intervention (in this case to receive the tailored-farm-specific advice). We used as covariates for matching those variables that were proxies for management of the farm and demographic descriptive factors of the farm and were collected during the farms receiving the advice. Those covariates were: region, number of sows, number of finishers, years of experience, number of employees and oldest building. At the time of the preparation of this abstract AMS data for the control farms of the years 2010, 2011 and 2012 were available. We matched treated farms with control farms

accounting for the dates of visit 1 and visit 3. For example if a farm had its 1st visit in 2010 and 3rd visit in 2012 we matched them with control farms with data from 2010 and 2012. This resulted with 14 advised farms that could be matched with control farms. Finally, the Genetic matching algorithm in "R" (CRAN project) was used to calculate the average difference-in-difference of the technical parameters and its standard deviation.

The economic benefits were estimated with Pigs2Win (www.remiweb.be). Pigs2Win is an economic simulation model that uses 40 input parameters which include: key technical efficiency parameters (average daily weight gain (g/day), feed conversion ratio, mortality of the finishers (%) and mortality of the piglets (%)), input prices and costs during the farrowing and finishing period and transform them in output (kg slaughtered finished pigs) (Meensel *et al.*, 2012) which are translated in monetary benefits. We used data from 13 typical farms which represent the Flemish scenario as default scenario. The mean difference and the standard deviation of the key technical parameters were inserted together with the estimated costs of implementing biosecurity and increasing the vaccination use to calculate the difference in gross margin.

Results

From the 51 closed farms that applied for participation in the study 49 farms participated during the whole study period. Finally 663 pieces of advice were provided in the second visit to the 49 farms. The majority of the advice focused on improving internal biosecurity (50.4%), followed by advice to implement external biosecurity measures (17.6%). Guidance provided to improve and achieve a sensible use of antimicrobials accounted for 17.4%. Finally 14.5% of the advice given was related to improve the current vaccination and anthelmintic therapy. From the given advice 73.9%, 64.1%, 63.8% and 42.7% was implemented by the farmers to improve respectively the effectivity of vaccination and anthelmintic therapy management, the responsible use of antimicrobials, internal and external biosecurity.

Within internal biosecurity the most commonly provided advices to farmers was to record the mortality reason (12.3%) and change of needle between pens (9.3%). The rate of implementation of these measures by farmers was high (82.9% and 64.5% respectively). Cleaning and disinfecting the carcass storage properly after carcasses have been removed (19.7%) and the creation of a hygiene lock in the quarantine stables (17.1%) were the most commonly given advices to increase the external biosecurity score. To promote a responsible use of antimicrobials guidance was provided focusing on stopping with standard treatment (26.7%) and the use of the correct dose and duration for the treatment (13.8%). The implementation rate was 54.8% and 56.2%. The most commonly given advice on the vaccination policy was related with changing the currently vaccination scheme (35.6%), and specifically to improve the vaccination strategyof PRRSV (11.5%). 58.8% of the farmers who received this advice implemented the new PRSSV vaccination scheme. 34.6% of provided intervention strategies was related to changing the present anthelmintic policy. The implementation rate of this advice was 69.4%.

The preliminary results of the economic analysis shown that the average difference-in-difference of the key technical parameters was +11.4 g/day for average daily weight gain, -0.1 for the feed conversion ratio, -1.3 for the average weaned piglets per sow per year, +1.5% mortality of the piglets and -1.5% for the mortality of the finishers for the advised farms compared to the control farms. Only the average weaned piglets per sow per year were significant.

The average costs of implementing biosecurity strategies and changing the vaccination policy per advised farm per year were €3,572.70 (range:€32.80, €12,863.10). The average costs per sow per year were €10.37 (range: €0.12 - €30.44) and €0.84 per finisher pig per year (range:€0 - €3.32).The average gross margin and its 95% confidence interval was +17%(-9.6, 44) for farms that have received the advice compared to farms that did not receive advice.

Discussion

The preliminary results of this study present some limitations. First the propensity score analysis relies on the election of the covariates to match the farms. We were restricted to use those variables collected in the advised farms this may have biased the results. Moreover, propensity score matching involves assuming that the 'control' farms have not received the specific tailored-advice which aimed to improve biosecurity, management and reduce antimicrobial use in farms. Probably the control farms have received advice from private veterinarians and advisors but not the specific-tailored advice and guidance provided by the researcher, therefore we think that the assumption holds true.

Secondly, we do not have exact data on when the farmers implemented the advised measures afterthe second visit. It could be argued that the farmer has implemented those measures just before the third visit was scheduled to 'pass' the test of the third visit and therefore we could not see significant changes on the technical parameters. However, we think that is not very plausible because farmers agreed with the researcher on the 3rd visit's date.

Thirdly, we could not obtain farm specific gross-margins due to the lack of the farm accountancy data of the advised farms. Fourthly, to calculate the total costs we did not include the change of use in antimicrobials because, when this abstract was submitted, we did not have the complete results of the reduction of treatment

incident. Interim results of 40 farms analyzed showed that farms had reduced antimicrobial consumption by a 50%. We would expect a reduction on the farrowing and finishers' costs spent by the farmers that have followed the advice.

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Pandora: a quick and practical first-line risk screening tool for parasitic and pathogenic micro-organisms

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Abstract

Before prevention or early eradication measures on potentially invasive and hazardous species (macroscopic or microscopic) can take place, it is essential to first identify those species that pose the highest risks. Given the huge and still-increasing number of animal species that become transported and may host emerging infectious diseases, such a prioritization must allow for a high number of species to be assessed in a relatively short time, using risk assessment tools. Pandora is a new first-line risk screening tool for parasitic and pathogenic micro-organisms, with respect to their potential (re)emergence capacity and impacts. Impacts are relative to a given target area and may refer to health impacts (on domestic or wild animals and plants, and humans), trade and public perception.

Pandora is a semi-quantitative risk assessment protocol, based on general epidemiological principles from existing schemes (OIE, EPPO, WHO). After having defined the pathogen, area and targets at hand, the user is asked to score the likelihood of pathogen release, transmission and exposure, and to score the likelihoods and effect sizes for consequences towards targets. Pandora provides ample guidance, suggested data sources and examples to assist the user in performing the analysis. The answers are ordinally scaled, and this allows to put these risks into scores, and thus, to classify pathogens accordingly. This may support policy makers in the prioritization of preventive control measures, and also point to caveats in our knowledge on particular pathogens.

Pandora draws on the same concepts as the Harmonia+ procedure for alien animals and plants (D'hondt *et al.*, submitted/under review). It was realized by a consortium of eight Belgian scientific institutes, each providing their expertise on components of the protocol. Experts that tested the protocol overall granted the Pandora protocol medium to high scores for clarity, consistency, completeness, novelty & usefulness. It is currently being used for an assessment of the recently emerging African Swine Fever Virus. Pandora can be consulted at http://ias.biodiversity.be/harmoniaplus

Introduction

One can generally distinguish 3 methodologies in risk assessment: fully qualitative, fully quantitative and semi-quantitative. No single method has proven applicable in all situations and all involve a certain level of subjectivity (OIE, 2004). The qualitative method involves a reasoned and logical discussion of all available information in a group of experts. Quantitative risk assessments often produce more reliable results as compared to qualitative risk assessment. However, this involves modelling and thus requires the availability of sufficient and high quality numerical data and mathematical skills (OIE, 2004; Tomuzia *et al* , 2013). Both methods are suitable for the majority of import risk assessments for domestic animals or derived commodities (OIE, 2004/2014), cultivated plants (FAO, 2006; EPPO, 2011), for translocation/pest risk assessments for wild animals or plants (FAO, 2006; EPPO, 2011; OIE, 2012;IUCN, 2013). Such assessments are generally performed one at a time.

However, before prevention or early eradication measures on potentially invasive species (macroscopic or microscopic) can take place, it is essential to first identify those species that pose the highest risks. Given the huge and still-increasing number of macroscopic species that become transported and may co-transport emerging infectious diseases, such a prioritization must allow for a high number of species to be assessed in a relatively short time. When a large number of hazards (e.g. pathogens, chemical substances, invasive pest species, risk factors/drivers of emergence) have to be comparatively ranked or evaluated for knowledge/data gaps, or when there is a limited time frame (emergencies or rapid screening) to produce an answer, the semi-quantitative risk assessment is more often used in practice. This type of risk assessment is based on experts scoring a number of criteria followed by simple calculations on these ordinal scores (multiplication, summation, weighting). The final output equally supports diverse policy decisions, particularly in prioritization of management/surveillance actions or in adaptive allocation of budget, e.g. for research.

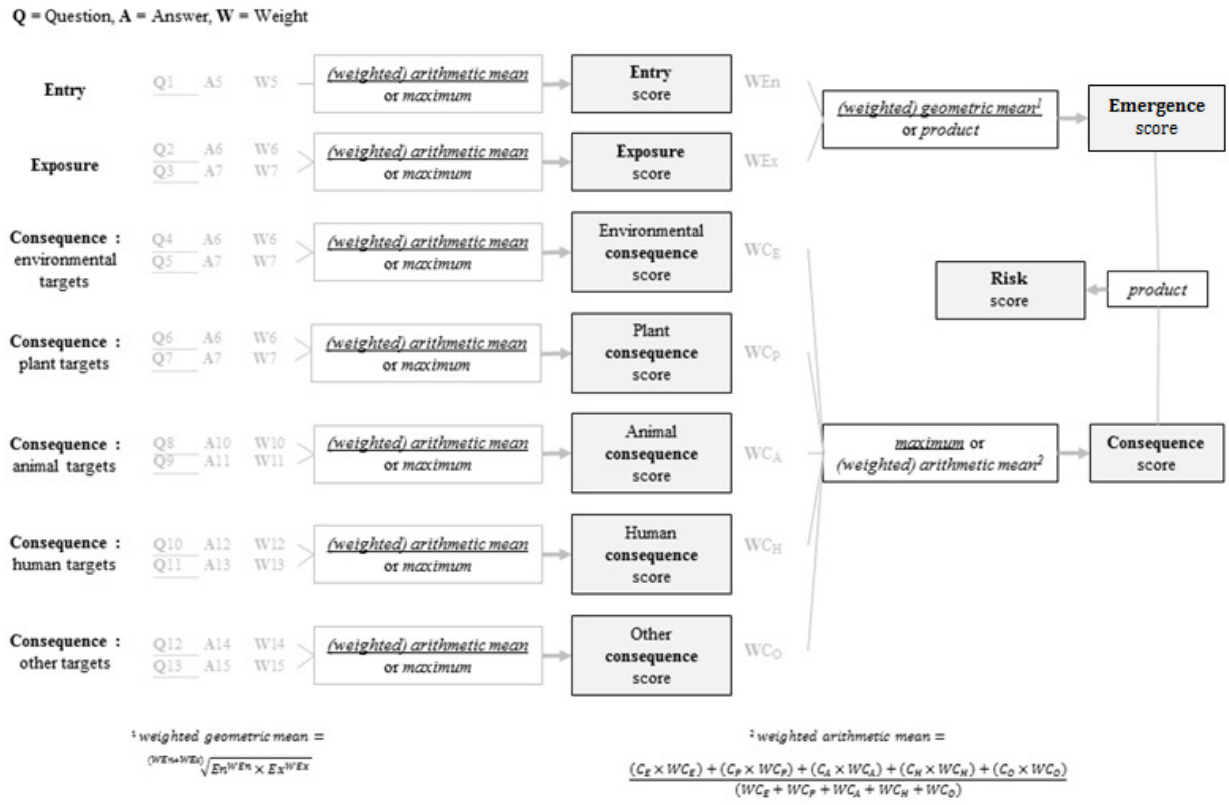
Materials and methods

Pandora is a semi-quantitative risk assessment protocol for Emerging Infectious Diseases. The tool is based on generally accepted notions of risk in epidemiology and biological invasion (OIE, 2012/2014; EPPO, 2011; WHO,

2008; IUCN, 2012): infection pathways entail both release and exposure (OIE, 2014), and risk is regarded as the product of probability and severity/magnitude of consequence/impact (Kinney and Wiruth, 1976; ISO, 2009; OIE, 2014). This standardized risk screening protocol is fully described in D'hondt *et al.* (submitted/under review) and may support policy makers in the prioritization of preventive control measures for different pathogens. It may also highlight knowledge gaps. One may consult an online version of Pandora through http://ias.biodiversity.be/harmoniaplus, which can be used freely and is open source.

When using Pandora (Figure1) in a predefined setting with given pathogen(s), area and targets of interest, a user (expert) is asked to answer a set of up to 13 questions that are considered important for understanding the risk of a specific pathogen in a specific geographic area. The questions are grouped into selectable modules, each of which refers to a distinct stage of infectious disease epidemiology: "Entry"includes 1 question on likelihood of pathogen(re)introduction), "Exposure" consists of 2 questions on maintenance and transmission to/exposure of targets, the 4Consequence modules on "Environmental targets"(wild animals and plants, habitats and ecosystems), "Animal targets"(domesticated: production and companion),"Human targets" and "Plant targets"(cultivated plants) each include 2 questions on individuals and populations,and finally "Other targets" involves 2 questions on international trade and tourism, and on public perception and attention.

Fig 1. Pandora semi-quantitative risk analysis framework



The questions within each module are scored by choosing from a set of either three or five pre-defined, alternative answers that fit an ordinal scale (type: 'very low/negligible' < 'low' < 'medium' < 'high' < 'very high'). To minimize any ambiguity with regard to the questions and their potential answers, every single question of the Pandora protocol comes with ample guidance that includes definitions, suggested data sources, cut-off values and examples. By converting the chosen ordinal answers to the questions into numerical scores (negligible: 0.05; low: 0.25; medium: 0.50; high = 0.75; very high: 1.00), and then performing some basic calculations on these, Pandora allows to express comparative risks on a numerical [0.00 – 1.00] interval.Any component of risk is liable to uncertainty, which in fact lies at the very base of performing risk analyses (Leung *et al.* 2012). For every relevant question, an assessor using Pandora+ is therefore asked to provide a level of confidence with his/her answer provided ('low','medium', 'high'), as well as any relevant remarks and references so support the answer. Following the guidance of Mastrandrea *et al.* (2011), the level of confidence is regarded as a combination of the average robustness of pieces of evidence, and the degree of agreement between these pieces. The Entry and Exposure module scores are then combined into the aggregated Emergence Score, by simple multiplication of the probabilities or by a geometric mean which allows individual weighting of these infection pathway processes. The scores from the different Consequence module scan equally become aggregated into a general 'Consequence score', either by taking the (potentially weighted) arithmetic mean if the user considers risks to be additive, or by taking the maximum of any module score if the user considers the highest risk as defining (precautionary "worst

case" approach).Finally, the 'Emergence score' and 'Consequence Score' can be multiplied to yield an 'Overall Risk Score' for the pathogen at hand, following the general 'risk' definition (probability * consequence).

Results and discussion

The Pandora protocol allows a considerable amount of flexibility. One does not necessarily need to follow the full mathematical framework, as shown in Figure 1. Instead, it is of paramount importance that the output should meet the stakes of the end-users. For instance, it may be of interest to present the scores for the different Consequence domains separately, rather than as an aggregated score, as to respect the different interests and competences from the stakeholders. Pandora alternatively allows stakeholders or experts to allocate different weights (of importance) to different questions and modules, to highlight certain parts of the emergence process or particular consequences they consider more important, through re-aggregation of the expert scores with more emphasis on particular field(s) of interest. Weights should ideally be set independently of, and prior to, expert scoring and may then result in more objective/realistic semi-quantitative output(Krause *et al.*, 2008; Discontools, 2012).

All risk assessments are subjective to some degree. However, through the use of arbitrary scores, weights, combined with expert opinion, semi-quantitative methods may be less transparent and may give a misleading impression of superior objectivity in comparison to a qualitative assessment (OIE, 2004; McKenzie *et al.*, 2007; Discontools, 2012). This is why Pandora offers the two approaches in a mutually non-exclusive way. As such, the standardized semi-quantitative outcome can be validated through the qualitative information and remarks provided by the experts, and through external validation against other approaches (especially fully quantitative ones), and peer review as advised by McKenzie *et al.* (2007).

Pandora draws on the same concepts as the Harmonia+ procedure for exotic animals and plants (D'hondt *et al.*, submitted/under review). These protocols were realized by a consortium of eight Belgian scientific institutes, each providing their expertise on components of the protocol. Since the risk of an alien plant or animal species (dealt with by Harmonia+ protocol) is often linked to the risk of pathogens or parasites being hosted by that species, we created a slightly adapted version of Pandora that is restricted to one specific host organism. This so-called Pandora+ protocol thus evaluates the risk of a particular pathogen to be introduced by one particular host species, and bridges the other protocols, as its final output may feed directly into a question in the Harmonia+ assessment.

In a first round of protocol validation, experts who tested the Pandora protocol to assess Classical Swine Fever virus, Bluetongue virus and *Echinococcus multilocularis* in raccoon dogs, overall granted the Pandora(+) protocols medium to high scores for clarity, consistency, completeness, novelty and usefulness. Since then, a risk assessment of the recently emerging African Swine Fever virus was performed, where Pandora has produced satisfactory semi-quantitative and qualitative output, comparable to other recent ASFV risk assessments (in preparation).

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Effectiveness and cost efficiency of passive, syndromic and active surveillance components for early detection of an emerging vector borne disease in cattle: Bluetongue as case study for Belgium, France and the Netherlands

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Introduction

An effective and efficient early detection of an emerging vector borne disease as well as quick recovery in order to substantiate freedom from infectionremain a constant challenge in animal health surveillance. In this context and following the major economic consequences of the Bluetongue outbreaks, regulation 1266/2007/EC last amended by 456/2012/EC (EU, 2012) prescribes the main surveillance components to implement in cattle population within each country. These components consist of active surveillance components (mainly characterized by yearly cross sectional surveys or sentinel serological/virological screening) and passive surveillance components (clinical surveillance/reporting).The minimum guidelines regarding sample size as well as the maximum tolerable prevalence with a set confidence level are described. The present study aims at evaluating the relative efficacy of each ongoing surveillance component in place in France, Nederland and Belgium in 2007 for proving freedom of infection or early detection of vector borne disease in the cattle population in accordance with the official standards, using Bluetongue as case study. The added value of using syndromic surveillance (milk production data, fertility data, and mortality data) as early warning systems for surveillance of vector borne disease was also investigated together with the cost of each surveillance component.

Materials and methods

In a first stage the different surveillance components in place in Belgium, in France and the Netherlands for Bluetongue in 2006 and 2007 were evaluated in terms of effectiveness for substantiating freedom of infection. Methods of Martin *et al.* (2007) were used for this purpose, taking into account; the minimum expected prevalence, population and sampled data, the sensitivity of the diagnostic process. Simulations were carried out to estimate the herd and component sensitivity at country level.

In a second stage, effectiveness of the existing surveillance components for early detection and the added value of using syndromic surveillance (mortality, dairy or fertility production data) were estimated. The methods of Martin *et al.*, were adapted to take into account the timeliness of detection. This was done using epidemic curves obtained by Reed Frost models. Because only surveillance data from the Flanders Belgium and the Netherlands were available, data within these regions were used and extrapolated at country level.

In a last stage the cost efficiency of each surveillance component in Belgium was investigated by comparing the obtained sensitivity gained divided by the cost of running the given surveillance component.

All simulations were carried out in ModelRisk® 3.0 (Vose software)with 10000 iterations per simulation.

Results

Preliminary results show that despite the differences in herds densities within each country during 2007 (Belgium 36000 herds; the Netherlands: 44200 herds; France: 190000herds) and herd structure, the surveillance components in place in each county performed well for proving freedom of infection after a whole year of surveillance (average sensitivity (Se) values around 99%), except for the sentinel component with a mean Se of ±70%. The high Se values are mainly explained by the high number of herds processed and the fairly good diagnostic test characteristics used in these surveillance components.

In terms of early detection, it's mainly the passive clinical component and syndomic components that performed best (Fig. 1). In Belgium, using fertility data was effective for early detection. No alerts were triggered using dairy and mortality data in Belgium, therefore no values were available for these components in Belgium. In the Netherlands, fertility and milk production data were effective for early detection as well as mortality data but values were slightly lower.

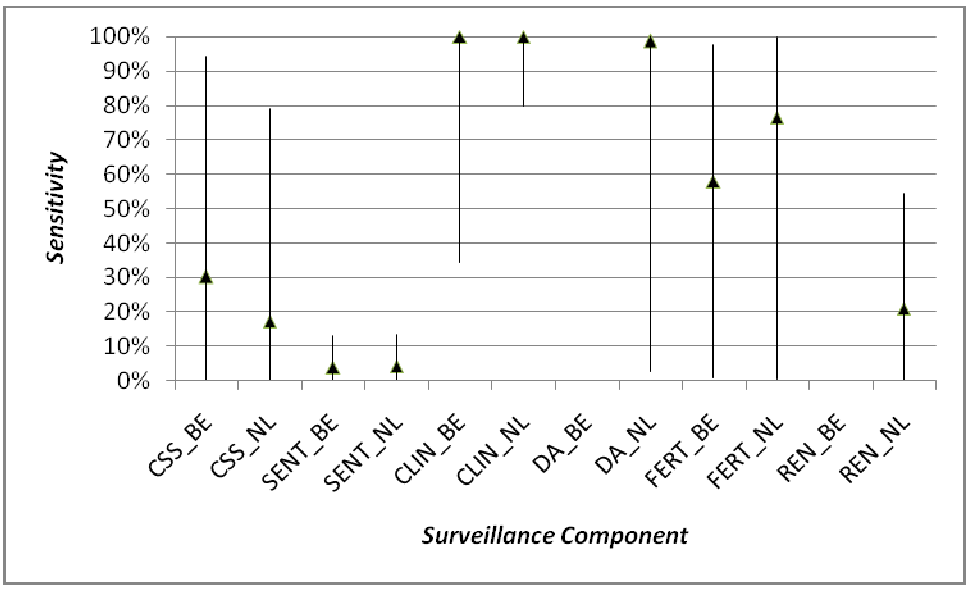

Figure 1: Component (CSS(Cross Sectional); SENT(Sentinel); CLIN(Passive Clinical); DA(Dairy); FERT(Fertility); REN(Mortality))sensitivities for early detection obtained in Belgium (BE) and the Netherlands (NL).

Preliminary results based in the simulated cost revealed that passive clinical surveillance is the most cost efficient component. The second 2 most cost efficient components were syndromic surveillance, solely based on fertility data, and they early cross sectional survey. The latter, despite being a good cost/sensitive option, because it was not repeated during the year, it hampered early detection. Sentinel surveillance would be the cheapest option ensuring relatively good level of detection and all through the year.

Discussion

From the present study it can be concluded that implementing syndromic surveillance on a routine base for early detection and in addition using the existing traditional surveillance methods once suspicions are raised would probably be the most effective and best cost efficient option.

The inputs and assumptions of the present study were based on past bluetongue outbreak datawhich provided reliable estimates for the simulations. However, care must be taken towards some inputs, for instance to estimate the epidemic curves, transmission values of the Bluetongue epidemic found in literature were used, but these estimates might change according to the disease under investigation.

Lack of specificity and pour positive predictive value due the false alarms and the difficulty of differentiating a baseline trend behind the back ground noise constitute the major limitations of syndromic surveillance, as this will lead to extra cost linked to confirmation testing generated in order to rule out the suspicious cases. Despite this, syndromic surveillance offers a very seducible option in a context of economic recession and scarce resources as it enables rapid diagnostics of deviations from trends, using readily available data.

To conclude it can be stated that results of such models, though only simulations, provide insight towards possible alternatives for optimising surveillance. Also by taking into account the efficacy of detection, field work, and financial resources, these models respond to required objectives laid down by the international standards for evaluation of surveillance programs.

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A trend analysis of antimicrobial resistance in indicator commensal bacteria from livestock in Belgium (2011-2013)

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Introduction

Antimicrobial resistance has become a major public health concern leading to the set-up of national and European surveillance programs to follow-up its evolution. Non pathogenic bacteria of the intestinal flora that are present in most animals and in humans are considered as good indicators to monitor antimicrobial resistance. In this study we performed a trend analysis of the prevalence of antimicrobial resistance in commensal *Escherichia coli* and *Enterococcus spp*. (*E. faecium* and *E. faecalis*) based on data of three consecutive years (2011-2012-2013) in Belgium. We also evaluated the level of multi-resistance (resistance against at least three antibiotics by the same strain) and its trend over the same period.

Material and methods

Sampling and laboratory testing

Representative faecal samples from cattle (veal calves and young beef cattle), slaughter pigs and broiler chickens were taken at the farm or at the slaughterhouse within a nationwide surveillance program of the Federal Agency for Safety of the Food Chain (AFSCA-FAVV). Isolates of *E. coli* (n=2504) and Enterococcal strains (n=1380) were obtained at the two Regional laboratories ARSIA and DGZ and sent to the National Reference Laboratory (CODA-CERVA) for confirmation of identification and for susceptibility testing using a micro-dilution technique (Sensititer®). A panel of 14 antimicrobials specified by the European commission was used for *E. coli* and a custom panel including 12 antibiotics was used for *Enterococci* isolates. For each strain and each antimicrobial substance, the Minimal Inhibitory Concentration (MIC) was read and recorded in a database. For each bacterial species and each antimicrobial separately, the percentage (with confidence intervals) of observed resistant strains was calculated per year and per animal category. Quantitative MIC values were converted in binary qualitative values (Resistant /Susceptible) based on the susceptibility breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Statistical analysis

Using SAS 9.2 software, several statistical methods were used to model the observed trends: (i) univariate models based upon categorical data (logistic regression, generalized logit models) or upon continuous data (models for interval-censored data, mixture models), (ii) multivariate model (Generalised Estimating Equation models or GEE). In addition, diversity indexes (entropy and weighted entropy) were calculated using R software to describe the degree of diversity of multi-resistance: a higher weighted entropy index reflecting a shift to multi-resistance against a larger number of antibiotics.

Results

E. coli

High levels of resistance against several antimicrobials were observed for the three consecutive years in all animal categories except in beef cattle. Decreasing trends were observed in veal calves and slaughter pigs for antimicrobials for which there was a low to moderate resistance prevalence. Increasing trends were observed in beef cattle for two antimicrobials (gentamicin, kanamycin) for which there was a low to moderate resistance prevalence.

The situation is worrying in broiler chickens: in this animal category, we observed a high resistance prevalence (≥ 60%) for half of the tested antimicrobials (7/14). Moreover, in this animal category, increasing trends of resistance were observed for 5 different antimicrobials (chloramphenicol, ciprofloxacin, colistin, florfenicol, nalidixic acid), including two substances for which there was high resistance prevalence (ciprofloxacin, nalidixic acid) **(Figure 1).**

Enterococci spp.

For these bacteria species, the number of tested isolates was sometimes insufficient to obtain significant trends. This was especially the case for the year 2011.

For *E. faecalis* resistance prevalence was high for 3 antimicrobials (erythromicin, streptomycin and tetracyclin) in all animal categories except in slaughter pigs for which only the resistance against tetracycline was high. Increasing resistance was observed mostly in strains from veal calves, including against 2 antimicrobials for which there was a high prevalence of resistance (erythromycin and tetracycline).

For *E. faecium* the resistance prevalence was generally low to moderate except for synercid for which there was a very high prevalence of resistance (> 80%) in all animal categories. Decreasing trends were observed for several antimicrobials in all animal categories except in veal calves for which there was an increasing resistance to 2 substances (erythromycin and tetracycline).

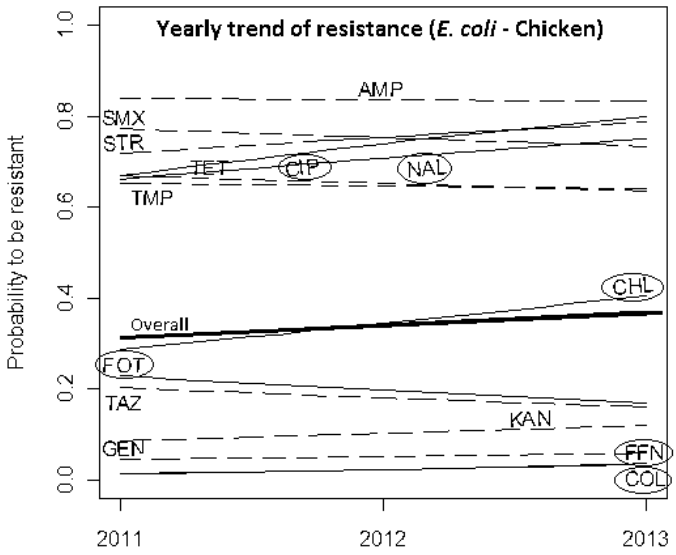

Fig.1 Yearly trend, per antimicrobial, of resistance in E.coli isolates from broiler chicken.
Five antimicrobials had a significant increasing trend and one a decreasing one
(dotted line= non significant; full line= significant; thick line= overall trend).

Multiresistance ***(Figure 2)***

In broiler chickens, the percentage of multi-resistant strains remained very high (>80%) during the three consecutive years both for *E. coli* and for *Enterococci*. In veal calves it was very high (>70%) during the three years for *E. coli*; it was increasing for *Enterococci* although the lack of data in 2011 makes such trend questionable. In slaughter pigs it was high for *E. coli* and decreasing for *E. faecium*. In beef cattle the level of multi-resistance was moderate.

The comparison of diversity indices (weighted entropy) shows that for *E. coli* strains, multi-resistance against a high number of antibiotics was higher in isolates from the veal calves animal category. The indices were lower for *Enterococci* isolates in all animal categories meaning that resistance to high number of antimicrobials was less frequent compared to *E. coli*.

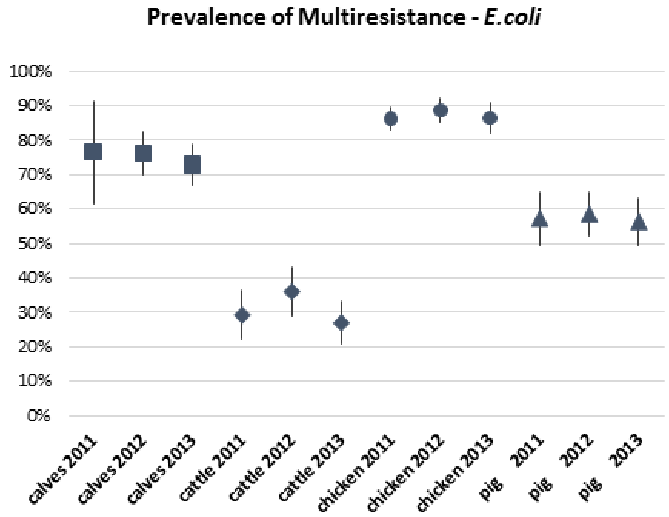

Fig.2 Yearly prevalence (with 95%C.I.) of multi-resistance in *E. coli* isolates, per animal category.
Prevalences were >50 % for all categories except for beef cattle.
No significant increasing nor decreasing trend was observed.

Discussion and conclusion

The models show that trends were sometimes diverging within the same animal species, with an increasing resistance for some antibiotics and decreasing for others without obvious explanation (e.g. florphenicol and cefotaxime in chickens) and the trends observed in *Enterococci* were sometimes conflicting with the trends observed in *E. coli*. (e.g. chloramphenicol in veal calves).

Nevertheless some of the results were more conclusive. A high to very high prevalence of resistance was observed for some of the tested antibiotics during the three consecutive years. Such high levels of resistance have already been observed previously both in pathogenic and non-pathogenic bacteria and results are comparable to those from other surveillance programmes in some other European countries such as in the Netherlands (Butaye *et al.*, 2001; Hendriksen *et al* , 2008a; Hendriksen *et al* , 2008a; Anonymous, MARAN 2014, Anonymous EFSA/ECDC 2014). The use of such antimicrobials should be carefully monitored especially in livestock species with intensive practices (veal calves, broiler chickens, slaughter pigs), for which the highest resistance prevalence were observed in our study. The situation is particularly worrying for *E. coli* isolated from broiler chickens with a high prevalence of resistance for several antimicrobials and yet, an increasing trend for some of them. It is also in this animal category that the level of multi-resistance was the highest, both in *E. coli* and *Enterococci* isolates. The prevalence of multi-resistance was also high for slaughter pigs and veal calves in *E. coli* isolates but no significant increasing/decreasing trend was detected in these animal species.

To conclude, the methodology and statistical tools applied in this study led to the observation of some significant trends and high prevalences of resistance after three years of monitoring; these results need to be confirmed by data covering a longer period. It is therefore recommended to continue the surveillance program to advise the public health authorities, based on objective data, in the measures to be taken to reduce antimicrobial resistance.

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Prediction of the infection status of pigs and pig batches at slaughter with human pathogenic yersinia spp. based on serological data

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Introduction

Pigs are identified as the main reservoir of human pathogenic *Yersinia enterocolitica* (Tauxe *et al.*, 1987). These *Yersinia spp.* represent 98.4% of the 7000 confirmed human yersiniosis cases in the European Union each year, and most of the remaining cases (1.6%) is caused by *Y. pseudotuberculosis* (EFSA and ECDC, 2013).

Pigs infected at farm level are the main source for the (cross-)contamination of pig carcasses at the slaughterhouse (Laukkanen *et al.*, 2009). The knowledge of the infection status of pig batches at slaughter may allow a distinction of non-infected and infected pig batches. A relation between the presence of *Yersinia spp*. in pigs at the moment of slaughter and the presence of antibodies, which can be obtained prior to slaughter, is therefore needed. The prevalence obtained by microbiology and serology in pigs at slaughter display a great variation between pig farms (Gurtler *et al.*, 2005; Laukkanen *et al.*, 2009; Novoslavkij *et al.*, 2011). The microbiological and the serological method also show some discrepancies. Depending on the age of the pigs to be sampled and the time of primary infection, a choice of method has to be made. Primarily, the presence of human pathogenic *Yersinia spp*. in pigs and the production of antibodies does not follow the same timeframe (Nesbakken *et al.*,2006; Vilar *et al.*, 2013). Secondly, the dilemma of analyzing tonsils or faeces is also depended on the time of infection. The carriage of enteropathogenic *Y. enterocolitica* last several months in the tonsils, whereas faecal excretion decrease within a few weeks p.i. (Nesbakken *et al.*, 2006).

The aim of this study is to provide a predictive value based on serology for the prognosis of the microbiological status of pigs and pig batches at slaughter age.

Material and methods

In 2012, 100 pig batches, all originating from different farms were sampled during slaughter. Two samples per pig were taken immediately after evisceration. Tonsils and a piece of the diaphragm pillar was cut from each pig. In total 7047 pigs (on average 70 pigs per batch) were sampled (Vanantwerpen *et al.*, 2014a; Vanantwerpen *et al.*, 2014b). The tonsils were diluted and 100 µl of each homogenate was plated onto a cefsulodin-irgasan-novobiocin (CIN) agar plate. Two typical colonies per plate were biochemically confirmed. Each colony displaying the typical morphology on CIN and the biochemical reactions on Kligler Iron Agar was verified by PCR based assays. The pieces of the diaphragm pillar (± 10g) were stored at -20°C. Two ml of meat juice was collected from each sample after thawing. The enzyme-linked immunosorbent assay (ELISA) Pigtype Yopscreen (Labor Diagnostik Leipzig, Qiagen, Leipzig, Germany) was performed according to the manufacturer's instructions. The amount of antibodies against Yops (*Yersinia* outer membrane proteins), expressed by the optical density (OD), was determined with a spectrophotometer. The cut-off value of 30 OD% was recommended by the manufacturer. Values lower than the cut-off value were considered as negative, values equal to or higher than the cut-off value were considered as positive. The results of both prevalence-determining methods were compared using a mixed-effects logistic regression at pig and batch level by using Stata/MP 12.1.

Results

Yersinia enterocolitica was present in 2009 pigs and 23 animals harbored *Y. pseudotuberculosis*. In one pig, both *Y. enterocolitica* and *Y. pseudotuberculosis* were recovered (Vanantwerpen *et al.*, 2014a). Of these pigs, 1872 also had a level of antibodies against *Yersinia* spp. above the proposed cut-off of 30 OD%. In total, there were 4851 pigs positive in at least one sample matrix.

Out of the 5016 pigs without *Yersinia* spp. in their tonsils, 2196 had no antibodies against these bacteria and 2820 pigs possessed the antibodies. The bacteriological negative pigs displayed a large variation in activity value. However, the majority of the bacteriological negative pigs (n=5016) yielded a low activity value. The number of pigs with an activity value between -5 and 5 OD% is 1105. Most pigs classified as bacteriological positive (n=2031) also have positive antibody titers. Applying the logistic regression, the microbiological contamination could not be predicted by the presence of antibodies at the pig level.

At batch level, 87 batches were infected with *Yersinia* spp. from which 78 batches had a mean within-batch OD% above 30 OD%. None of the negative batches for *Yersinia* spp. in the tonsils had activity values of more than 30 OD% in the meat juice. The mixed-effects logistic regression resulted in the following formula:

$$within-batch\ microbiological\ prevalence = \frac{0.444}{1 - e^{-0.063*(mean\ OD\% - 37.069)}}$$

Discussion

Since pork is a main source for human yersiniosis, it is important to reduce the prevalence of *Yersinia spp.* in pork as much as possible. A main strategy of the reduction of the risk of pig carcass contamination is the decrease of the number of infected slaughter pigs (Laukkanen *et al.*, 2009). The presence of *Yersinia* spp. in a batch is important regarding contamination of carcasses and is the basis when logistic slaughtering is applied. Therefore, knowledge of the infection status is needed before pigs are slaughtered. The comparison between microbiology and serology for *Yersinia* spp. at pig and batch level only results in a relation at batch level.

Pigs without human pathogenic *Yersinia* spp. in their tonsils, most (56.2%) are serologically classified as positive due to an activity value above the cut-off value of 30 OD%. The proposed cut-off of 30 OD% is not reliable to detect microbiological negative pigs. Decreasing the cut-off activity value leads to a higher detection of infected pigs, which is the most important. However, this also leads to indicating more non-infected pigs as infected.

A very low amount of the microbiological positive pigs (7.8%) presented an activity value below the proposed cut-off of 30 OD%. An explanation for the discrepancy is the biological difference between the presence of the pathogen and the serological reaction in the animal. Presence of the *Yersinia* spp. in the tonsils is due to a recent infection, whereby no antibodies will be detected in the serum of these infected animals. According to Thibodeau *et al.*(1999), tonsils can already be colonized a few hours post infection, while infected pigs are all seroconverted around 19 days p.i. (Nielsen *et al.*, 1996).

Based on serology results it is possible to detect infected batches prior to slaughter by using the presently proposed equation: when the mean activity value is more than 37 OD%, the batch is indicated positive. Knowing the microbiological prevalence prior to slaughter, infected pig batches can be delivered to the abattoir and slaughtered after non-infected pig batches to avoid cross-contamination in the slaughterhouse. It is more important to detect microbiological positive batches than pigs, since separating infected and non-infected pigs at the slaughterhouse is impossible. If pig batches are categorized in the slaughterhouse based on serological testing, it should be taken into account that slaughter pigs can still harbor *Yersinia* spp. in the tonsils, without seroconversion (9 of the 87 batches).

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POSTERS

High seroprevalence of Q fever in large animal veterinarians in southern Belgium

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Abstract

Q fever is a zoonosis occurring worldwide and caused by *Coxiella burnetii*. In Belgium Q fever is enzootic in the domestic ruminant population. The disease is submitted to a compulsory declaration in animals since the new Royal decree of February 2014, and in humans since 1971. The human infection results mostly from the inhalation of aerosols generated from contaminated animals and animal products. Human Q fever may range from subclinical infection to endocarditis and long term debilitation such as chronic fatigue syndrome. Q fever is a well-known occupational zoonosis, but so far no epidemiological studies have been conducted to establish its seroprevalence among the different risk categories in Belgium.

The aim of this work was to investigate the seroprevalence and characterize the immunological response after Q fever infection in large animal veterinarians in Southern Belgium. The study was performed after approval of the Medical Ethical Commission of the University of Liège.

Collected sera were initially screened using a commercial ELISA kit, allowing detecting phase II IgM and IgG antibodies. Results were confirmed using indirect immunofluorescence assay (IFA), which allowed the detection of phase I IgM and IgG antibodies, as well as to define the antibody titre of each immunoglobulin.

The results obtained from the two serological tests seemed to display a good agreement and a high seroprevalence was measured within the tested population. Furthermore, high phase I antibodies characterized the immune response of the seropositive large animal veterinarians.

Observance as a tool to assess the compliance of veterinary students to biosecurity rules in a necropsy room and at a slaughterhouse

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Abstract

In veterinary medicine, the concept of biosecurity relies on the implementation and respect of procedures that reduce the risk of introduction/spread of pathogens. The main objective of the study was to assess the usefulness of observance (ratio of the number of biosecurity measures fulfilled to the number of measures to be applied) to assess the compliance of veterinary students to biosecurity measures implemented in a necropsy room and at a slaughterhouse. Such assessment was performed by observing sessions of practical works performed in the necropsy room of the University of Liege (N = 122 observations), and by accompanying veterinary students during their practical works at the Liege slaughterhouse (N = 56 observations). A total of 40 biosecurity rules were compiled in a checklist applied to the necropsy room while 56 rules were considered for the slaughterhouse. Observations were performed by a single person ensuring standardization. The observance was expressed a percentage. A comparison between groups was also performed through a Welch's t test. The survey carried out in the necropsy room revealed that 22 rules were disregarded and the observance level reached 50%. At the slaughterhouse, 33 rules were disregarded, but only 20 of them were considered due to the impossibility of students to respect the 13 others. The level of observance reached 53%. These results confirm the observations made in human medicine. One group of students significantly committed more breaches at the slaughterhouse, while no group effect was observed for the necropsy room. The compliance to biosecurity rules is clearly intermediate, which should pave the way to a series of measures such as increasing awareness of students through education. The follow-up of observance along time could be a good way of assessing the evolution of compliance to biosecurity measures and should be perceived as an aspect of auto-control.

Modelling economic impacts of an epidemic/epizootic spread of West Nile virus in Belgium

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Abstract

The present study aimed at estimating, in a prospective scenario, the potential economic impacts of a West Nile virus epizootic and epidemic in Belgium, both for the equine sector and for the human health sector. The virus being still exotic to date, the modelling of risk areas, based on the habitat suitable for *Culex pipiens*, proven vector of the disease in Western Europe, allowed determining equine and human populations at risk. Characteristics of the disease based on European past experiences allowed estimating morbidities among horses and humans. The main costs for the equine sector were prevention measures such as vaccination and containment of horses indoors to avoid mosquito bites. Regarding the human health aspect, only short-term costs and losses were estimated for patients who developed the neuro-invasive form of the disease, as no vaccine is available yet. Losses attributed to the death of patients represented the major financial consequences of the West Nile virus epidemic in humans.

Experimental in vivo infection of pigs by a Belgian wild boar hepatitis E virus strain

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Abstract

Hepatitis E virus (HEV) possesses four genotypes. In Europe, genotype3 mainly circulates and its route of transmission is highly suspected to be zoonotic. The aim of this study is to investigate the potential transmission of a wild boar HEV strain (WbHEV) from wild boar to swine.

Seven pigs were divided in 3 groups (Gp) and inoculated by intravenous route with 0.45µm filtrates of livers from pig and wild boar. The Gp1 contained 3 pigs inoculated with WbHEV strain; the Gp2 (negative controls) contained 2 pigs inoculated with a HEV negative swine liver and the Gp3 (positive controls) contained2 pigs inoculated with a HEV swine strain. All the inocula belonged to genotype 3 subtype F. The pigs were euthanized and necropsied after 8, 9 and 10 days of infection. Samples were analysed by qRT-PCR, ELISA and histopathology.

No clinical signs were observed during the infection. The hepatic enzymes (ALT and AST) were not increased in each group. Bile and liver were PCR positive in Gp1 and Gp3 pigs. Sera were PCR positive in Gp1 from the 8thday until the euthanasia and in 1 pig from Gp3 at the euthanasia. Faeces were also PCR positive in Gp1 from the 4th day until the end and at the euthanasia for Gp3. All groups were seronegative in ELISA.

In this study, we developed a swine model of in vivo infection with a Belgian WbHEV strain and we showed for the first time that a WbHEV strain could replicate in swine. These results provide experimental data about the likelihood of the crossing of the wild boar–swine barrier in the context of outdoor breeding of pigs.

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Main diseases in diurnal birds of prey from three care centers for wildlife in Wallonia – Belgium

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Abstract

From 2007 to 2011, 244 diurnal birds of prey collected by three care centers for wildlife (CREAVES) were submitted to a veterinary team for diagnostic and treatment or necropsy. Diagnostics were conduct by clinical examination, radiography, endoscopy and laboratories exams (biochemistery). Necropsy were completed by parasitic evaluation and, in some case, laboratory examination (bacteriology, cytopathology and histopathology). The main species from the study were : *Buteo buteo* (82), *Falco tinnunculus* (71) and *Accipiter nisus* (64). They are presented by traumatic and non traumatic statistics. The rest of the cases (27) are presented in a table with the suspected or confirmed etiology. For *Buteo buteo*, 39 cases were traumatic (T) (48,75%), 33 had infectious or metabolic disease (IMD) (41,25%), 9 were undiagnosed (U) and 1 was juvenile (1,25%). For *Falco tinnunculus* T were 29 (30,99%), IMD were 22 (30,99%), U were 8 (11,27%) and 12 juveniles (16,90%). For *Accipiter nisus* T were 38 (59,38%), IMD were 18 (28,13%), U were 6 (9,38%), 1 was a juvenile (1,56%) and 1 was catch by a predator (1,56%). This first study in CREAVES demonstrate the scientific more value of lived animals relative to the necropsy of died animals in the wild and the CREAVES role of sentinel for sanitary survey in Wallonia.

Main diseases in nocturnal birds of prey from three care centers for wildlife in Wallonia-Belgium

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Abstract

From 2007 to 2011, 258 nocturnal birds of prey collected by three care centers for wildlife (CREAVES) were submitted to a veterinary team for diagnostic and treatment or necropsy. Diagnostics were conducted by clinical examination, radiography, endoscopy and laboratory exams (biochemistery). Necropsy were completed by parasitic evaluation and, in some case, laboratory examinations (bacteriology, cytopathology and histopathology). The main species from the study are: *Tyto alba* (84), *Strix aluco* (68), *Athene noctua* (63) and *Asio otus* (28). Results are classified by traumatic or non traumatic statistics. The rest of the cases (15) are presented in a table with the suspected or confirmed etiology. For *Tyto alba* : 27 cases suffered from traumatic lesions (T) (32,14%), 26 suffered from infectious or metabolic disease (IMD) (30,95%), 7 were undiagnosed (U) and 24 were juveniles (J) (28,57%). For *Strix aluco* : 21 suffered from T (30,88%), 11 suffered from IMD (16,18%), 8 were U (11,76%), 4 were victims of predation (5,88%) and the last 24 one were juveniles (35,29%). For *Athene noctua* : 28 suffered from T (44,44%), 13 suffered from IMD (20,63%), 4 were U (6,35%), 1of them was catched by a predator (1,59%) and the last 17 one were juveniles (26,98%). This first study in CREAVES demonstrate the scientific more value of lived animals relative to the necropsy of died animals in the wild and the CREAVES role of sentinel for sanitary survey in Wallonia.

Spatial and temporal interactions between livestock and wildlife in South Central Spain assessed by camera traps

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Abstract

The diversification of livestock farms into hunting estates in South Central Spain (SCS) may impede the success of *Mycobacterium bovis* eradication programs by facilitating transmission between wildlife and livestock. In this observational study we aimed to provide information of relevance about the nature and frequency of interactions (observed visits to study points) between livestock (cattle and domestic pigs) and wildlife (wild boar and red deer).

The study was conducted in an extensive cattle farm in SCS where the land is also used for game hunting. During a period of one year, camera traps (n = 16) were placed at a priori risk points for interspecies interactions: water (natural and artificial troughs), food placed on the ground for baiting wildlife, and pasture. To define indirect interspecies interactions, a critical time window for *M. bovis* to survive in the environment was selected based on the literature. Results suggest that wildlife frequented food and pasture points more often than water points, and that the number of visits increased through the dry season, peaking during the acorn season (October–January) and the deer breeding season (June–July). Direct interactions were rare (n = 10), as opposed to indirect interactions (n = 8992). Wildlife-followed-by-livestock interactions (n = 7714) occurred much more often than livestock-followed-by-wildlife (n = 1278) and were frequent at water points (66% water points, 17% food, 17% pasture).

Results also suggest that water points are a hotspot for indirect interactions and might therefore be a source of infection at the wildlife–livestock interface in the territory covered, particularly for *M. bovis*, as it is around water where the bacteria seem to survive the longest. Preventing aggregation and therefore reducing contact rates between domestic and wild animals especially at water points may be valuable for disease control in South Central Spain.

Using Somatic Cell Counts in milk to monitor udder health at cow and herd level

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Abstract

Individual cow somatic cell counts in milk (SCC) are widely used in the dairy industry to monitor the udder health of cows and herds. In New Zealand, bulk tank milk somatic cell counts (BTMSCC) ≥400,000 cells/mL results in demerit points for reduced milk quality and a reduction in the milk pay out price. Moreover, an increase in SCC in a single cow is associated with decreased milk production. Therefore, maintaining low SCC as well as low BTMSCC is important, not only to maintain milk quality but also for economic reasons. Farmers participating in herd performance testing by the Livestock Improvement Corporation (LIC) receive information on individual SCC of all cows. Each milk consignment is also tested for BTMSCC independent of LIC performance testing. The availability of this information provides an opportunity for farmers to use SCC and BTMSCC as hazard points for milk quality and production monitoring. Therefore, this study aimed to evaluate critical levels of SCC and BTMSCC that may be used by farmers to maintain high production efficiency and adequate milk quality.

The evaluation used herd test data of individual cows available from June 2001 to May 2002 (i.e. 2001/02 milking season). It included 522 randomly selected herds using regular herd testing services of Livestock Improvement Corporation (LIC), from three regions of the North Island (set 1). A second, herd-level data was generated from this pool. It included herd average somatic cell count, a quasi-bulk tank SCC (BTMSCC), calculated as geometric mean SCC of every cow for each test day and each herd. This BTMSCC was weighted by milk yield (volume) of individual cows on that day (set 2). These two datasets were used to evaluate the association between SCC and daily milk production, age, breed, and lactation stage (set 1), as well as the association between the proportion of cows with high SCC and BTMSCC (set 2). Regression models were developed to investigate lactation dynamics of

increases or decreases of SCC across a threshold of SCC of 200,000 cells/mL of composite milk between herd test days as a proxy for new intra-mammary infection (IMI) or recovery from IMI.

A third set included data from Fonterra BTM consignment testing for milk solids and BTMSCC. The dataset included approximately 60% of all dairy herds in New Zealand during the 2001/02 season (set 3)(Bissielo *et al.*, 2008)This dataset was used to evaluate the risk of BTMSCC ≥400,000 cells/mL depending on preceding BTMSCCs at varying intervals. Logistic regression with lag effects was used adjusting for region and lactation stage (days after first consignment as off 01 June 2001).

SCC increased with age and was higher in Holstein-Frisian and cross breeds than in Jersey. The geometric mean SCC dropped from 130,000 cells/mL to 60,000 cells/mL within 3 weeks of calving, then gradually increased again to about 200,000 cells/mL towards the end of lactation. The approximated rate of IMI continuously increased from 5 to 30% during lactation whereas the rate of recovery from intra-mammary infection (RIMI) steadily decreased from almost 60% at the beginning to 18% at the end of lactation. These trends suggest that the times immediate post-calving and close to drying-off are the most critical risk periods for udder health. The most substantial production loss was observed when SCC levels increased from <20,000 to 200,000 cells/mL but little beyond 200,000 cells/mL. For example, a production loss of 7.6% was associated with an increase from 50,000 to 100,000 cells/mL. Since about 80% cows had SCC <200,000, these trends suggest that efforts to decrease SCC below 200,000 cells/mL might potentially be rewarded with substantial production increase.

The proportion of cows in a herd with SCC ≥200,000 cells/mL increased steadily during lactation from approximately 15% at the beginning to approximately 40% at the end of lactation. However, the proportion of cows with SCC ≥1,000,000 cells/mL remained at a mean of approximately 3% throughout the course of lactation. This suggests that so called 'millionaire' cows (i.e. SCC ≥1,000,000 cells/mL) may be identified and removed at any time during lactation, and again, that monitoring and interventions are highly critical towards the end of lactation.

With a similar pattern as the proportion of cows with ≥200,000 cells/mL, monthly means of BTMSCC increased steadily during the milking period suggesting that the increase in BTMSCC was mainly influenced by the proportion of cows with SCC ≥200,000 cells/mL and to a lesser extent by 'millionaire' cows. This monthly increase in the BTMSCC was also reflected in the increase in proportion of herds with BTMSCC over the penalty limit, starting with proportions less than 2%, followed by a slow increase, and ending with an abrupt increase at the end of the lactation period of 6%.

There was a strong increase (from 0.3 to 9.9%) in the risk of BTMSCC ≥400,000 cells/mL when herds crossed the threshold of 30% cows with ≥200,000 cells/mL suggesting that herd managers would be well advised to keep the percentage of cows with elevated SCC clearly below this cutoff (e.g. <20%) to prevent the accumulation of penalties due to poor milk quality. These trends suggest that, at gross population average, contagious pathogens (increasing the proportion cows in the herd with SCC >200,00) have a much greater impact on BTMSCC and on the risk of BTMSCC related penalties than individual cows with high SCC (>1 million). Therefore research and development efforts should prioritise risk factors and infection dynamics of such pathogens, e.g. *S uberis, S. agalcatiae* and *St. aureus*.

The critical limit above which a current BTMSCC indicated an exponentially increasing risk of obtaining a BTMSCC ≥400,000 cells/mL 3 to 5 days later was around 200,000 cells/mL. Up to 200,000 cells/mL, the estimated risk of crossing over the 400,000 cells/mL demerit threshold was below 0.5%. The risk increased towards the end of the lactation (> 240 days of lactation), when a current BTMSCC of 200,000 cells/mL was associated with a 20% risk of passing the limit 3-5 days later.

In conclusion, SCC 200,000 cells/mL appeared to be a critical limit for BTMSCC, particularly towards the end of the milking period. The proportion of individual cows with SCC ≥200,000 cells/mL should be safely lower than 23% at any time to reduce the risk of demerit points due to BTMSCC ≥400,000 cells/mL. Milk production appeared to be optimal as long as cows maintained SCC <100,000 cells/mL. The described thresholds are recommended benchmarks for sustainable milk quality and economic returns.

Assessment of brucellosis management measures and vaccination campaign in two districts of Buenos Aires, Argentina

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Abstract

Bovine brucellosis is endemic in Argentina. The disease is more controlled in dairy than in beef facilities. Almost 50% of the dairy farms (6,500 out of 11,000) and approximately 10 % of beef farms are free from brucellosis. In addition, 2% of beef cattle in the country are seropositive to *Brucella*.

The National Control and Eradication Program establishes the compulsory sub-cutaneous vaccination of all 3 to 8 month-old females with 15-30 x 10^9 viable *Brucella* strain 19. In most cases vaccination is performed simultaneously with the Foot and Mouth Disease vaccination. The Argentine National Veterinary Service, SENASA, entrusts both vaccination campaigns execution to 310 Local Sanitary Entities (LSEs).

The objective of this study is to assess the vaccination campaign in two districts, Brandsen and Navarro, in Buenos Aires province, and to evaluate some farmers' and veterinarians' management measures concerning the disease.

Four different questionnaires were performed face to face to 105 farmers (52 dairy farms and 53 beef farms), 11 private veterinarians, 13 vaccinators and 2 people responsible for the LSEs. To evaluate the compliance between field practices of vaccination and the SENASA's regulation, serum samples of a subgroup of those studied farms were randomly taken from heifers vaccinated 21-50 days before. The buffered plate antigen test (BPA) was the diagnostic test chosen to verify the vaccine exposure.

As regards dairy farms, 11 (21%) were not officially free certified and 7 of them (63%) were in cleaning up process. Only 7 beef farms (13%) were officially free certified. Most veterinarians (80%) suggested serologic diagnose when having reproductive disorders, which were mainly due to *Neospora caninum*. These animals were sold to slaughterhouses. A total of 45 farmers (42%) bought animals, 27 (57%) of which came from not officially free certified farms and 77 (74%) consulted a veterinarian when having reproductive disorders. None of them quarantined these animals and only 4 (15%) ran brucellosis serologic tests. The individual identification of the vaccinated heifers was performed in 100 farms (95%) and only in 11 beef farms (10%) some heifers might have remained unvaccinated. A high percentage of the vaccinators (77%) calibrated the syringe before the vaccine injection, all of them temporally stored the vaccine in boxes with refrigerants and 40% agitated the vaccine bottle during the vaccination process. If some vaccine dropped when vaccinating, 11 vaccinators (85%) performed the vaccination again but none of them wrote it down in the vaccination records. The LSEs audited the performance of the vaccinators at least once per campaign. The authors are still taking the serum samples so the BPA results will be available in the next weeks.

Preliminary results suggest that farmers and veterinarians applied management practices regarding brucellosis, though some of them should be improved. In addition, although the vaccination campaign was globally well performed, some improvements concerning the individual identification should be applied to beef farms, mainly in those with breading season throughout the year where the age of the heifers cannot be guaranteed.

Unexpected Brucella suis biovar 2 infection in a dairy cow in Belgium: Epidemiological aspects

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Abstract

A spill over infection with *Brucella suis biovar* 2 (BS2) in a dairy cow was detected during the Belgian national brucellosis screening program in spring 2012 (positive bulk tank milk indirect ELISA). All animals of this "reactor" farm were further serologically tested. One animal classified positive by SAT-EDTA and two independent indirect ELISAs, was slaughtered on April 23, 2012. This was a non-pregnant dairy cow (last calving in March 2011) older than four years, born in the farm that showed no clinical sign of brucellosis. Bacteriological examination was conducted on the following samples collected at the slaughterhouse: spleen, uterus, lymph nodes (supramammary, retropharyngeal, iliac and submandibular) and the udder. *Brucella spp.* were only cultured from the spleen and the uterus and were further identified as BS2. This case was epidemiologically related to exposure to BS2 infected wild boar offal discarded in a pasture during the 2011 hunting season.
In livestock, the different *Brucella* species have preferred (but not exclusive) animal hosts. In bovines, in addition to *Brucella abortus*, infections caused by *Brucella melitensis* and *Brucella suis* have been previously reported. These cases are always epidemiologically linked to the presence of a reservoir of *Brucella spp.* in their preferential hosts. Infection associated with *B. suis* in cattle is considered to be a "spill over" from a wildlife reservoir. In Europe, wild boars (*Sus scrofa*) and hares (*Lepus europaeus*) constitutes the reservoir of BS2. Only two cases of BS2 infection in cattle have been described previously in the literature in Denmark and France, presumably related to BS2 infected hares and wild boars, respectively. Since this report, two other cases were reported in Europe, one in Poland (result of bacteriological investigations in cattle slaughtered as a result of positive serological reactions for brucellosis) and one in France (under investigation).The results of the presented study indicate that BS2 can infect cattle, and plays a role in the epidemiology and need to be considered in the control of bovine brucellosis. Lessons to be learned: Firstly, preventive measures must be implemented by hunters (awareness campaign, education on biosecurity and responsibility) and farmers (double fences for "at risk" pastures). Secondly, typing of *Brucella spp.* is necessary to trace back the infection. Thirdly, a sound epidemiological inquiry must always be done in case of positive results in order to identify or exclude *B. abortus* infections as well as to investigate possible BS2 infections. Fourthly, our bacteriological results (i.e., the absence of isolation of BS2 from the samples collected at the abattoir from 111 additional bovines) suggest that stamping out is not necessary because BS2 is not likely to be transmitted between cattle given that cattle are spill over hosts, not preferential hosts for BS2 (i.e. the basic reproduction number, R0<1). Lastly, from a veterinary public health perspective, according to the literature BS2 has a very low residual pathogenicity in humans.

Brucella melitensis at the wildlife-livestock-human interface in the UAE

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Abstract

Brucellosis has been reported in almost all domestic animals in the Middle East and especially in goats and camels (1). In the emirate of Abu Dhabi (UAE), 55.1% of the 247 domestic farms sampled in 2010 were *Brucella* seropositive (2). *B. melitensis* has been reported only once in a Nubian Ibex (*Capra ibex nubiana*) in Dubai (3). In addition, there are no recent data accessible on the species and biovars circulating in the UAE. Both *B. melitensis* biovar 1 and 3 were reported on human cases from Tawam Hospital, Al Ain (Abu Dhabi) hence were co-circulating in the UAE in 1996 (4). From 2000 to 2003, 6,5 % of the 998 patients admitted in this same hospital were *Brucella* seropositive (5). The livestock management system in the UAE is in favour of *Brucella* spreading with the coexistence of several livestock species and the under-diagnosed and/or underreported animal and human positive cases. *B. melitensis* is rarely reported in wildlife (6) but direct contact between free roaming livestock and wild ungulates is likely in the region. In addition distant transmission via biological (dogs, foxes) (7,8) and mechanical vectors such as insects (9) is possible.

In this study we investigate the brucellosis seroprevalence within a large herd of scimitar horned oryx (SHO) (*Oryx dammah*) and try to trace the source of infection. SHO are extinct in the wild since 2000 and conservation programmes rely heavily on captive stocks for possible future re-introduction. Among the 480 juveniles/subadults SHO and the 400 adults SHO tested, 75% and 95% of them were *Brucella* seropositive respectively based on a single rose Bengal test. Sand gazelles housed within the same collection but 2 km away from the oryx herd are also infected, 15 of which were tested and were all seropositive. Clinically most the male oryx suffer from orchitis while sand gazelles present mainly hygroma at the tibio-tarsal and metacarpo/tarso-phalangeal joints. *B. melitensis* biovar 1 was isolated from both oryx foetal stomach content and metacarpal fluid from a gazelle and genotyped using multiple-locus variable-number tandem-repeat (MLVA) at CODA-CERVA (10). Both strains have a similar genetic profile to the BfR25 strain previously isolated from a host specific goat in the UAE. Supposedly, oryx and/or gazelles initially acquired brucellosis infection from livestock, and only later did it start circulating among the wildlife collection. Moreover, *Brucella spp.* that has not been genotyped has clinically contaminated 3 ungulate keepers handling contaminated materials in this same collection. All were cured following the specific Word Health Organisation treatment' recommendations.

Holistic and ecosystem based approach should be used to tackle *Brucella* transmission and maintenance in heavily contaminated environment. Efforts in identifying species and subtyping of *Brucella* isolates are paramount for any preventive (awareness campaign) and epidemiologic surveillance-control programme in *Brucella*-endemic area (11,12). Active surveillance of susceptible animals and occupational health screening of the workers will give a more accurate picture of the *Brucella* foci in the country and will help defining the appropriate control strategy. In heavily infected countries, mass vaccination programmes seem inevitable but require proficient veterinary services. Implementation of a strategic approach of the enzooty on the scimitar horned oryx will require preliminary studies on diagnosis tests, host/pathogen/environment interactions, vaccine assessment and appropriate protocols.

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Clinical sentinel surveillance of equine West Nile fever, Spain

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Abstract

West Nile fever (WNF) is a viral zoonotic infection caused by a mosquito-borne flavivirus of the *Flaviviridae* family. According to a comparative study, the passive surveillance of horses by equine veterinarians appeared to be the most cost-effective system in the European context of WNF. Clinical data issued from a passive epidemiosurveillance network from September 2010 to December 2011 on horses in Spain were statistically compared and used to develop a predictive diagnostic decision tree, both with the aim to improve the early clinical detection of WNF in horses. Although clinical signs were variable in horses affected by WNF, four clinical signs and the month of occurrence were identified as useful indicators to distinguish between WNF related and unrelated cases. The signs that pointed out a presumptive diagnosis of WNF in horses were cranial nerves deficits, limb paralysis, photophobia and nasal discharge. Clinical examination of horses with neurological signs that are not vaccinated against WNV could provide important clues for the early clinical detection of WNF, and therefore, serve as an alert for possible human viral infections. The study of the clinical pattern of WNF in horses is of importance to enhance awareness and better understanding, and to optimise surveillance designs for clinical detection of WNF in horses in advance of epidemic activity affecting humans.

Schmallenberg virus in Belgium: impact in sheep and cattle herds

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Abstract

Schmallenberg virus (SBV) emerged during summer 2011. SBV was shown to be responsible for an acute unspecific syndrome in cattle and reproductive diseases in domestic ruminants. To date, the real impact of SBV in Belgium remains unknown. Therefore a study, via a telephone questionnaire, was conducted for cattle and sheep farms. Questions related to reproduction parameters and clinical signs observed in newborn and adult animals were designed.

In cattle, the impact of SBV was shown by the rate of malformed calves born during the epidemic and represented 2.32% of gestations, with 2.2% attributable to the SBV infection. In addition, based on the farmers' statement, an increased percentage of aborted calves was calculated in 29% of farms, while this was 32% of farms for the percentage of stillborn calves and 36% of farms for the percentage of malformed calves.Interestingly, abortions and stillbirths did not seem to be a clear sign of SBV infection for the cattle farmers. Even though, individual and high differences between herds did exist. In addition, it was calculated that 21% of the farmers perceived a decrease of the fertility, probably due to embryonic mortality. Twenty-six percent of farmers also declared to have observed clinical signs that can be related to an SBV infection in adult animals the year of the outbreak. In calves, the most observed clinical signs were scoliosis, twisted limbs and big head. Almost 69% of the first suspicions by the farmer of SBV infection were due to calves with malformations and/or stillborn.

In sheep, the impact of SBV was higher than in cattle with 2.89% of lambs born aborted, 9.39% of lambs born stillborn and 5% of lambs born malformed the year of the outbreak. In addition a percentage of 2.56% for aborted lambs, 6.75% for stillborn lambs and 5% for malformed lambs were attributed to the SBV infection. It was also calculated that 31%, 60% and 45% of farmers had an increased proportion of lambs aborted, stillborn or malformed, respectively, the year of the outbreak. Specifically, torticollis, twisted limbs and big head were the most related malformations within the different studied systems. The reason the most frequently invoke by the farmers to suspect the presence of SBV in the farm was the birth of a malformed/stillborn lamb, which represented 82.76% of the sheep farmers.

In conclusion, the results of this study suggest that the impact of SBV was particularly high in Belgian cattle and sheep farms with relation to reproductive disorders encountered during the epidemic. This work demonstrated that the birth of a malformed calf was an essential condition for the farmer to suspect the presence of the virus in his herd and potentially send samples to the National Reference Laboratory for further analyses. This study also emphasized the critical need for further knowledge about the vectors (e.g. *Culicoides spp.*) and its risk factors given the possible resurgence or emergence of *Culicoides*-associated diseases.

Experimental superinfection in calves previously immunized with bluetongue virus serotype 8

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Abstract

Calves previously infected with bluetongue virus serotype 8 (BTV8) were challenged with BTV1. The inoculum appeared to be contaminated with BTV15. To allow comparison additional BTV1 and BTV15 single serotype experimental infections were performed. Virological, serological (including cross-neutralisation assays) and clinical parameters were followed and compared to the initial BTV8 experimental infection.

Eight Holstein calves had been infected with BTV8, including 4 animals previously vaccinated against BTV8. Five animals were kept as controls. About 5 months later, the 8 infected calves were challenged with BTV1 infectious blood. Each animal received $10^{6,15}$ $TCID_{50}$ of virus, half intravenously and half subcutaneously. An incidental contamination of the inoculum with BTV15 was discovered during the course of the study: the inoculum contained respectively $10^{6.8}$ and $10^{7.6}$ copies of segment 2 cDNA per ml of blood, for BTV1 and BTV15.

Subsequently, two groups of three calves were infected with a unique serotype, BTV1 (10^{6} $TCID_{50}$/animal) and BTV15 (10^{4} $TCID_{50}$/animal). Viral RNA was detected by serotype specific RTqPCR, in blood and organs. Calves were euthanatized 21 days post infection (dpi) (following superinfection) or 35 dpi (following single serotype infection) and necropsied.

Most of the calves showed a more severe clinical picture when superinfected with BTV1/BTV15 than with BTV8 alone.

Residual BTV8 viraemia could be detected in non-vaccinated animals until the end of the experiment (thus up to 181days after the previous BTV8 challenge). BTV1 could only be detected inconstantly, and generally at lower copy number than BTV15. On the contrary, BTV15 could be detected in both vaccinated and non-vaccinated groups, until the end of the experiment. At viraemic peak, S2 BTV15 copies number / ml was about 1000 fold higher than BTV1 copies number. BTV1 only gave rise to low titres of neutralising antibodies, whereas anti-BTV15 neutralising antibodies titre increased regularly until the time of slaughter.

BTV8 RNA still could be detected in organs of two non-vaccinated calves; BTV1 RNA could only be detected in the spleen of one calf and BTV-15 RNA in 16 organs belonging to 7 different calves.

BTV1 and BTV15 single serotype infections caused only very slight clinical signs. RNAemia evolution of these two serotypes, following single serotype inoculation, was quite similar and reached levels in line with BTV8 single infection and BTV15 RNAemia after superinfection. BTV1 and BTV15 RNA could be detected in 33 and 53 % of the tested organs.

By contrast to *in vivo* viral replication, *in vitro* growth curves showed a fast replication of BTV1, a delayed one for BTV15, BTV8 being intermediate, in VERO and BPAEC cells. Only a minor cross reactivity could be demonstrated between BTV1, 15 and 8.

In this study BTV1/BTV15 superinfection has been clearly pathogenic. Interference mechanisms might be at the origin of the low replication of BTV1 in the course of superinfection. BTV15 could replicate in calves notwithstanding the contemporary circulation of BTV1 and/or BTV8, in a similar manner as if it was the unique inoculated serotype.

Infectious bronchitis virus infections of chickens in Belgium: epidemiological data paving the way to improved control measures

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Abstract

Infectious bronchitis virus (IBV) is the cause of a very common and economically important disease of chickens. In young birds IBV mostly leads to respiratory problems although some strains are nephropathogenic. Mortality in infected flocks may rise to 30%. In layer and breeder chickens, egg production and quality declines are noted.

IBV was first observed in 1931 in Massachusetts. This original IBV was therefore called the Massachusetts type. Being a single stranded RNA virus, IBV appeared highly susceptible to mutation. Variations in the S1 spike protein which is projected on the surface of the virus particles, have led to the emergence of dozens of variants worldwide. Variants may exhibit a varying susceptibility to protection afforded by vaccine strains, thereby hampering control of the disease by vaccination. Formerly variants were identified on the basis of virus neutralisation assays (serotyping) but nowadays these tests have been replaced by molecular techniques (genotyping). Available information on the prevalence of IBV variants in Belgium was fragmentary. It was therefore the aim of the present study to examine the prevalence of IBV in chicken flocks and to identify the IBV types involved.

From April 2012 to June 2014, cloacal and/or tracheal swab samples were collected from 230 broiler, breeder or layer chicken flocks exhibiting signs that might indicate an IBV infection. The samples were submitted to a RT-PCR procedure in order to detect the presence of IBV RNA. When positive, approximately 400 base pairs (bp) encoding for a hyper-variable region of the S1 protein were sequenced. Distinction between vaccine strains and field strains was made on the basis of the sequencing results, the Ct threshold values obtained, the vaccination history and the intensity and nature of clinical signs. The amino acid composition of the S1 protein was deduced from the sequenced region.

Nineteen percent of the samples examined did not reveal any RNA from IBV and was therefore considered negative. In 35% of the samples only RNA from IBV vaccine strains was found. In 16 % of the samples, a very small quantity of RNA was present, which rendered the strains untypeable. The remaining 30% of submissions contained RNA from IBV field strains, thus indicating a field infection. Within the series of IBV field isolates, the 4/91 (793B) type of strains was most prevalent, representing approximately 46% of the cases. It was recovered predominantly from layer and broiler breeder flocks in the second half of the production period. The variant type QX accounted for 35% of the IBV field isolates. It was found most often in broiler and in layer flocks. Thirteen percent of the field strains represented the variant type D274. These strains were demonstrated almost exclusively in broiler breeder flocks. Classical Massachusetts strains were found on 2 occasions (3 %) in broiler breeders. Finally the UKR/27/2011 type and the CK/CH/Guandong/Xindadi/0903 type were both found once (± 1.5 % each), in a flock of layers and in a flock of broiler breeders, respectively.

Comparing the composition of the S1 protein for the region examined between field strains and homologous vaccine strains (when available) an average of 3.2 amino acid substitutions was found, however ranging from zero to 11.

The results indicate that IBV infections are highly prevalent in Belgian commercial chicken flocks. IBV related problems occurred especially in poorly vaccinated flocks and/or flocks at a higher age. This underlines the necessity of not only providing chickens with a strong vaccinal immunity against IBV but also of maintaining it during the entire life of the birds.

At least 6 types of IBV field strains appeared to be circulating simultaneously. IBV vaccination schedules should therefore afford a broad protection against a wide range of IBV types. This can be achieved by applying vaccines according to the protectotype concept. Briefly, improved protection can be obtained against many IBV strains by using a combined vaccination programme incorporating 2 antigenically different IBV vaccines, as it has been amply elaborated for Massachusetts and 4/91 vaccine strains(1,2). This approach could also help to overcome an eventual reduced level of protection when field and homologous vaccine strains differ in multiple amino acids of the S1 protein.

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Association between microbiological and serological prevalence of human pathogenic Yersinia enterocolitica in pigs and pig batches

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Abstract

Pigs are the main reservoir of *Y. enterocolitica*, and the microbiological and serological prevalences of this pathogen differ between farms. The infection status of pig batches arriving at the slaughterhouse is largely unknown.

Moreover, information about a possible link between the presence of *Y. enterocolitica* bacteria and the presence of antibodies is missing. Understanding the association between the microbiological and serological prevalence could help to predict the contamination of the pigs prior to slaughter.

Pigs from 100 different farms were sampled at the abattoir. Tonsils and pieces of diaphragm were collected from 7047 pigs (on average 70 pigs per batch). The tonsils were analyzed using a direct plating method and confirmed with a multiplex Polymerase Chain Reaction (*ail, yst, virF*). When at least one colony was detected, the sample was indicated as positive. The meat juice of the pieces of diaphragm was analyzed by Enzyme Linked Immuno Sorbent Assay Pigtype Yopscreen (Labor Diagnostik Leipzig, Qiagen, Leipzig, Germany). A used cut-off of the activity value was 30 OD%. The results of these prevalences were compared using a mixed-effects logistic regression at pig and batch level.

Yersinia enterocolitica was found in 2009 pigs, of which 1872 also had antibodies against *Yersinia spp*. A total of 2820 pigs possessed antibodies without positive microbiology. According to the logistic regression, the microbiological contamination could not be predicted by the presence of antibodies at pig level.

At batch level, an association was observed (microbiological prevalence $=0.444/(1-e^{-0.063*(\text{activity value}-37.069)})$), which means that a batch is microbiological positive when its mean activity value is more than 37 OD%).

The given formula could predict whether a pig batch contained infected pigs before they arrived at the slaughterhouse. This way, infected batches could be slaughtered last so cross-contamination in the slaughterhouse could be avoided or diminished.

Autochthonous Tick-Borne Encephalitis Virus (TBEV)–seropositive cattle in Belgium: A risk-based targeted serological survey

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Abstract

Tick-borne encephalitis virus (TBEV) is the most important arthropod-borne virus in Europe. The Western subtype of this pathogenic neurotropic flavivirus is carried by *Ixodes ricinus*. Tick-borne encephalitis has become a considerable public health risk in several European countries, with currently 3000 hospitalized cases per year. The risk of TBEV-introduction into Belgium remains high and the presence of infected wildlife in Belgium is suspected. Domestic animals such as dogs or cattle can serve as excellent sentinels for TBEV-surveillance in order to install an early warning surveillance component for this emerging zoonotic disease of public health importance.

In a targeted, risk-based and cross-sectional sampling design, serological screening was performed on Belgian cattle (n=650), selected from the 2010 Belgian national cattle surveillance serum bank. Hereto, the three most Eastern located provinces of Belgium (Liège, Luxemburg, Limburg), which are geographically situated closest to known and/or recently emerging TBEV-endemic, were targeted. These areas are also currently known as endemic for Lyme disease (*Borrelia burgdorferi*), another disease transmitted by the same tick.

Bovine sera, obtained during the cross-sectional winterscreening campaign of 2010-11, were tested at the TBEV Belgian National Reference Centre at the WIV-ISP, by gold standard TBEV seroneutralisation test, based on the rapid fluorescent focus inhibition test (RFFIT) protocol. Using a conservative >1/15 cut-off titer for SN test, 17 bovines were seropositive and six had borderline results (1/10 < titer < 1/15). The accuracy of the RFFIT-SNT was confirmed in a mouse inoculation test and by West Nile virus and Rabies virus serology. There was a positive correlation between the neutralizing antibody titer, present in the serum samples and determined by SN, and the median survival time in mice during experimental challenge studies in mice inoculated intranasally with a mix of virus and serum. Lesions consistent of viral encephalitis were demonstrated in histopathology.

The overall bovine TBEV-seroprevalence in the targeted area was estimated between 2.6 and 4.3% and freedom could no longer be substantiated. Bovines with borderline results were often located close to confirmed seropositive animals. The geographical locations roughly coincided with the known Belgian hot spots for Lyme disease.

This risk-based cross-sectional serological survey in cattle, obtained through "one health" cooperation confirms the presence of infected foci in Belgium for the first time. Given the relevance of TBEV for the food chain through consumption of unpasteurized milk and cheese and through its considerable public health burden in other European countries, further surveillance in cattle, other sentinels, ticks and humans at risk is recommended to further determine the location and size of endemic foci and the risk for public health.

Effects of ZnO and colistine on animal health and production parameters in weaned piglets

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Introduction

The aim of this study was to identify if ZnO supplementation in weaned piglets has an effect on the piglet's health, production parameters and reduction in the use of antibiotics, in comparison with colistine supplemented piglets and a negative control group.

Animals, material and methods

Three commercial pig herds were selected. Within each herd, two successive weaning rounds were included. In each round, four treatment groups of each 60 weaners were investigated. During the first two weeks of the nursery period, one group received colistine (Promycine® 400 IU/mg, premix, VMD, Belgium) in the feed, another group colistine (ColistineEurovet® 400000 IE/ml, Eurovet, Belgium) in the drinking water, a third group ZnO (Gutal®, 2500 ppm, Huvepharma, Belgium) and a fourth group was a negative control group. The backhand of the piglet was scored per pen every week. During the entire nursery period, feed intake, antimicrobial usage and mortality were recorded. On day 7 and 14, feces samples were taken for enumeration of total and hemolytic *E. coli*. Pigs were weighted individually on the day of weaning, at day 14 and at the end of the nursery period to determine daily weight gain (DWG) and feed conversion ratio (FCR).

Results and conclusion

The results are shown in **table 1.** The piglets from the ZnO group showed significantly ($P<0.03$) better DWG during the first two weeks of the nursery period in comparison to the other groups. Total *E. coli* was significantly lower for the colistine in drinking water group compared to the ZnO and the control group ($P<0.05$). The negative control group had the highest percentage of positive samples for hemolytic *E. coli* (73.5%) ($P<0.05$). The ZnO group scored the lowest number of dirty backhands ($P=0.033$). The treatment incidence showed clear differences between the two colistine groups compared to the ZnO and the control group ($P<0.01$). In conclusion, this study documented that ZnO can be used as an alternative for colistine in weaners, as it showed equal or better production results combined with lesser dirty backhands and a reduced use of antibiotics.

Table 1: Results of the measured parameters in each treatment group.

Dependent variables*	Colistine in feed	Colistine in drinking water	ZnO in feed	Control group
DWG 1st period	108[a]	98[a]	144[b]	105[a]
DWG 2nd period	383[a]	396[a]	408[a]	400[a]
DWG total period	281[a]	285[a]	309[a]	290[a]
FCR	1.6[a]	1.53[a]	1.57[a]	1.6[a]
Total *E. coli*	6.7[ab]	6.2[a]	6.8[b]	7.1[b]
% hemolytic *E. coli*	45.8[a]	45.7[a]	34.3[a]	73.5[b]
% mortality/wasters	2.9[a]	3.4[a]	5.7[a]	5.3[a]
% backhand scoring	2.3[a]	2.6[a]	0.3[b]	2.7[a]
Antibiotic usage	0.4592[a]	0.4656[a]	0.0020[b]	0.0038[b]

* Results for DWG (growth/gram/day) in the first period (first two weeks of the nursery period), second period (from day 15 until the end of the nursery period), the total nursery period, FCR, total *E. coli* (log transformation of cfu/ml), hemolytic *E. coli* (% positive samples), mortality/wasters (% died piglets or wasters removed from the trial), backhand scoring (% dirty backhands) and antibiotic usage (treatment incidence).

Description of post-mortem lesions in slaughter pigs in Belgium between 2011 and 2013

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Introduction

Slaughterhouse results are a good indication of the general health status of pig herds. They can be used as an important tool to assess the importance of specific diseases (e.g. *Mycoplasma)* or they can also be used to monitor the level of infection for certain (respiratory) disorders.

The current study was conducted as a part of the ProHealth-project. This is a project which aims to better understand multifactorial diseases in the European pig and poultry production. The aims of the current study are to describe the prevalence of important lesions in slaughterpigs in Belgium.

Material and Methods

A set of existing data was gathered from two Belgian pig slaughterhouses from 2011 to 2013. Only fattening pigs originating from Belgian pig farms with quality label ("Certus") were included. Slaughterhouse A was located in the Province of Antwerp and slaughterhouse B in West Flanders. The data contained all Belgian slaughter pigs that were slaughtered in this period. Details about the herd of origin, the number of pigs delivered in each batch and information about the post-mortem findings were included. The post mortem findings contain information on prevalence of livers with white spots and prevalence of livers that were disapproved for white spots. Also information about lungs and the thoracal cavity were present: prevalences of pneumonia, fissures and pleuritis. Fissures are typical lesions and can be considered as "scar tissue", these lesions are an indication of a previous *Mycoplasma*-infection.

Results

In total 23 095 batches of Belgian fattening pigs were slaughtered in both slaughterhouses. Details about the post-mortem findings were present for 21 281 batches (92.1%). This made a total of 2.98 million fattening pigs originating from 628 different pig herds.

The average (min-max) number of fattening pigs per batch was 140 [25;720]. An overview of the results is shown in **table 1**.

Table 1: Post-mortem findings (as averages expressed in %) between 2011 and 2013.

	2011		2012		2013		Overall	
Slaughter- house[1]	A	B	A	B	A	B	A	B
Pneumonia	24.9	35.8	22.6	31.4	24.6	27.1	24.0	31.4
Fissures	4.6	1.9	4.4	1.1	5.4	0.8	4.8	1.3
Pleuritis	14.2	23.0	13.9	18.6	16.4	19.3	14.8	20.3
White spots	7.0	2.9	3.9	3.1	2.8	2.6	4.6	2.9
Disappr. livers[2]	1.9	2.0	1.4	2.4	1.4	1.8	1.6	2.1

[1] A and B represents resp. slaughterhouse A and slaughterhouse B.

[2] Disappr. livers: the percentage of livers not suitable for human consumption.

The yearly overall prevalence of pneumonia rangedfrom 35.8% (highest in 2011 in slaughterhouse B) towards 22.6% (lowest in 2012 in slaughterhouse A). The prevalence of fissures was constantly higher in slaughterhouse A. The prevalence of pleuritis on the other hand was highest in slaughter-house B. The prevalence of white spots and disapproved livers tended to decrease during the subsequent years.

Conclusion and Discussion

The current dataset contains > 10% of all Belgian pig herds and can therefore be considered to give a representative overview for the Belgian pig production. The prevalences of pneumonia and pleuritis are in accordance with other publications. Meyns et al. (2011) described a prevalence of 23.9% for pneumonia and 20.8% for pleuritis. Also Maes et al. (2001) described similar levels of pneumonia (24%) and pleuritis (16%). The average prevalences of white spots, caused by the migration of certain stages of the intestinal parasite, *Ascaris suum*, are similar with other publications (Ondrejkova et al., 2012). Further research will investigate temporal variations, correlations between different lesions and associations with management and herd factors.

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Prescription patterns of antimicrobials by small animal veterinarians in Belgium

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Abstract

The use of antimicrobials in animals contributes to the development and maintenance of antimicrobial resistance. The aim of this study was to describe the prescription pattern of antimicrobials by small animal veterinarians in Belgium.

A questionnaire was send to veterinarians and could be filled in online from 20 December 2013 till 31 January 2014. Data were collected on the veterinarians, their practices, the sources of information they use regarding antimicrobials and their prescription habits. The veterinarians were asked which diagnostic tests they would use and which general therapy and medicines they would prescribe in six commonly occurring clinical cases related to dogs (acute diarrhoea (1), pyodermia (2), tracheobronchitis (3)) and cats (upper respiratory tract infection (4), lower urinary tract infection (5), subcutaneous abscess (6)). Eighty-six veterinarians started filling in the questionnaire, but the number of answers varied between the clinical cases due to discontinuation of the questionnaire. The main sources of information concerning antimicrobials and responsible use were said to be scientific literature, graduate and post-graduate courses and representatives of pharmaceutical companies.

In the dog clinical cases regarding acute diarrhoea (1), pyodermia (2) and tracheobronchitis (3) respectively 16%, 88% and 95% of the veterinarians prescribed an antimicrobial therapy whereas this is assumed to be unnecessary in a first consultation for the described cases . The most frequent prescribed antimicrobials were metronidazole (7%), cephalexin (43%) and amoxicillin/clavulanic acid (57%) in clinical case 1, 2 and 3 respectively. In clinical case 1, 2 and 3 respectively 35%, 98% and 54% of the veterinarians performed a correct diagnostic approach. In the cat clinical cases concerning an upper respiratory tract infection (4), lower urinary tract infection (5) and subcutaneous abscess (6), respectively 87%, 60% and 98% of the veterinarians prescribed an antimicrobial therapy, which was assumed to be unnecessary in a first consultation for the clinical case 4 and 6. In all cat clinical cases, amoxicillin/clavulanic acid was most often prescribed (clinical case 4, 5, 6 respectively 40%, 21%, 38%). In clinical case 4, 5 and 6 respectively 90%, 17% and 94% of the veterinarians performed a correct diagnostic approach. In dogs, 31% of the total number of canine prescriptions concerned amoxicillin/clavulanic acid, 19% cephalexin and 13% doxycycline. In cats, 31% of the total number of feline prescriptions concerned amoxicillin/clavulanic acid, 17% cefovecin and 9% amoxicillin.

In conclusion, there is room for improvement regarding prescription patterns of Belgian small animal veterinarians: 1. The diagnostic approach can be optimized. 2. The prescription of antimicrobials in cases where an antimicrobial therapy is not indicated should to be decreased. 3. Since cefovecin is classified as a critically important antibiotic by the WHO and OIE there is a need to decrease the prescription of cefovecin and 4. When treating a patient, in all cases more emphasis should be put on the general- and non-antimicrobial therapy to support the animal, certainly when an antimicrobial therapy is not indicated.

Antimicrobial use in pigs, broilers and veal calves in Belgium

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Abstract

Given the risks associated with antimicrobial resistance and its link with antimicrobial use, it was aimed to compare available data on antimicrobial use in the Belgian pig, broiler and veal calf production. Allowing for comparisons of the data available from three peer-reviewed scientific articles, the unit of measurement for antimicrobial use was the Treatment Incidence (TI), defined as the number of animals per 1000 treated daily with one 'defined' (DDDA) or 'used daily dose animal' (UDDA). Moreover, an extrapolation of farm-level data to national-level data was attempted according to the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) methodology, to estimate the amount of antimicrobials used in Belgium per species. Although, among the three species, the highest TI was observed in veal calves (TIDDDA=414, TIUDDA=379), it was estimated based on the extrapolation, that most antimicrobials were administered to pigs (159.4 tons). Thus, the most rapid decline in the total use could potentially be achieved by targeting the pig sector. Methodologically, a need for harmonized monitoring programs was also revealed during the process of data collection for the comparisons and calculations performed for the purposes of this article.

Bacterial insertion sequence IS256 among Staphylococcus epidermidis from animals

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Abstract

Staphylococcus epidermidis is one of the most prevalent causes for implant-associated and nosocomial infections. In veterinary medicine, *S. epidermidis* is one of the main etiological agents of ruminant intramammary infections and it can be involved in diverse infections in companion animals. The main defined virulence factor associated with *S. epidermidis* is its ability to form biofilm and colonize biomaterials. In the biofilm formation, the polysaccharide intercellular adhesion (PIA) encoded by the *icaABCD* operon is the major component mediating intercellular adhesion. The *ica* operon was shown to be more prevalent in clinical *S. epidermidis* isolates than in community isolates. Similarly, the IS*256* involved in the phase variation of biofilm formation is a marker gene to differentiate between invasive and non-invasive isolates. In Belgium, methicillin-resistant *Staphylococcus epidermidis* (MRSE) was found to be a major constituent of the methicillin-resistant non-*Staphylococcus aureus* staphylococci flora in pig nostrils (Vanderhaeghen et al., 2012). MRSE has been also found in lower rates in bovines in Belgium (Vanderhaeghen et al., 2013). Research on the biofilm properties of *S. epidermidis* isolates from animals is scarce. The aim of this study was to determine the prevalence of the *ica* operon among animal *S. epidermidis* isolates, as well as their possibilities to form biofilm and their population structure.

A collection of 103 *S. epidermidis* from nasal samples of livestock [including isolates from pigs (n=70), dairy cows (n=7), beef cows (n=1), veal calves (n=14), and broiler chickens (n=11)], was investigated for the presence of the gene *mecA* and the *icaABCDR* operon by PCR. The *ica*-positive isolates were additionally tested for the presence of the IS*256* and other genes involved in biofilm formation (*bhp*, *aap*, *embp*) by PCR, as well as for *in vitro* biofilm formation on Congo Red Agar (CRA) and their adherence to plastic tissue culture plates (Christensen et al., 1985). Randomly selected isolates of the collection were subjected to multilocus-sequence typing (MLST) in order to assign sequence types (STs) and clonal complexes (CCs).

Seventeen out of 103 (16.5%) isolates [from pigs (n=6), dairy cows (n=2), veal calves (n=5), and broiler chickens (n=4)] were *icaABCDR* operon positive. 64.7% of these *ica*-positive isolates was *mecA* positive, and thus considered MRSE. All *ica*-positive isolates carried the gene *embp*, nine carried *aap*, nine carried *bhp* and seven the IS*256*. The IS256, the *aap* and *bhp* genes appeared in different combinations among the *icaABCDR* and *embp*-positive isolates [*bhp*-*aap* (n=3), *bhp*-IS*256* (n=2), *aap*-IS*256* (n=3), only *bhp* (n=4), only *aap* (n=3), or only IS*256* (n=2)]. IS*256* was only detected in pig and bovine isolates, while the other genes were detected in isolates from all three animal species. Only four of the *ica*-positive isolates formed biofilm on CRA plates. These four isolates were also strongly adherent to plastic tissue culture plates. The remaining 13 *ica*-positive isolates were strongly adherent (n=9), weakly adherent (n=2), or non-adherent (n=2) to plastic tissue culture plates. Only one of the non-adherent isolates was IS*256* positive, while the remaining IS*256* positive isolates strongly or weakly adhered to plastic tissue culture plates. The isolates belonged to the CC2. The pig isolates were ST100, the bovine isolates analysed were ST2, ST7, ST16 or ST22, while the broiler isolates were ST7, ST357 or ST487.

Few isolates were *ica*-positive, but they carried the genes *embp, aap* and *bhp* at similar rates to those reported for human community and nosocomial isolates. Few isolates were IS*256* positive, but as far as we know this is the first description of IS*256* among pig and bovine isolates. Previously, the IS*256* has been rarely detected in poultry staphylococcal isolates, and has been associated to the presence of transposons and genes mediating antimicrobial resistance. The isolates belonged to CC2, which has been described as the most prevalent CC among nosocomial and community *S. epidermidis*. Our findings suggest that at least part of the *S. epidermidis* population among healthy animals may have a human origin. Further studies should be performed in other to assess the zoonotic potential of these isolates.

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Antimicrobial resistance and population structure of Staphylococcus aureus recovered from pigs in Belgium

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Abstract

Staphylococcus aureus is a common facultative pathogen that has since long been recognized as a burden in both human and veterinary medicine. *S. aureus* is well known to frequently resistant to antimicrobial agents which may lead to complications in the treatment of its infections and increase the cost of treatments. During the last decade, an increasing number of studies reported the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals. Most studies have focused on the asymptomatic carriage of MRSA among pigs, in which clonal complex (CC) 398 is the dominant lineage. During 2013, a survey was performed in different pig farms randomly selected over Belgium, with the aim of monitoring the current epidemiology and antimicrobial susceptibility of livestock-associated (LA-) MRSA among asymptomatic pigs.

From 328 farms nose swabs were taken from 20 animals and pooled. MRSA was isolated using the standard method proposed by the European Food Safety Authority (EFSA). MRSA identification was performed using the triplex 16S rRNA-*mecA*-*nuc* PCR. All isolates were characterised by means of susceptibility testing by a microbroth-dilution method using epidemiological cut-off values (Eucast), SCC*mec* typing, *spa*-typing and by the *sau1-hsdS1* clonal complex (CC) 398 PCR. CC398 PCR negative isolates were subjected to multi-locus sequence typing (MLST) in order to assign sequence types (STs) and CCs. Selected isolates were subjected to DNA microarray-based typing for detection of resistance and virulence genes.

MRSA was detected in 215 farms [65.6% (95% CI: 60.1%-71%)] out of 328 farms sampled. Most isolates (n=207) were positive for the *sau1-hsdS1* CC398 PCR. The remaining eight isolates were ST9 (one isolate), ST80 (two isolates), ST239 (one isolate) or ST398 (four isolates) as demonstrated by MLST. A total of 22 different *spa* types were identified. The *spa* types t044, t337 and t4150 were found in the ST80, ST9 and ST239 isolates, respectively. Nineteen *spa* types were found among the CC398 isolates, but most were t011 (n=180, 85%). Regarding to the SCC*mec* typing, most isolates carried SCC*mec* V, and less carried SCC*mec* IV or III. More than 90% of the isolates were epidemiological resistant to tetracycline and trimethoprim and high resistance rates (between 66% and 45%) were also found for ciprofloxacin, clindamycin, erythromycin, kanamycin and gentamicin. Lower epidemiological resistance levels (between 30% and 10%) were detected for streptomycin, fusidic acid, sulfamethoxazole, quinupristin/dalfopristin, tiamulin, rifampicin, chloramphenicol and mupirocin. All isolates were susceptible for vancomycin. More than 90% of the isolates were multi-resistant, and half of them were resistant to at least seven different antibiotics.

Microarray analysis showed that most genes were homogeneously distributed among the CC398 isolates. The non-CC398 isolates carried additional virulence genes, as the *egc*-like cluster with enterotoxins genes (*seg, seh, sei, selm, seln, selo, selu*). Interestingly, the ST80 strain analysed by microarray carried the leukocidin Panton-Valentine (*lukPV*) and *lukED* genes. Regarding to antimicrobial resistance genes, all CC398 isolates investigated carried the tetracycline resistance gene *tet*(M). Most CC398 isolates carried the *bla* operon (*blaZ, blaI,* and *blaR*) encoding for penicillin-ampicillin resistance and the tetracycline resistance gene *tet*(K). Some CC398 isolates carried genes encoding resistance to the macrolide-lincosamide-streptogramin group [*erm*(B), *erm*(C), *lnu*(A), *vga*(A)], aminoglycosides (*aacA-aphD, aadD, aphA3, sat*) and/or chloramphenicol (*fexA*). One *fexA* positive isolate was additionally positive for the multi-resistance gene *cfr*.

The MRSA prevalence among pigs in Belgium remains similar to previous studies performed on 2007 and 2009. As has been demonstrated before, the CC398 isolates were highly multi-resistant. However, in this survey there is a larger diversity in *spa*-types than ever detected before. Moreover, in this survey we have detected the European clone ST80-IV, which corresponded to the main community-acquired (CA-) MRSA clone in Europe. The ST80-IV had the Panton-Valentine leucocidin and had emerged recently as a cause of healthcare-associated infections. The recovery of this CA-MRSA from livestock indicates that one should remain vigilant to the evolution of LA-MRSA CC398.

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Sow and litter factors determining colostrum yield

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Abstract

Colostrum intake by piglets is crucial as well neonatal, as well later in life. Colostrum supplies energy, bio-active compounds (e.g. growth factors) and maternal immunity. In the current context of reducing antibiotics, achieving a good maternal immunity through a sufficient colostrum intake is primordial. As colostrum yield by sows is independent of the litter size and litter size is increasing, insufficient colostrum yield is a well-recognized problem in nowadays high productive pig industry.In addition to insufficient colostrum yield, there is also a huge variation in colostrum yield. Till now, factors determining the high variability of colostrum yield are poorly understood.

Therefore, the present study investigated whether and to what extent sow (breed, parity, gestation length), litter (size, average birth weight, heterogeneity, interval between birth and first suckling) and parturition factors (duration, intervention, stillbirth) influence colostrum yield across 10 different commercial herds despite their herd-specific policy.

In total 100 sows of 5 different breeds and their 1 455 live born piglets were included. Sows' colostrum yield was calculated as the sum of colostrum intake by their piglets. Colostrum intake by the piglets was estimated by the regression equation by Devillers et al. (2004). The associations between sow, litter and parturition factors on the one hand and colostrum yield on the other hand were investigated using linear mixed regression models with herd included as random factor.

Colostrum yield averaged 3 500 ± 110 g. Sows with a gestation length of 113 days had a higher colostrum yield (4,178 ± 506 g) than sows with a gestation length of 114-115 days (3,342 ± 107 g) (P = 0.04). An interaction between the litter birth weight of suckling piglets **(LW_{SP})** and gestation length **(GL)** was observed (P = 0.03). In sows with a GL of 114-115 days colostrum yield increased with higher LW_{SP} (P = 0.009). Compared to sows with GL of 116 days, colostrum yield of sows with a GL of 114-115 days increased with 148 gram for each unit increase of LW_{SP}. A shorter interval between birth and first suckling of the litter **(t_{FS})** was related with a higher colostrum yield (P < 0.01). When t_{FS} lasted 1 min longer, colostrum yield decreased with 11 g. This study did not observe any influence of parturition management on colostrum yield. Eighteen percent of the variation in colostrum yield between sows in the present study was explained by the final model. The relative contribution of GL, the interaction between LW_{SP} and GL and t_{FS} to the total explained variation was 26; 28 and 46% respectively.

This study clearly demonstrated that t_{FS} is a major factor involved in colostrum yield. Further research is needed to elucidate the observed significant influence of GL (per se) and (combined with the effect of the) litter birth weight of the suckling piglets on colostrum yield by the sow.

A cross-sectional and longitudinal study on the risk of BVDV re-infection in BVDV-free cattle herds in Belgium

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Abstract

Bovine viral diarrhoea virus (BVDV) is worldwide spread and can cause considerable economic losses. In Belgium BVDV prevalence is high and up to date control is on a voluntary basis at the herd level, consisting of three essential measures: removal of persistently infected (PI) animals, biosecurity and monitoring. Vaccination is proposed as an additional optional measure. Although BVDV control strategies are well understood, studies demonstrate a considerable risk of BVDV re-infection. Therefore the aim of study was to evaluate the risk of BVDV re-infection and identify risk factors for re-infection in Belgian cattle herds.

The target population for this study comprised all cattle farms in Belgium assumed free from BVDV, but with a history of BVDV circulation in the herd. A total of 61 herds were selected and visited during which young stock was sampled for serologic examination and a face-to-face interview with the farmer was conducted. The questionnaire was designed to collect data regarding BVDV management and control at the herd level. The sampling of young stock served as an indirect screening for BVDV infection in the herd and a farm was classified as infected when more than 20% of the animals sampled tested positive for BVDV antibodies. Potential risk factors for a herd being re-infected were identified by means of a multivariable logistic regression model.

Of the 61 farms considered for the cross-sectional study, 26 farms with a negative serologic examination during the farm visit and no application of BVDV vaccination were selected for monitoring for BVDV re-infection for 18 months. For this, three additional serologic examinations were performed every 6 months. When re-circulation of BVDV was suspected, possible causes were explored using a farm-specific questionnaire investigation and additional sample collection if necessary.

In 11 of the 61 herds (18%) at least 20% of the young stock tested BVDV-seropositive. Farms monitoring the BVDV-status had a significant lower probability of being BVDV re-infected (OR 0.09; 95% CI 0.02; 0.55). There was also a decrease in risk when the neighbouring cattle farm was located further away (OR per km 0.06; 95% CI 0.00; 0.96). An increase in the probability of infection was detected when farmers participated to auctions and/or competitions (OR 140.27; 95% CI 3.00; 6559.65).

In the longitudinal study BVDV-seropositive animals were detected in 6 of the 26 farms (23%). On half of these farms the re-infection resulted in the birth of at least one PI animal. In two herds self-clearance was noticed: no virus-positive animals were detected following screening of all animals and the following serologic examinations were negative. The cause of re-infection was confirmed in one herd: purchase of a PI animal in gestation. Fence-line contact with cattle from other herds on pasture was suspected as a cause of re-infection but could not be confirmed.

The results of the cross-sectional and the longitudinal study demonstrate a high risk of BVDV re-infection in regions where the virus is endemic. This study illustrates the importance of monitoring the BVDV-status. Six herds having a negative serologic examination during the first visit eventually became BVDV-infected, indicating the BVDV-free status should be confirmed on a regular basis. In a very densely populated livestock area such as Belgium neighbouring herds can be as risk factor for BVDV infection. Contact with other cattle at auctions, competitions or purchase should be avoided.

Description of BVDV-antigen results during a 5-year period of voluntary BVDV control in Northern-Belgium

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Abstract

In January 2015, the Belgian cattle industry will start with an obligatory BVDV program. Bovine Viral Diarrhoea Virus (BVDV) is widespread in Belgium and responsible for major economic loss in the cattle industry. For more than a decade, Animal Health Care Flanders (DGZ Vlaanderen) propagates BVD control mostly through systematic detection of persistently infected (PI) cattle and their consequent removal, apart from other measures. Nevertheless BVD-infection remains widely spread, illustrated by recent studies (Sarrazin et al., 2013) and demonstrates non-systemic efforts of control do not succeed at a national level.

This abstract describes BVD-antigen analyses performed in Northern-Belgian cattle herds during a five-year period (August 2009 – August 2014). Information was collected combining laboratory data and I&R data of sampled animals, on the condition of a correct and linkable animal identification. Lab data consisted of all BVDV-antigen analyses during this time-period performed by Erns antigen-capture ELISA (Idexx) and BVDV-RT-PCR (both individual as pooled) (Adiagene& LSI). Descriptive results were generated at the sample and animal level. A subset of animals that 'tested more than once' was created and used to classify animals in: naive (negative), transiently infected (TI) and persistently infected (PI). Of the latter, age of first detection was calculated.

Preliminary results show a total of 392.447 samples were analysed during this time-period on 11.208 cattle herds. A total of 373.624 number of cattle were tested for BVD antigen. Positive antigen test results were detected in 1.926 cattle herds. Ignoring the number of animals tested in the herd, this means BVDV infection was demonstrated in 17,1 % of herds with BVD-antigen investigations.

Ninety-five % of cattle (356.512) were tested once in their life-time of which 0,87% of animals (3.091) tested positive. 17.112 animals were tested 'more than once' (irrespective of the animal's location) of which 7,97% at least once antigen positive (17.112). 888 animals were classified PI and 476 were classified TI. TI-animals were detected mostly during herd screenings. The average age of first detection of classified PI animals (398,1 days) and was considerably lower than in TI-animals (611,7 days). 25% of detected PI animals were older than 545 days. The oldest detected PI animal was 7,2 years old. In total, the 880 classified PI-animals together lived a 'collective 969,1 years' before initial detection.

In interpreting these results, it should be stressed, the generated numbers cannot be seen as prevalence data as only a minority of the annual cattle population was tested (on average 1.3 million number of cattle in Northern-Belgium). Additionally, testing is non-systemic and mostly performed in cattle herds experiencing BVD problems; data therefore reflects the voluntary BVD-control approach in Northern-Belgium.

Nevertheless, the detected number of antigen positive animals and affected cattle herds in this abstract illustrate this voluntary BVD-approach is highly cost-inefficient! When accounting for the nature of the BVD virus and it's high infectious nature of PI animals creating new PI animals in naïve pregnant animals, it is clear, the sector is in need for a more structural approach. Census-testing of all new-born animals is a crucial part of this, since nowadays PI-detection occurs only in a very late stage (over a year). An 'underestimation' of 880 classified PI animals were responsible for a 'minimum collective risk-period of 969,1 years'. One PI-animal in herd will mostly infect all in-contact animals just in a 6-week period!

This abstract aids the well-known fact Belgium is in need of a systemic BVDV-control program, as the voluntary approach simply does not work.

Modeling the spatial distribution of Fasciola hepatica in dairy cattle in Europe

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Abstract

Liver fluke in dairy cattle caused by Fasciola hepatica, a trematode parasite, which has Galba truncatula as an intermediary host, has a high economic impact on dairy cattle production worldwide in general, and in Europe in particular. Identifying areas at risk may contribute to improve the management of this disease and reduce its cost. To date most attempts to model the spatial distribution of liver fluke were limited to countries or regions within countries and no pan-European models have been published at a sufficiently detailed spatial resolution.

As part of the GLOWORM project, funded through the Seventh European Framework Program (FP7), a systematic sampling strategy was developed and bulk milk samples were collected from 3,359 dairy cattle farms in selected regions from Belgium, Germany, Ireland, Poland and Sweden. All samples were analyzed using a standardized bulk milk ELISA test to measure the antibodies against the excretory-secretory products of F. hepatica and the results were expressed as an Optical Density Ratio (ODR). Based on this database, representative for a large part of Europe, a pan-European distribution model at a 5X5 km pixel resolution was computed using the VECMAP™ software package.

In this paper we first discuss the development of the spatial sampling strategy and how the area in Europe representative for the sampled area was defined. We then compare spatial model outputs generated using the VECMAP™ software package. Finally particular attention is given to how the choice of ODR thresholds may impact on results and recommendations are made for future improvements.

Prevalence of Mycoplasma gallisepticum in commercial poultry, hobby poultry and wild birds in Belgium

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Abstract

Mycoplasma gallisepticum is a pathogenic species for poultry and responsible for major economic loss. It is a common cause of chronic respiratory disease and can result in a variety of different symptoms such as coughing, nasal discharge, sinusitis, airsacculitis, decrease in egg production, and increase in embryo mortality in layers, reduction of weight gain and downgrading of carcasses in broilers. Because of the possible vertical transmission, infected breeder flocks should be depopulated in order to prevent further spread. Despite high hygienic measures, outbreaks in breeder flocks still occur and to date the exact route of spread into the breeder farms is not known. Because knowledge about the prevalence of *M. gallisepticum* in poultry holdings (other than breeders) is currently lacking in Belgium, the aim of this study was to determine the prevalence of *M. gallisepticum* among layer, broiler and turkey holdings, as well as in other bird species (backyard poultry, racing pigeons and wild birds) that are commonly present in Belgium and could possibly act as reservoirs for this bacterium.

A random selection of poultry herds, proportional to herd density per province, was made based on the active holdings present in the Belgian National Animal Identification and Registration System (Sanitel) data base owned by the Federal Agency for the Safety of the Food Chain (FASFC). At each layer holding (n=87) and turkey holding (n=17) 60 blood samples were collected and serum was analyzed using a rapid plate agglutination test (MG-RPA test, Soleil diagnostics). Positive sera were confirmed with a blocking ELISA test (Svanovir® Mg-Ab kit, Boehringer Ingelheim Svanova). Due to the slow immune response that occurs during mycoplasma infection and the shorter life span of broilers, real-time PCR was performed to test the presence of *M. gallisepticum* DNA (ADIAVET™ Myco AV, Biomérieux) on 12 tracheal swabs per broiler holding (n=102). Samples of other origins include: blood samples of racing pigeons (n=56) taken during consultation at the Faculty of Veterinary Medicine (University of Ghent); fifty-six owners of backyard chickens (n=460); sera from 2012, taken from wild crows and geese (n=192); tracheal swabs from wild birds (n=100) collected by hunters during hunting season of September - January 2013; and finally,seven bird rescue centers were visited and blood samples and tracheal swabs (n=197) were taken from different species of wild birds. All serum samples were analyzed using the Svanovir® Mg-Ab kit and all tracheal swabs were analyzed with RT-PCR (ADIAVET™ Myco AV, Biomérieux).

Two layer holdings (2.3%; 95% CI: 0.6-8%) were positive after confirmation with ELISA with a within herd prevalence of 39.2% (95% CI: 30.9-48.1%) and 47 out of 5220 serum samples (0.9%; 95% CI: 0.7-1.2%) tested positive. Eight broiler holdings (7.8%; 95% CI: 4-14.7%) were positive with a within herd prevalence of 34.4% (95% CI: 25.6-44.3%) and 33 out of 1224 tracheal swabs (2.7%; 95% CI: 1.9-3.8%) tested positive. Seventeen turkey holdings were sampled and all serum samples were negative for *M. gallisepticum*. Fifty-six serum samples from racing pigeons tested negative for *M. gallisepticum*. 73.2% (95% CI: 60.4-83%) of backyard flocks were *M. gallisepticum* positive with a within flock prevalence of 48.8% (95% CI: 43.6-54.1%) and 169 out of 460 serum samples (36.7%; 95% CI: 32.5-41.2%) were positive. One sample (wood pigeon) obtained during hunting season (1%; 95% CI: 0.2-5.4%) and 4 samples (two herons, one duck, and one magpie) obtained from bird rescue centers (2%; 95% CI: 0.8-5.1%) tested positive for *M. gallisepticum*. Ninety-six samples from crows and 96 samples from geese, collected in 2012, were negative for *M. gallisepticum* antibodies with ELISA.

The prevalence of *M. gallisepticum* in commercial poultry is rather low, although in broilers a higher prevalence is found than in layers. This could be due to analysis method (RT-PCR vs serology). In backyard chickens there is a very high seroprevalence of *M. gallisepticum* which indicate that they may act as a reservoir for this bacterium which may disseminate into commercial poultry holdings. Further isolation and typing of strains will be used to determine the possible routes of transmission.

Prevalence of Mycoplasma synoviae in broilers, hobby poultry and wild birds in Belgium

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Abstract

Mycoplasma synoviae has been considered a less important pathogenic mycoplasma species for poultry than *Mycoplasma gallisepticum*. However, the importance of this bacterium and its relevance from a clinical and economic viewpoint has increased since *M. synoviae* arthritis, amyloid arthropathy, and eggshell apex abnormalities have been reported. Because of vertical transmission of mycoplasma species, there is an official eradication program for *M. gallisepticum* in breeder stock. Since the implementation of such a program, a decreasing incidence of *M. gallisepticum* has been observed. However, for *M. synoviae*, there is currently no such eradication program which may be a continuous cause of dissemination from breeder stock into layer and broiler holdings.

During a prevalence study, data on presence of *M. synoviae* in broiler holdings, backyard flocks and wild birds was gathered.

A random selection of broiler herds, proportional to herd density per province, was made based on the active holdings present in the Belgian National Animal Identification and Registration System (Sanitel) data base owned by the Federal Agency for the Safety of the Food Chain (FASFC). At each broiler holding (n=102) 12 tracheal swabs were collected and analysed using a commercial real-time PCR kit (ADIAVET™ Myco AV, Biomérieux). Fifty-six owners of backyard chickens (n=460) were visited and of each owner maximum 10 chickens were sampled. Tracheal swabs from wild birds (n=100) were collected by hunters during hunting season of September – January 2013; and three bird rescue centers were visited and tracheal swabs (n=90) were taken from different species of wild birds common in Belgium. All tracheal swabs were analyzed with RT-PCR (ADIAVET™ Myco AV, Biomérieux).

Twenty-seven broiler holdings (26.5%; 95% CI: 18.9-35.8%) tested positive for *M. synoviae* with a within herd prevalence of 48.8% (95% CI: 43.4-54.2%) and 158 out of 1224 tracheal swabs (12.9%; 95% CI: 11.1-14.9%) were *M. synoviae* positive. Fifty-four out of 56 owners of backyard chickens had positive flocks resulting in a seroprevalence of 96.4% (95% CI: 87.9-99%) of backyard flocks with a within flock prevalence of 78% (73.9-81.6%). Three hundred and fifty-one out of 460 tracheal swabs (76.3%; 95% CI: 72.2-80%) were positive for *M. synoviae.*

Four samples (one crow and three wood pigeons) obtained during hunting season (4%; 95% CI: 1.6-9.8%) tested positive for *M. synoviae.* Three samples (one peacock pigeon and two wood pigeons) out of 90 tracheal swabs collected in bird rescue centers (3.3%; 95% CI: 1.1-9.3%) were *M. synoviae* positive.

The prevalence of *M. synoviae* in broilers is high which could be related to the lack of eradication programs and the possibility of vertical transmission from breeder stock into commercial poultry holdings. In backyard chickens the seroprevalence of *M. synoviae* is extremely high which indicates that they may act as reservoir for this bacterium from which dissemination to poultry holdings is possible. The birds, other than chickens, positive for *M. synoviae* were mainly pigeons. The significance of this remains unclear. Further analysis on samples from wild birds in bird rescue centers is ongoing and isolation and typing of strains will be used to determine the possible routes of transmission.

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Salmonella on Belgian breeding and rearing farms

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Introduction

The aim of the study was to gain insight in the prevalence of Salmonella on breeding and rearing farms in Belgium and to define a practical sampling method for assigning qualifications to herds by bacteriological sampling. In addition, the effect of pooling as well as the usefulness of the biocheck in relation to Salmonella in pigs was evaluated.

Materials and methods

Between April and December 2013, 40 breeding and 9 rearing farms were sampled monthly and followed up during 6 months. On each sampling round 2 pair of environmental swabs and 2 pair of overshoes were taken in the farrowing-, insemination- and gestation units of the breeding farms. In the fattening unit 2 pairs of hand swabs were taken. In the rearing farms, farms that house only gilts, the same number of samples was taken spread over the different compartments. Overshoes and swabs were pooled per 2 pairs. On 46 herds, in the last sampling round, only overshoes were taken. Per unit or compartment 2 pairs of overshoes were pooled and 2 pairs were examined separately. Bacteriological analysis for Salmonella was performed by a standard enrichment method according to ISO-6579 Annex-D (MRSV). Salmonella strains were serotyped at CODA-CERVA. In the fattening unit the association between serology and bacteriology was studied. Therefore the serological results of blood samples taken during the project for the Belgian Salmonella Action Plan were taken into consideration. On each herd the biocheck questionnaire (www.biocheck.ugent.be) was completed and the standard use of antibiotics and acids was registered.

Results

Among the 2177 analyses which were carried out during the project, Salmonella spp. were isolated 334 times in 36 different herds. This corresponds to an overall proportion of positive samples of 15.34% (IC95%: 11.53-20.13) and a herd prevalence of 73.47% (IC95%: 59.33-84). The medium within herd prevalence was 29%. The 3 most frequently isolated serotypes were Salmonella Derby, Salmonella Typhimurium including monophasic variants of Salmonella Typhimurium strains and Salmonella Livingstone (respectively 47%, 21% and 13% of the analyses and 61%, 47% and 25% of the positive herds).
A farm was considered as positive when at least one sample was positive for Salmonella spp. Most of the herds (62%) had a changing status over the different consecutive sampling rounds. The probability for a herd to have 2 or 3 consecutive positive statuses was 0.31 (min:0.18-max:0.51) and 0.24 (min:0.18-max:0.51).
Statistically more positive samples were found using overshoes compared to hand swabs (Odds Ratio: 2.45 [IC95%:1.77-3.38]). No statistically significant difference was found between pooled and non-pooled overshoes. The correlations between the bacteriological results of the 4 sampling units are small to moderate. The highest correlation was observed between the insemination and gestation units (rho=0.54).
Standard administration of acid and antibiotics was applied on 15 and 26 herds respectively. There was no significant relationship between the use of them and the Salmonella status of the herds. No significant correlation between the Salmonella status and the results of the Biocheck could be found.

Discussion

In conclusion, these results indicate a high prevalence of Salmonella on the Belgian breeding and rearing farms. The most optimal way to determine the Salmonella status considering a minimum within herd prevalence of 10% is by taking 12 pools of 2 pairs of overshoes spread over all the different units. At least 3 consecutive sampling results are needed to get a clear view of the Salmonella status.