EARLY DETECTION AND OUTBREAK

Cattle Health Surveillance in the Netherlands
GD - Veekijker - the re-active surveillance component

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Abstract
Since 2003, the Animal Health Service Ltd (AHS) has implemented a national cattle health surveillance system. Objectives of the surveillance system – as defined by the stakeholders are:

- to monitor well known but for The Netherlands exotic OIE list diseases
- to detect new or emerging diseases
- to analyze trends and development of cattle health aspects over time.

To reach these objectives a number of complementary surveillance components have been developed to collect information either passively or actively. Great emphasis is put on the "GD-Veekijker", a free telephone helpdesk that farmers and veterinarians can use as a source of information to solve herd health problems. The principal objective for surveillance is early detection of diseases. The re-active component "GD-Veekijker" is introduced and illustrated with some results.

Keywords: re-active surveillance, detection emerging diseases, detection new/exotic diseases.

Introduction
The AHS is a private organisation supporting animal health and food safety by supplying knowledge, diagnostics, voluntary disease-free programs and national surveillance. Since the 1960's the different county-locations of AHS had consultative helpdesks for private veterinarians and farmers. The information gathered by the specialists was used for private monitoring purposes. In 2002, AHS organised its activities on 1 location and was asked by private and public stakeholders to set up a national cattle surveillance system. This was implemented in 2003. Objectives of the surveillance system – as defined by the stakeholders - are:

- to monitor well known but for The Netherlands exotic OIE list diseases
- to detect new or emerging diseases
- to analyze trends and developments of cattle health aspects over time.

To reach the objectives three complementary surveillance components have been developed to collect information:

1. Re-active collection of information on symptoms and signs in cattle
2. pro-active prevalence studies on endemic cattle diseases
3. pro-active combination of data from six nationally operating organizations (I&R, herd improvement, milk quality, compulsory herd-health checks, disease-free certification and incinerator)

Materials and methods
"GD-Veekijker = Cattle-watch": For this re-active component private practitioners are invited to report information on (un) known or changing symptoms and signs in cattle to a nationally operating group of 5 ruminant health specialists: "GD-Veekijker". When deemed necessary, farms are visited by these specialists in order to pursue more detailed information. To stimulate the telephone calls, feedback of information for practioners is communicated by means of a monthly AHS-magazine and a restricted website. Each case handled is entered into a computer database (MORP). Results of all samples send to the AHS laboratories are entered into a laboratory database (LIMS).

Each week the reported information and results on AHS laboratory submissions (clinical chemistry, bacteriology, parasitology, immunology, virology, toxicology and post-mortem) are discussed by specialists in various fields of expertise (veterinary medicine, pathology, laboratory, epidemiology and statistics) for their relevance as indicators for an emerging disorder or early detection of a highly contagious non-endemic disease.

Unusual incidents are investigated by AHS specialist in a pilot study. If considered sufficiently important a team of specialists from AHS, with links to other institutes, can be formed to investigate the problem and provide expert advice to the stakeholders.

AHS reports results and findings quarterly to the stakeholders with an advice on possible actions. Thereby stakeholders can adapt policies over time or instantly in case of an emergency.

The performance of this surveillance system will be illustrated with results on emerging OIE-list diseases (bluetongue), new disorders (pithomycotoxicosis), zoönosis (salmonella) and intoxications (Pb).

Result
The examples of surveillance results are selected from a list of events that were recorded in the Netherlands between 2003 and 2010.

Early detection of a notifiable disease (Bluetongue)

In the first week of August 2006, practitioners in a small area of the Netherlands started reporting to "GD-Veekijker" the following symptoms in sheep flocks: fever, reluctance to stand, anorexia and "bottle-jaw". Normal differential diagnosis like haemonchose, photosensitization or ecthyma could be excluded by laboratory tests or did not respond to treatment.

Although the nearest confirmed bluetongue cases were more than 1500 km's away, when more cases were notified and sheep owners indicated that colleagues just

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across the border in Belgium experienced the same symptoms the suspicion of Bluetongue arose and was notified on August 14th 2006 to the governmental veterinary service. The diagnosis was confirmed by CVI-Lelystad (the Netherlands) and the European reference laboratory Pirbright, England on August 17th 2006 [2].

Detection of a new disorder (pithomycotoxicosis)
In the 3rd and 4th quarter of 2005, 10 practitioners from across the Netherlands reported 13 farms with problems of severe 'photosensitization' in either heifers, dried-off cows or suckling cows. Affected animals had extensive sunburn, aborted and some were culled prematurely. A pilot study was started to find cause(s) for these signs. All reported herds were visited by a "GD-VeeKijker" specialist. Serum analyses revealed severe liver damage in all affected animals. Overall, the findings were mostly related to 'facial eczema' [1]. Evidence was found that the condition was caused by fungal toxins of *Pithomyces*. On grass samples of the affected farms, *Pithomyces chartarum* spores were found and subsequently the fungus was cultured by the Central Laboratory for Fungal cultures (CBS, Utrecht). Presence of this fungus had not been described before in the Netherlands [3].

Detection of a zoönosis (Salmonella)
AHS has a voluntary monitoring program for salmonella on dairy farms. The program monitors the presence of antibodies in bulk milk of participants 4 times a year. Antibodies in bulk milk can be detected when >10% of the lactating cows have had the infection in the last 6 months. On a closed dairy farm, which processed all milk on-farm and sold it as dairy products, salmonella antibodies were detected in bulk milk. The AHS-protocol was started to detect Salmonella carriers. All Salmonellas that are cultured at AHS are anonymously sent to the public health laboratory RIVM-Bilthoven, the Netherlands for typing. AHS was contacted by the public health service as the type found on the dairy farm corresponded with the type found in a massive human outbreak (>100 human cases). Investigations on the dairy farm resulted in the salmonella-type being found in very low numbers in the cheese.

Detection of intoxications (Pb)
GD-Veekijker was consulted for nervous signs and death in beef-cows. The source of the infection proved to be grass silage from a shooting ranch. Although the use of Pb-ammunition is prohibited on shooting ranches, until 2008, participants of the Olympic Games were still allowed to use Pb-ammunition. Two other farms with the same clinical symptoms were found near another shooting ranch and Pb-ammunition could be found in their meadows bordering the shooting area. Government restrictions were placed on these farms to assure that the beef of these cattle was excluded from the food chain.

Discussion
Apart from the official government activities, cattle health surveillance in The Netherlands is now directed and implemented by a core group of specialists in various veterinary disciplines employed by one organization, and situated in one location. This benefits the collection, aggregation and interpretation of the information. Regular meetings between specialists of different background (veterinary medicine, pathology, epidemiology and statistics) on information and results of the different surveillance components can be efficiently planned with little burden on human and time resources. Where experts of various disciplines are cooperating closely, it requires the capacity to 'zoom out' from their field of expertise and to consider viewpoints from other professionals. This benefits both the specialists and the surveillance program. A possible disadvantage of having people from only one institute is to have a restricted view on information. For this reason, close cooperation is established with other national and governmental institutes on animal and public health research. Additionally, surveillance components are evaluated regularly by external experts (Dutch Faculty of Veterinary Medicine, Utrecht University; the Central Veterinary Institute-Lelystad and Wageningen University and Research Centre).

Further improvements are made by regular evaluation of all surveillance components. In our opinion, the system would benefit from international cooperation, especially with neighboring countries with comparable aims and systems.

Those that have reported (the farmer and/or local private veterinarian) are given an answer to the question raised in order to support them to cope with the herd-health problem. By providing such a direct feed back, farmers and practitioners feel acknowledged and rewarded for reporting adverse-health events now and in the future. Motivation of these so called 'eyes and ears' of disease surveillance is pivotal to any surveillance system.

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Syndromic surveillance using veterinary laboratory diagnostic test requests

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Abstract
The development and implementation of systems for real-time monitoring of health data has been limited in veterinary medicine by the lack of computerized, automatically collected data. We describe the construction of a surveillance system for early detection of cattle diseases based on laboratory requests, and explore algorithms for automated classification of data into syndromic groups. Classification rules resulted in high accuracy (99.95%), and allowed detailed documentation of the medical knowledge input in the model, improving communication with experts contributing to system development.

Keywords: syndromic surveillance, cluster detection, aberration detection, time series, classification, laboratory-based surveillance

Introduction

Traditional surveillance, which in general depends on laboratory confirmation and reports of diseases by clinicians and laboratories [1], suffers from chronic underreporting, a long time lag between outbreak onset and reaction, and a low sensitivity as a result of the high specificity of these methods [2].

Using the tools provided by informatics, real time surveillance systems have been developed to make use of pre-diagnostic data already available and automatically collected, such as patient's chief complaint upon emergency visit, or laboratory test orders [3]. The assumption is that that these data are sensitive to changes to the level of disease in the population, containing an early, though weak, signature of a disease outbreak [4]. Due to the lack of specificity, this new type of surveillance targets general groups of diseases, or syndromes, and it is therefore referred to as "syndromic surveillance" [5].

Currently, considering the limited historical use of computerized records in veterinary practice, especially in livestock medicine, diagnostic laboratories appear to be the most readily available source for syndromic surveillance in animal health. While the use of laboratory results suffers from the delays previously noted, laboratory test requests are a potential source of syndromic data [6]. While recorded later in the disease process than clinical data, laboratory data are more specific. Considering the infrequent use of data standards in veterinary medicine, the use of laboratory data in livestock medicine can offer the further advantage of a centralized data source, in many situations covering large areas without the need for data integration across multiple databases.

In the province of Ontario (Canada), the Animal Health Laboratory (AHL) at the University of Guelph is the primary laboratory of choice for practitioners submitting samples for diagnostics in food animals. The AHL is a full service veterinary diagnostic laboratory that serves livestock, poultry and companion animal veterinarians. The AHL has a LIMS (lab information management system) that is primarily used for reporting of results of diagnostic tests, however, it is also used for data retrieval for surveillance purposes. Taking advantage of this centralized, computerized, routinely collected source of data, a syndromic surveillance system is being developed to monitor daily test requests submissions as an indicator of disease trends in the field. The initial phase of implementation is focused on cattle sample submissions.

Syndromic surveillance systems in public health are usually based on emergency department visits. Syndrome classification in this case can be performed by direct mapping billing disease codes into syndromes, or by using text mining tools in order to identify syndromes based on free-text entered during triage (chief complaint). In veterinary medicine, where the field of syndromic surveillance is still incipient, less work has been dedicated to the problem of classifying data into syndromes in an automated manner. Systems monitoring clinical data more frequently collect data already classified into syndromes by the veterinarian, or have not yet reported a plan for automated classification of data. Shaffer (2007) [7], working with laboratory data, reported mapping test request codes directly into syndromic groups, with the list of tests mapped into each group being defined with the help of clinicians and laboratory staff.

This paper reports our experience with syndrome definition and data classification using veterinary laboratory data, as this is the first step into preparing the data for temporal (or spatiotemporal) monitoring and aberration detection [8].

Materials and methods

Data source

Test requests are entered in the AHL database daily. Individual tests are entered as unique data entries, with a case code given to all samples from the same herd. The nature of the diagnostic sample is identified in the database by two fields: one in which an option is chosen by the laboratory staff from a pre-set list (for instance blood, feces, brain tissue, *etc.*); and a free-text field used to enter the sample type description given by the client.

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The diagnostic tests are identified by text codes pre-set in the system, for instance "IHC_IBR" refers to the Immunohistochemistry test for infectious bovine rhinotr3acheitis.

Syndrome definition

A review of historical data from *circa* three years at the AHL was performed. Because veterinarians do not often give detailed history information, the identification of syndromes was based on the type of diagnostic test requested, and the type of sample submitted. The classification was therefore not based directly on the signs observed by the veterinarian, but on the organ system targeted for diagnosis. A potential list of syndromic groups was compiled and then reviewed by two pathologists (including the head pathologist at the AHL) and a field clinician. After their review, all historical data available were manually classified into syndromic groups to serve as training examples to the automated processes to be implemented next.

In each submitted case (one disease event in one herd), multiple numbers and types of samples may have been collected. Without the practitioner's description regarding the primary signs observed and potential diagnosis, defining one single syndrome per case would represent a loss of information. Therefore, without disregarding the fact that each collection of samples from the same herd represents one single health event, syndromic classification was performed for each individual sample submitted. Each case, if considered as the health event of interest, may therefore be a member of as many syndromic groups as those found to be associated with its samples.

Algorithms for automated syndrome classification

Based on the list of syndromic groups constructed with the help of experts, a list of all diagnostic test codes that could be directly mapped into a syndrome group was established. This is especially the case of serological tests, in which the veterinarian specifies the pathogen or disease to be confirmed, giving clues as to the signs observed during clinical examination.

This mapping was built as a model in RapidMiner 5.0 (Copyright 2001-2010 by Rapid-I and contributors), an open source data mining software package. The initial mapping model assigns "Unknown" to all test codes for which a mapping rule cannot be determined. This is the case of test requests such as "bacterial culture", which are not informative of the signs observed or organ system targeted by the veterinarian.

For all the latter, text mining was used to separate all words found in the fields describing the sample type (break points are any non-letter character). A list of all mined words in the historical data was manually reviewed to select the medically relevant words, and create a list of tokens to be used when classifying new data. For all unclassified data, therefore, new binary variables were created to document whether each of those tokens was found in the sample type description.

In order to implement automated classification of these data, we tested the performance of different classification algorithms in using this list of binary variables to determine the syndromic group of each submitted sample. Manually classified historical data were used to train each classifier, which was then applied to the other half of the data. Algorithm classification was then compared to manual classification in order to determine its accuracy (percentage of entries classified correctly by the algorithm).

The most common algorithm used for syndrome classification in syndromic systems is the naïve Bayes classifier, often used when the input data is emergence chief complaint (free-text typed in by nurses) [9]. We tested this in addition to the following classifiers: Artificial Neural Networks, Support Vector Machines (SVMs), Rule Induction, and Decision trees.

Results

Cases

The 3 years of historical data (July 2007 to June 2010) contained a total of 24,331 cases, which totalized 234,313 samples.

Syndrome definition

Based on evaluation of the 3 years of historical data, and input from pathologists, the following syndromic groups were defined:

- Abortion
- Circulatory
- Eyes and ears
- GIT
- Haematopoietic
- Hepatic
- Mastitis
- Musculoskeletal
- Nervous
- Reproductive
- Respiratory
- Skin
- Systemic
- Urinary

The following categories were added to group entries for which the organ system could not be determined, based on the type of laboratorial test requested:

- Environmental or Feed analysis
- Bacterial susceptibility
- Clinical Pathology
- Toxicology

Algorithms for automated syndrome classification

From all samples submitted, 75.7% could be directly mapped into syndromic groups based only on the diagnostic test requested. For the remaining 24.3% of the data (56,941 samples) a range of classification algorithms were explored. Both Artificial Neural Networks and Support Vector Machines are algorithms primarily designed for use with continuous (rather than categorical or binary) data. Both required transformation of the syndromic categories into numerical attributes and their parameterization, even using automated tools, was cumbersome.

Naïve Bayes, the algorithm most commonly reported in syndromic surveillance systems in public health, resulted in an accuracy of around 78%. However, this algorithm exhibits low interpretability (how well humans grasp the way the classifier works [10]). The classifier was provided as input the list of tokens and their binary occurrence per sample in the training data. A model is generated and applied to new data, but it cannot report exactly how it is making use of the medical knowledge input.

More importantly, while working with the Naïve Bayes classifier, it was not possible to establish syndromic groups in cases where the training data were sparse, as the inclusion of groups with low participation in the data resulted in loss of accuracy. In other words the choice of how detailed the classification could be, using this classifier, was based in the number of records for each category in the historical data; rather than the medical relevance of each syndromic group. Important categories such as "Nervous" and "Musculoeskeletal" needed to be merged together to achieve reasonable accuracy when using the Naïve Bayes classifier.

Nonmetric methods, such as Decision Trees, provide a "natural way to incorporate prior knowledge from human experts" [10], in this case pathologists. Decision tree methods applied to the data, however, achieved at most an accuracy of around 67%, and the models appeared to be unstable: slight changes in the training data resulted in a completely new set of rules for establishing the tree branches. This type of behavior in terms of training set sensitivity has been documented for Decision Trees [10].

Improvement in the accuracy was achieved by incorporating classification based not only on single variables, but also on relationships among then. In this case, rule-based methods are recommended over Decision Trees [10]. We therefore chose to use RapidMiner to mine the text, create tokens, and write a model based on a Rule Induction algorithm (Repeated Incremental Pruning to Produce Error Reduction [Ripper, 11]). We then manually edited the rules linking the presence of certain tokens to the final syndromic group, guaranteeing the correct incorporation of medical knowledge and the stability of the algorithm. Rules were of the type: simple mapping, such as "if sample=brain, syndrome=Nervous"; word exclusions, such as "if sample=ear, but the word 'tag' is NOT present, then syndrome=Eyes and ears"; and word combinations, such as "if sample=liver, then syndrome=Hepatic, but if sample=liver+heart, then syndrome=Systemic".

The use of rules allowed the syndromic classification to be as detailed as judged important by the group of experts, including less frequent sample types, such as 'skin', and also accounting for common abbreviations. Moreover, it allowed the establishment of certain "precedence" parameters. "Abortion" for instance, is the only syndromic group that truly corresponds to a disease sign, not an organ system, and therefore it was considered important that the presence of any token related to abortion (such as placenta, fetus, *etc.*), should determine the classification into this syndromic group, regardless of the test ordered or other sample type descriptions.

In most cases, if no medically relevant token is found within the sample type description, the Syndrome type is considered to be "Nonspecific". Using custom rules, however, it was possible to combine the information on the tests requested with the information on the sample type. For instance, if the test request is for "Clostridium difficile", the syndrome type is directly defined as "GIT". But if the test requested is "Clostridium Fluorescent Antibody", the syndrome is defined based on the sample type submitted (for example brain, feces, *etc.*), and when no sample type description is included, the syndrome types is defined as "Musculoeskeletal" (rather than "Nonspecific").

Most importantly, the use of custom set rules achieved 99.95% of accuracy in classifying the data, based in comparison to the manually classification.

After classifying all sample submissions individually, and then counting as cases all different syndromes found in the same herd investigation, the final number of cases in the historical data was 33,731, versus 24,331 initial herd investigations: 5,354 groups of samples contained two or more syndromic groups.

Discussion

Establishing real-time monitoring of animal health data depends on establishing reliable models that capture medical knowledge, and that can be applied in an automated manner. Such models should be efficient, but also comprehensible by end users. We achieved high accuracy using a Rule-based automated syndrome classifier, which needed to be manipulated manually at the construction phase, but resulted in clear interpretability of the decisions and the resulting classification.

The use of custom set rules, however, limits the possibility of automated updates of the model in the future. Further research will be needed to establish internal validation rules, possibly based on the results available from historical data, in order to define automated ways to update the model without user input.

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Development of an Animal Health Monitoring System Based on Abattoir Condemnation Data

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Abstract

Animal disease surveillance is typically limited to specific diseases whereby the identification of the etiological agent relies on laboratory test results. When efforts are focused on the eradication of known endemic diseases, traditional sampling and testing regimes have proven largely successful. In contrast, such surveillance systems do not work well when confronted with emerging disease events. When novel disease agents emerge in animal populations, it often takes time to identify and control them. By the time a novel disease is recognized, disease transfer outside of the index area may be well underway. In order to achieve early warning of possible disease emergence, analysis based on methodical observation of disease syndromes at specific control points may be beneficial. While not designed to identify a particular disease agent, such monitoring systems provide signals of animal health anomalies, thus allowing earlier investigation and intervention by animal health professionals.

Keywords: abattoirs, condemnations, surveillance, syndromic.

Introduction

This paper describes a potential augmentation of animal health surveillance by the United States Department of Agriculture (USDA). USDA's Food Safety and Inspection Service (FSIS) inspectors evaluate each carcass that passes through abattoirs; determine whether a carcass requires condemnation and which condemnation category is assigned to the carcass; and record condemnation data in the FSIS electronic Animal Disposition Reporting System (eADRS). Inspectors are trained to report individual dispositions and evidence of overt foreign animal disease, but do not necessarily identify anomalies in the data otherwise. Through partnership with its USDA sister agency, the Animal and Plant Health Inspection Service (APHIS), FSIS shares data that could be used for routine monitoring of animal condemnations at points of slaughter enabling early warning of the possible emergence of an animal disease. This paper describes validation of early warning methods by a retrospective analysis of a documented 2001 swine erysipelas outbreak that occurred in the States of Iowa (IA), Minnesota (MN), and North Carolina (NC). Based on the outcome of this exploratory work, a pilot project using systematic weekly monitoring of IA swine abattoirs for erysipelas, pneumonia, and septicemia condemnations has been implemented.

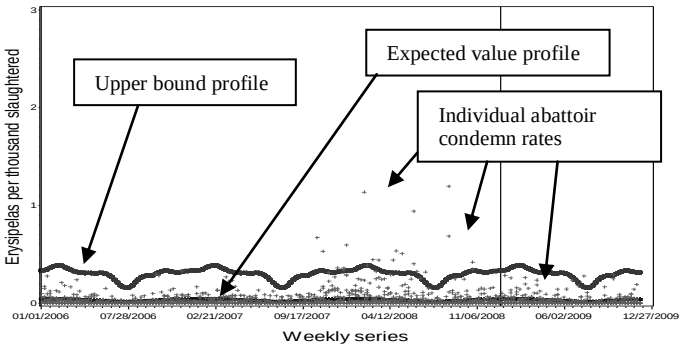
Materials and methods

FSIS eADRS data from 14 IA swine abattoirs that consistently processed swine from 2006-2009 were evaluated. Condemnation data from eADRS, along with information on the species, week of slaughter, and total number of animals slaughtered that week were reviewed. Condemnation categories indicating swine erysipelas, pneumonia, and septicemia were evaluated. Data processing was performed using SAS version 9.1 and an Excel-based "Alerting Algorithms Tool" developed and made publicly available by Dr. Howard Burkom [1].

Three methods for gleaning signals of potential concern from the data are identified. For the first two methods, baselines of expected condemnation rate values (EVs) and upper bound values (UBs) are calculated using 2006-2008 eADRS data. Distributions of condemnation rates for large and small abattoirs are clearly different and therefore, the data were sorted into two abattoir size strata. UBs were set at four standard deviations above the EVs for each abattoir size stratum. Outlier observations in the upper tails of the distributions were eliminated from the baseline data to avoid inclusion of data points that might reflect outbreak conditions. Both EVs and UBs were created weekly in order to account for observed seasonality in condemn rates. Once baseline UBs were established, weekly 2009 condemnation rate data were compared to the UBs to identify signals of concern; i.e., weeks where condemn rates were higher than UB values.

While Method 1 (M1) and Method 2 (M2) are similar in construct, M1 reflects individual abattoir condemn rates and is more likely to create signals of adverse animal health conditions (see Figure 1), while M2 considers condemn rates at the stratum level and results in fewer signals of possible concern (see Figure 2). M1 can produce

Figure 1: Method 1: Erysipelas condemns per thousand slaughtered, Jan 2006 -Nov 2009 FSIS eADRS data



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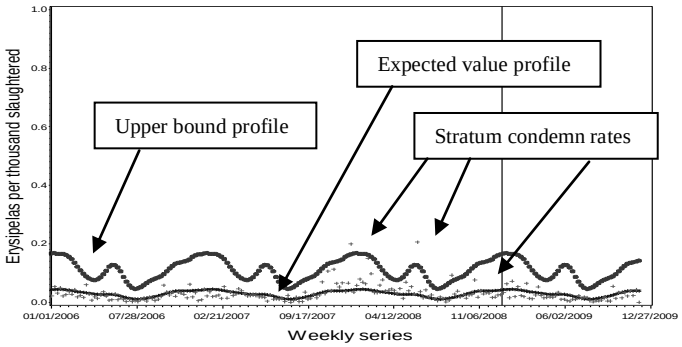
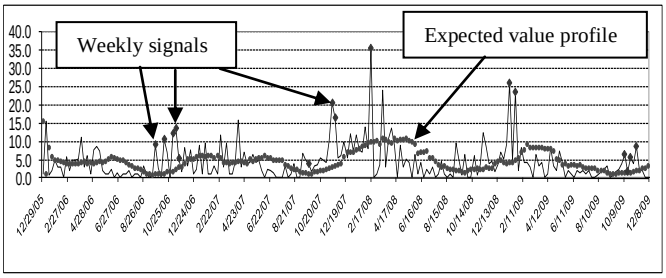
Multiple signals for each week and size-stratum combination, up to a maximum of one signal per abattoir per week, while M2 can only produce one signal per week and size-stratum combination because condemn rates are calculated at the abattoir size-stratum level. Often, when a single abattoir signaled under M1, there was no corresponding M2 signal. A signal resulting from M2 may indicate a very high condemn rate in an individual abattoir or smaller spikes in condemn rates in multiple abattoirs.

Figure 2: Method 2: Erysipelas condemns per thousand slaughtered, Jan 2006 -Nov 2009 FSIS eADRS data



The approach used for Method 3 (M3) is a modification of the 'C3' version of the Early Aberration Reporting System developed by the United States Centers for Disease Control and Prevention which is used widely by state and local public health departments [2]. This method is based on a cumulative summation (CuSum) routine, which compares normalized current-week condemnation counts with the mean of a moving baseline period 4 to 15 weeks prior to the current period (see Figure 3).

Figure 3: Method 3: Erysipelas condemn counts for a large abattoir, Jan 2006-Nov 2009 eADRS data



A lag of 3 weeks was used between the current week and the baseline period. While Methods 1 and 2 compare abattoirs' performances against baselines constructed using historical information from all abattoirs in the study, M3 measured changes in one abattoir's performance against the previous performance of that same abattoir. If condemnations in an abattoir creep upward for a few consecutive weeks, there may be an M3 signal for that abattoir prior to a signal under M1 and M2.

Signals based on each of the three methods provide a slightly different analytical perspective and are evaluated contemporaneously. To create an overall weekly signal score, the numbers of signals resulting from each method are summed. When the weekly signal score reaches a predefined level, a potential swine health anomaly in abattoir catchment areas may be indicated.

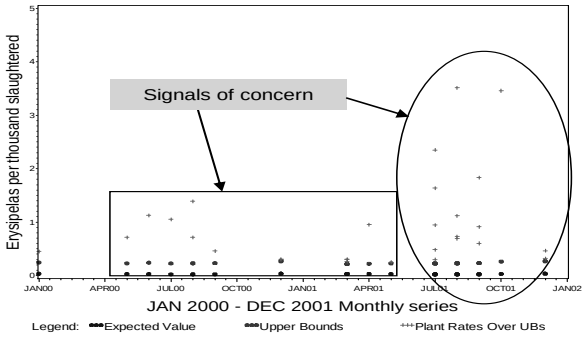
Methods validation: The methods outlined above were applied to legacy FSIS condemnation data in an attempt to retrospectively determine if early warning of a documented erysipelas outbreak could be obtained. Widespread knowledge of the 2001 swine erysipelas outbreak did not occur until it reached its peak in July and August 2001. In the post-outbreak analysis, Engle charted erysipelas-positive diagnostic laboratory data and FSIS erysipelas abattoir condemnations between January 2000 and December 2001 [3]. Based on casual observance of the plotted data, he postulated that systematic monitoring of erysipelas condemns at slaughter could have resulted in a warning of an outbreak during the autumn of 2000. Using the methods described above, it appears that warning of a possible impending outbreak could have been issued as early as October 2000, 10 months prior to the epidemic period.

For the methods validation exercise, a 1997-2001 series of monthly IA, MN, and NC FSIS eADRS data representing abattoir erysipelas condemnations was used. Prior to 2004, eADRS data are available by month rather than by week. M1 and M2 baselines were constructed using 1997-1999 data, and 2000-2001 condemnation rates were compared against the baseline UBs. The CuSum-based M3 uses a one-month lag period, and the three months prior to the one-month lag as the baseline period. As previously described, signals from each of the three methods are summed to create a final signal score.

Result

Indicators of excessive erysipelas condemnations based on M1, M2, and M3 are presented in Figures 4, 5, and 6, respectively.

Figure 4: Method 1; Erysipelas condemns per thousand, large abattoirs, 2000-2001 IA, MN, and NC eADRS data



For ease of analysis, data on Figures 4 and 5 are only displayed for months during which individual abattoir condemn rates (Figure 4) and aggregated abattoir size-stratum condemn rates (Figure 5) exceeded the UBs. Signals associated with the 2001 epidemic period beginning in July 2001 are observable on the M1 and M2 data plots (see reference ovals in Figures 4 and 5). Signals of lesser magnitude in the months leading up to July 2001 are also observable and are detailed within

the rectangles overlaid on both plots. Similar pre-epidemic signals are observed on most of the M3 charts (see Figure 6 for a representative M3 abattoir profile).

Figure 5: Method 2: Erysipelas condemns, large abattoirs, 2000-2001 IA, MN, and NC eADRS data

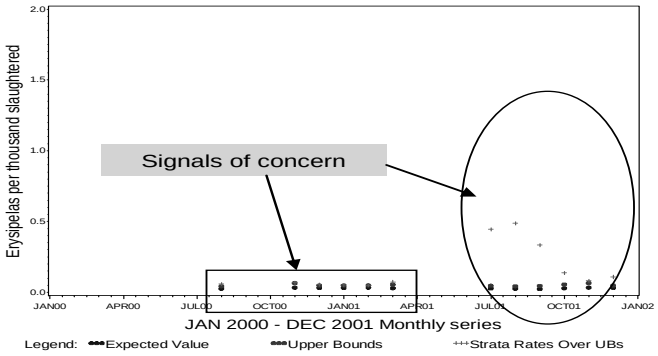
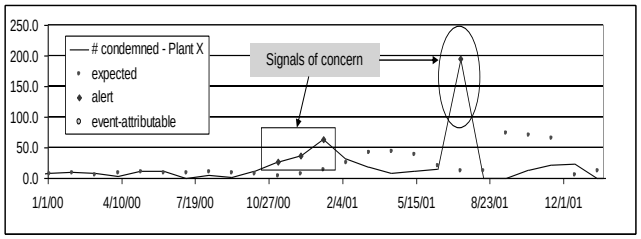


Figure 6: Method 3: Erysipelas condemn counts for a large abattoir, 2000-2001 IA eADRS data



Summing M1, M2, and M3 signals, signal scores were created for each month in 2000 and 2001. With the perspective of hindsight, we know that the outbreaks were widespread during July and August of 2001. During these months, signal scores are '11' and '16', respectively. Using the July and August 2001 signal scores as a reference and using a signal score value of '8' as a warning threshold, it would have been reasonable to have issued a warning of the potential for swine erysipelas outbreaks during October, November, and December 2000 and again in March 2001 when the signal scores reached '8', '12', '8', and '8', respectively.

Discussion

The exploratory work and validation exercise described herein lays the foundation for continuous weekly monitoring of FSIS abattoir condemnation data for the purpose of identifying potential adverse animal health conditions at points of slaughter. Currently, APHIS-Veterinary Services (VS) monitors IA swine abattoirs on a weekly basis for excessive erysipelas, pneumonia, and septicemia condemnations.

FSIS abattoir inspectors evaluate animals presented for slaughter for a broad set of predefined ante- and post-mortem conditions. Condemnation categories include 'infection,' 'inflammation,' 'icterus,' 'central nervous system disorder,' 'pyrexia,' and many more. Other than swine erysipelas, which is perhaps the only condition for which a specific disease may be identified by visual observation at slaughter, most categories are not disease-specific, but represent non-disease-specific abnormalities. Although specific diseases cannot be diagnosed visually by abattoir inspectors, the observance of categories with abnormally high condemns may allow for the initiation of a disease investigation earlier than would be possible by conventional surveillance. Currently, work is underway at VS to explore application of this type of 'observational' or 'enhanced passive' surveillance to other data streams including laboratory test requests and disease syndromes identified at livestock markets and by practitioner networks. Signals of concern at any of these points of observation may be cross-validated with other data streams in order to identify potential disease situations. Furthermore, information documenting the absence of excessive condemnation rates or levels could possibly be used as auxiliary information for providing documentation of freedom from certain diseases.

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West Nile virus in Europe: a comparison of surveillance system types and sizes in a changing epidemiological context

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Introduction

Recent surveillance data and outbreak reports suggest that WNV circulation patterns in European countries could change. An endemic WNV circulation is now suspected in Italy [1] and Hungary [2]. Moreover, while a high level of diversity in WNV strains have been described in Europe, their pathogenic properties and impact on public and animal health have not been characterized [3, 4]. In the light of these new epidemiological events, the risk of WNV emerging in Europe as a serious threat for horse and human health appears real but remains unpredictable. This uncertainty justifies the establishment of appropriate surveillance systems. We used a recently published metapopulation model [5] to estimate the optimal dimension of equivalent surveillance systems dedicated to the detection of WNV circulation and compared the performance of these systems in three epidemiological contexts: a base situation with a recurrent, low-level WNV circulation and two opposing situations: one in which there is a very low level circulation and one in which there is an epidemic.

Materials and methods

Five surveillance systems were considered. The passive surveillance systems were based upon horse populations and sentinel veterinarians reporting clinical WNV cases in their practices. We assumed that 10% of horses infected by WNV would present neurological disorders [6] that could be detected by the sentinel veterinarians. The two passive systems were distinguished from each other by the specialization of the sentinel veterinarians and the corresponding sensitivity of the sentinel function: in the first, specialized equine veterinarians reported every WNV neurological case; in the second, non-specialized equine veterinarians reported only 10% of WNV neurological cases. With regard to active surveillance systems, three types were considered: (i) the detection of seroconversions in sentinel horses or chickens and (ii) the detection of WNV genome in trapped mosquito pool assumed to contain 50 insects [7, 8]. For each of the three active surveillance systems, we considered that sampling or trapping were performed on the 1st and the 15th of each month, from May 1 to November 1. We calculated the minimum number of subjects (horses, chickens or mosquito pools) each type of surveillance system had to cover annually to ensure WNV detection at a 95% confidence level. The five surveillance systems then were compared by considering the calendar date of the first WNV detection and the annual laboratory costs. We assumed hat the laboratory diagnostic of equine cases detected by the passive systems, as well as the serological tests carried out on sera coming from the active systems (horses or chicken), was performed through a seroneutralisation test. The sensitivity and specificity of this test were assumed to be perfect. The calculation of the minimal sizes of the surveillance systems was based upon the cumulative density function $P(t<T)$ of the calendar T date at which the 1st detection event occurs This cumulative density function was defined according to N, the size of the surveillance system. Solving the equation $P(t<T) = 0.95$ for N thus allowed us to determine the minimal size of a surveillance system for the detection of WNV circulation with a confidence level of 95%.

For passive surveillance systems, $P(t<T)$ is the cumulative density function of the calendar date at which the 1st clinical case is detected: $P(t<T)=1-1(1-Se*0.1*F_h(T-\Delta I))^N$ where:

- *Se* is the sensitivity of the sentinel function (10 or 100%),
- is the probability for a contaminated horse to show clinical signs,
- ΔI is the duration of the incubation period, set to 8 days [9],
- -N is the number of horses monitored,
- F_h (T) is the probability for a horse to be contaminated before calendar day T.

The same methodology was used for active surveillance systems.

The function Fh (T) was obtained numerically from a mathematical model of WNV circulation between western Africa and southern Europe [5]. This model is a metapopulation model in which three sites are considered (wet African area, dry African area, European area), which are shared by host and vector populations and which are linked by bird migrations. On each site, the infection dynamic is modelled using an S-I-R model for hosts coupled with an S-E-I model for vectors. In order to simulate situations of very low level circulation and epidemics , the vector-host ratio of the European area [5] was adjusted to obtain the desired annual incidence rates in horses (62% or 1%). Model calibration consisted of estimating these site-independent bite relative risks as well as site-specific vector-host ratios [5]. The limit cycle predicted by the model was used to compute Fh (T) and the corresponding functions of active surveillance systems.

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In this base situation, the annual infection rate in horses is 3% [5]. In addition to this base situation, surveillance systems were compared in two opposite epidemiological situations: an epidemic situation, characterized by an annual infection rate in horses of 62%, as described by Nielsen *et al*. in California, a low-level WNV circulation situation, in which the annual infection rate in horses was arbitrarily set at 1%.

In both cases, a single parameter of the model was modified: the vector-host ratio of the European area [5]. This ratio was increased (or decreased) until the desired yearly incidence rate in horses (62% or 1%) was obtained. The two corresponding limit cycles were then used to calculate F_h (T).

Result

In the base, low-level but recurrent circulation context, a passive equine surveillance network composed of specialized equine veterinarians would need to monitor 1,033 horses for the detection probability to reach 95% . If the annual incidence of neurological syndromes in horses is 1% [10], and if these syndromes are identified as suspect cases, the annual number of suspicions reported by sentinels would be 11. To obtain the same detection performance with non-specialized sentinel veterinarians, 10,338 horses need to be monitored. Under an active surveillance system, the same detection threshold would be reached by sampling approximately 100 sentinel horses or 160 sentinel chickens every 15 days. Searching for the WNV genome in 20 vector batches trapped every 15 days would allow the same performance to be reached.

The median date of the first WNV detection would occur slightly earlier under a passive equine surveillance network than an active surveillance system. Under an active system based on horses, 1,326 serological exams, would need to be carried out annually; the same figures for a chicken-based system would be 2,093 exams. For the active surveillance based on vectors, 260 PCR exams would be needed on mosquito batches.

In a putative epidemic context, the vector-host ratio corresponding to a yearly incidence rate in horses of 62% was estimated to be 3.9. The five monitoring systems would allow the detection of WNV circulation with a 100% probability. The median date of WNV circulation detection would occur one to one and half months earlier than in the base context, however, passive systems would be more efficient. On average, the detection date under a passive system would occur in mid-July compared to early August for the three active systems.

In a very low level of transmission level context, the vector-host ratio corresponding to a yearly incidence rate in horses of 1% was estimated to be 2.2. In a third of the cases, monitoring systems would be unable to detect this circulation, with the active mosquito trapping being the most efficient. WNV circulation would be detected first one month later than in the base situation. Again, passive systems would be slightly more efficient.

Discussion

In the base context, the detection performances of the passive surveillance of a thousand horses by specialized equine veterinarians appeared equivalent to the bi-monthly follow-up of seroconversions in a hundred sentinel chickens or horses, as well as to trapping about twenty pools of mosquitoes twice a month to detect the WNV genome. The passive surveillance of horses appeared, however, to allow virus circulation to be detected slightly earlier than active surveillance systems at a comparatively very low cost.

In both passive systems, the median date of first detection (August 27) is coherent with what actually happened in France in 2000, where the first acute neurological disorders in horses were detected by a veterinary practitioner on August 24 and 28 [11], and in Italy in 2008 [12], where the first signs of infection were detected on August 20. The estimated median dates of first detection in an epidemic context are consistent with what was observed in 2010.

In the very low level circulation situation, PCR on mosquito pools allowed a slightly higher proportion of detection than other systems for a similar median date of first detection. In that case, again, a passive surveillance system would be a cost effective alternative.

Our estimations were performed with the assumption that, in the area subject to WNV surveillance, the infection risk was spatially homogeneous for horses, chicken and mosquitoes. Moreover, it was assumed that a sufficiently large naive horse population existed in the area. In cases of sporadic transmission and low mosquito infection rate, detection capacity may be improved largely by focusing mosquito trapping on locations known to be potential “hot spots” [13, 14]. The sizes of surveillance systems we obtained here therefore should be used as a base that must be adapted to local contexts.

In order to simulate situations of very low level circulation and epidemics , the vector-host ratio of the European area [5] was adjusted to obtain the desired annual incidence rates in horses. Results show that a small decrease of this vector-host ratio may lead to an apparent disappearance of the virus, and to a decrease of vigilance. Conversely, for the epidemic situation, the adjusted vector-host ratio was 3.9 vs 2.6 for the base situation. This result suggests that a small variation of the vector density, one caused by any climatic event or environmental change influencing vector population dynamics, may lead to an outbreak. Recent Italian and Greek outbreaks clearly demonstrate that such a scenario is plausible, justifying the need for an appropriate surveillance system capable of taking timely measures to protect public health

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Authors would like to thank Dr Thomas Balenghien for its help in vector trapping methodology. This study has been facilitated through the International Network for Capacity Building for the Control of Emerging Viral Vector Borne Zoonotic Diseases (Arbo-Zoonet) supported by the European Union under grant agreement no 211757.Monitoring wildlife diseases: syndromic surveillance for the detection of unusual events

The ecological surveillance of West Nile virus in Catalonia: in continuous evolution

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Abstract

An ecologic surveillance system for West Nile was implemented in Catalonia in 2007. This system consisted of different components: active and passive avian surveillance, follow-up of chicken sentinels, cross-sectional surveys in feral equines, follow-up of equine sentinels, passive equine surveillance, and entomological surveillance. Until 2010 these activities have been continuously adapted to improve the efficiency and the sensitivity of the surveillance. Between 2007 and 2010 the active WNV infection was not detected in any component.

Keywords: West Nile, surveillance, North-Eastern Spain.

Introduction

West Nile Virus (WNV) is a widespread zoonotic pathogen that maintains its transmission cycle principally between mosquitoes and birds. The circulation of this flavivirus has been frequently evidenced around Europe and the Mediterranean Basin. Recent outbreaks, such as epidemics in Greece or Rumania in 2010, have caused serious neurological disease in humans and equines and even fatal cases [1, 2].

In the south of Spain, its activity has been also recently detected in humans and horses and previous circulation of WNV had been also confirmed in diverse avian species and horses in this area [3, 4, 5].

To prevent the potential impact of epidemics in public and animal health there is a current need of implementing surveillance systems in areas at high risk in Europe and neighbouring countries [6].

In Catalonia (in north-east of Spain), which is close to neighbouring areas where WNV has been previously evidenced [1], exist important wetlands that serve as resting sites for many birds migrating between regions of Africa and Europe and which are exposed to a high density of culicids. In this region has been implemented an ecologic surveillance system for WNV since 2007.

The work describes the different strategies carried out between 2007 and 2010, the results obtained from each component, and discusses the strengths and weaknesses detected.

Materials and methods

The ecologic surveillance for West Nile implemented in Catalonia consists of avian, equine and entomological surveillance. These components were mainly implemented during the period of activity of adult culicids in the main wetlands considered at risk.

The **active avian surveillance** consisted in serological surveys. All the sera were tested by a competitive ELISA (cELISA), and confirmed by virus-seroneutralization test (VNT). Between 2007 and 2009 these samples were obtained from the bird ringing schemes that were run in chicks of *Phoenicopterus roseus*, *Larus audouinii,* and *Larus michahellis* in areas considered at risk. In 2010 the strategy of sampling was modified, and sera samples of a broad variety of different species were obtained from the wildlife rehabilitation centers who sampled birds from the whole region during all the period of study.

In 2008 the **serological sampling of sentinel chickens** was initiated as a new component. These samples were collected from backyard holdings located close to areas at risk. In each holding 5 chickens were bimonthly sampled between May and November.

The **passive avian surveillance** included investigations of birds found dead from peaks of mortality detected over the entire territory. Tissue of encephalon was collected from carcasses and subsequently tested through a specific real time RT-PCR (RRT-PCR) for WNV.

The **equine surveillance** was based on periodical **serosurveys in sentinels, cross-sectional surveys in feral horses** bred ourdoors in the wetland areas, and **passive surveillance**. In relation to sentinel equines, a total of 17 holdings were followed up. In each holding sera samples were bimonthly collected from 4 seronegative unvaccinated horses, subsequently were screened by cELISA and confirmed by VNT.

The passive surveillance in equines was based on the investigation of those suspicions that showed neurological clinical signs. The sample of election was cerebrospinal fluid (CSF) or nervous tissue (mainly medulla oblongata). These samples were taken in collaboration with veterinarians and the services of carcass disposal that collected the nervous tissue at the moment of picking up and removing deceased horses. The sera and CSF samples were tested by cELISA, and CSF and nervous tissue by RRT-PCR.

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The **entomological surveillance** was carried out in areas considered at high risk. Between 2007 and 2009 a homogeneous scheme of trapping was used in the different wetlands. This system allowed comparing the results among these areas. CDC light traps baited with CO_2 traps were set out fortnightly. These traps were located in three different sites based on the type of habitat: natural areas with vegetation of coastal wetlands with high density of wild birds, rural areas with crops (rice fields and others) and with high density of domestic animals, and urban or periurban areas. The specimens captured were identified and pooled into groups up to 40 mosquitoes, according to the date, site of collection and species. All these pools were processed to detect viral genome for the *Flavivirus* genus. This detection consisted in viral RNA extraction according to manufacturer (Qiagen) instructions, followed by a generic RT-nPCR. Subsequently, the positive pools for flavivirus were specifically tested for WNV by RRT-PCR.

In 2010 the scheme of the entomological surveillance changed. A continuous monitoring using different types of traps was carried out. The goal was to determine the species and the dynamics of culicidae population in wetland areas. The viral detection in vectors was uniquely planned in the event of previous detection of WNV in vertebrates.

Results

The follow-up of the different components of surveillance implemented in Catalonia along this 4-year period is shown in Table 1 and Figure 2.

Table 1: Summary of the ecologic surveillance for West Nile implemented in Catalonia (2007-2010)

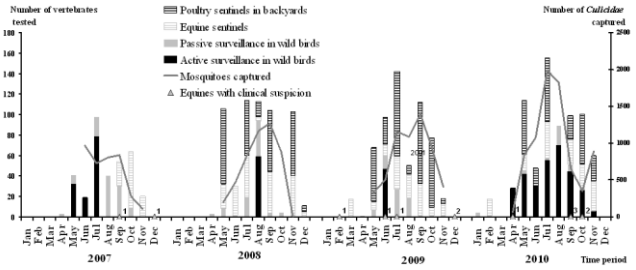
		Wild birds		Chicken sentinels	Equine		Mosquitoes captured
	YEAR	Active surveillance	Passive surveillance	Nr. of animals	Nr. of sentinels	Clinical suspicion	Nr. specimens
Areas at high risk	2007	130	19		47		3748
	2008	59	21	105	43		4827
	2009	47	39	110	48		5829
	2010	78	10	125	67		7679
	Total 07-10	**314**	**89**	**340**	**205**		**22083**
Rest of Catalonia	2007		90		20	2	
	2008		54		18		
	2009		29	10	18	5	
	2010	223	29			6	
	Total 07-10	**223**	**202**	**10**	**56**	**13**	
TOTAL in CATALONIA		**537**	**291**	**350**	**261**	**13**	**22083**

Avian surveillance in wild birds

Between 2007 and 2010, 828 wild birds belonging to 71 species of 18 orders were tested for WNV. In total 537 sera were collected by active surveillance to detect the presence of antibodies, and 291 were collected by passive surveillance and tested by RRT-PCR for WNV.

Between 2007 and 2009 none of the samples tested gave a positive result. In 2010, although none of the samples resulted positive by passive surveillance, 5 sera of species belonging to order *Accipitriformes* resulted positive by cELISA and VNT. Three of these positive birds were Short-toed Eagles (*Circaetus gallicus*) (1/20, 1/40, 1/60), one was a Black kite (*Milvus migrans*)(1/10) and one was a Red kite (*Milvus milvus*) (1/15). All of them are migrant species in the period of nesting in Catalonia that come from African countries. None of these positive birds was sampled in areas considered at high risk in Catalonia.

Figure 2: Temporal implementation of the different components of surveillance for WNV



In chicken sentinels

A total of 690 sera coming from 36 different holdings located in areas exposed to a high density of culicid population were tested by cELISA from 2008 to 2010. All of them resulted negative.

Equine surveillance

In total 572 equine sera coming from 17 sentinel holdings were tested between 2007 and 2010. Sixty-seven horses in 2007, 61 in 2008, 66 in 2009 and 67 in 2010 served as serological sentinels for WNV. All serological tests resulted negative except one indigeous that was positive to cELISA and VNT (1/10), and negative to the ELISA for Ig M. None of the feral horses included in cross-sectional serosurveys gave positive results.

Clinical suspicions in equines

Between 2007 and 2010, thirteen equines with clinical signs of encephalitis were reported and tested. All of them were negative to WNV by real time RT-PCR.

Entomological surveillance

A total of 22,083 mosquitoes were captured during this 4 –year period. Between 2007 and 2009, 14,404 mosquitoes belonging to 15 different species were pooled in 902 groups. Sixty one pools resulted positive to *Flavivirus* genus, although none of them gave positive to WNV.

The most abundant species captured were: *Culex pipiens (40.1%), Ochlerotatus caspius* (37.5%), *Culex modestus* (12.3%), and *Anopheles atroparvus* (4%).

Discussion

From 2007 to 2009 none of the samples obtained from the components of ecological surveillance for WNV in Catalonia resulted positive. These results are in contrast with previous serosurveys performed in humans in the area of influence of Ebro Delta [7]. However it should be noticed that during this period the active surveillance in wild birds was mainly focused, both space and time. Thus, the results can not be inferred to the global population of wild birds in Catalonia. In contrast in 2010 the serological surveys conducted in wild birds from May to November in different species have shown that the WNV incursion is highly probable. This kind of sampling has demonstrated to be efficient to detect avian reservoirs and points out the need of amplifying the surveillance to other areas not considered at risk.

In relation with the negative results from the passive surveillance in wild birds, no peak of mortality caused by WNV infection was detected. However, unlike the WNV epidemic in the United States, observing the behavior of previous outbreaks occurred in other European areas, peaks of mortality in wild birds have not been a good indicator of the early circulation [8, 9]. Thus, the fact that WNV have not been detected in dead birds in Catalonia is not a sufficient indicator to discard viral circulation.

Regarding the use of chicken sentinels, this method has proved to be useful to the early detection. This is the case of northern Italy in 2005 where 90% of sentinel birds (56/62) seroconverted without being detected in any other population [10]. From 2008 to 2010 a total of 350 birds were used as sentinels, and none has been detected as positive. These results are in agreement with other components and indicate that in recent years the WNV circulation in domestic birds has been null or insignificant.

The detection of WNV in horses indicates a high rate of viral amplification in an area and poses a potential danger to public and animal health [8]. In Catalonia the unique indication of WNV circulation in equines was detected through serology in 2010. One of the equine sentinels resulted positive to IgG (1/10) and negative to IgM, indicating an old infection or cross-reaction. This result corroborates the importance of maintaining a continuous equine surveillance in the region to detect active circulation.

Regarding entomological surveillance, previous experiences showed that this is not a very sensitive component to detect the presence of WNV in the initial stages [10]. Nevertheless, this component provides itself valuable information to both identify the possible risk of spread in an area and in the event of outbreak to plan control measures The results of entomological monitoring in these recent years show that species such as *Culex pipiens*, or *Culex modestus*, *Ochlerotatus caspius*, which are known as competent vectors for WNV, are widely distributed in Catalonia. Moreover, the introduction and wide spread of *Aedes albopictus* in this region constitutes an important concern due to its vectorial capacity and its aggressive behaviour in human biting. In relation with the WNV genome monitoring in vectors it should be pointed out that the circulation WNV has not been detected; however other flaviviruses with an unknown importance in public or animal health were frequently detected mainly in *Aedes caspius* and *Culex pipiens* in areas with a high density of wild birds.

From our results we believe that components as theserological avian surveillance from the wild rehabilitation centers, the active and passive equine surveillance, and the continuous entomological monitoring could serve as the best indicators of the risk of WNV spread in our region. Anyway, on the basis of our experience, the surveillance for WN should be maintained as a flexible system under constant evalution. And its design requires a continuous adaption according to the epidemiological situation, the disponibility of resources and the technical capacities of the network of professionals involved in each task.

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Surveillance and control of classical swine fever in Bulgaria, a country with a high proportion of non-professional pig holdings

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Abstract

Control and eradication of classical swine fever (CSF) in countries with a high proportion of non-professional pig holdings poses additional challenges for the veterinary services since such holdings may act as a reservoir for CSF virus and as possible source of infection for commercial farms. Bulgaria has about 60 000 non-professional holdings in which 34% of the domestic pig population are kept.

Bulgaria started in 2007 a new strategy for surveillance and control of CSF, based on the categorization of pig holdings according to their biosecurity standard. Basically three categories of holdings where identified: (i) holdings with high or appropriate biosecurity measures comprising large industrial farms or smaller family farms involved in trade, (ii) holdings with low or no biosecurity measures comprising smaller family farms involved in local trade and back yard farms and (iii) traditional outdoor pig herds (East Balkan Pigs).

Once the holdings were categorized, adequate CSF surveillance programmes manageable by the local and central veterinary service, could be designed for the different types of holdings. Additional tools including the electronic identification system for holdings and pigs and a check-list for clinical examinations were introduced to facilitate the surveillance activities.

In this communication the categorization of farms is presented as a successful tool for CSF surveillance and control in different categories of pig holdings. Under conditions like in Bulgaria categorization of farms appeared to be also a reliable method for improving the biosecurity level and an essential tool for CSF surveillance and control in non-professional pig holdings.

Keywords: Classical swine fever, non-professional holdings, surveillance.

Introduction

Classical swine fever (CSF), also known as *Hog Cholera*, is a fatal viral disease affecting pigs and wild boar. CSF is notifiable to the World Organisation for Animal Health (OIE) and causes major economic losses especially in countries with an industrialised pig production system [1]. When outbreaks of the disease occur in domestic pigs, a broad set of eradication measures have to be applied in the Member States of the EU according to the Council Directive 2001/89/EC on Community measures for the control of classical swine fever [2]. Eradication programmes in recent decades have been successful to a great extent in many European countries including Bulgaria.

However, countries like Bulgaria with a high proportion of non-professional pig holdings, also addressed as back yard holdings, face additional difficulties in surveying and controlling the disease. In Bulgaria about 250,000 pigs (34%) are kept in about 60,000 non-professional holdings while 450,000 pigs (64%) are kept in 61 industrialized farms. The role of non-professional pig holdings in the CSF problematic is of great epidemiological significance since such holdings may act as a reservoir for CSF virus and as possible source of infection for commercial farms. Despite their average small size and pig density and therefore their apparent unimportance, non-professional holdings can play an important role in the dynamics of a CSF epizootic. For instance, Bulgaria experienced during recent years six CSF outbreaks in 2006, one in 2007 and the last one in 2008, all of them in non-professional holdings with low biosecurity measures. Additionally two outbreaks were detected in free ranged pig herds in the Eastern part of the country in 2007.

Rearing pigs in non-professional holdings is very common and has tradition in the rural areas in most of the new and acceding Member States. In these countries, this type of rearing still is a significant part of the agricultural practices. It represents an important source of meat supply for the population in the countryside and often generates a valuable cash income. Apart from that, non-professional pig holdings play an integral role in recycling of food and kitchen waste as pigs on these holdings are fed amongst others with leftovers from the kitchen.

Usually, non-professional holdings have a low standard of biosecurity and are much more difficult to control by the veterinary services. In particular surveillance activities, as for example for CSF, are demanding and challenging.

We present a feasible approach how CSF surveillance in domestic pigs can be performed in a country with a high proportion of non-professional pig holdings.

Materials and methods

Three main steps where conducted towards facilitating a pragmatic surveillance programme for CSF in the domestic pig population.

1) Categorization of all domestic pig holdings on the basis of biosecurity measures in place. The following basic criteria of biosecurity were used for categorizing the holdings:

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- production cycle (closed or open),
- keeping system (indoor/partially outdoor; access to foreign personnel; access to other pigs or feral pigs),
- appropriate means of disinfection at the entrance and exit of buildings,
- fences around the holdings,
- appropriate hygienic measures for persons coming in contact with pigs,
- trade patterns (free markets or direct contact of the owner with potential customers),
- use of swill feeding,
- outdoor keeping, including traditional free ranging systems,
- number and categories of pigs,
- regular veterinary controls.

2) Introduction of an electronic information system for registration of animal holdings and identification of pigs. The basic elements of the information system are:

- registration of all pig holdings by categories;
- registration of all owners;
- registration and identification of all pigs;
- registration of all movements of pigs and traceability;
- registration of sampling and laboratory testing;
- registration of all clinical examinations;
- registration of the health status of pig herd and individual pigs;
- operation and access to the electronic information system by official and private vets concerned.

3) Introduction of a standardized check-list for clinical examination of pigs in the framework of active surveillance for CSF.

Based on the above three components an appropriate CSF surveillance scheme for the different types of holdings was implemented.

In holdings with high biosecurity the survey is conducted as follows:

- in large industrial farms monthly clinical examinations by private vets (12/year) and clinical examinations by official vets every two months (6/year); serological investigations to detect 20% antibody prevalence with 95% confidence;
- in small commercial farms with high biosecurity monthly clinical examinations by private and official vets (24/year) and serological investigations to detect 10% antibody prevalence with 95% confidence.

In holdings with low biosecurity the survey is conducted as follows:

- in commercial farms with low biosecurity clinical examinations by private vets every two months (6/year) and clinical examinations by official vets every three months (4/year); serological investigations to detect 10% antibody prevalence with 95% confidence;
- in backyard holdings with poor biosecurity where pigs are kept for own consumption only clinical examinations by private vets every three months (4/year) and clinical examinations by official vets on 10% of the backyards every three months (4/year). Samples for laboratory testing are taken only in case of suspicion.

Holdings with free ranging pigs (East Balkan pigs) are surveyed as follows:

- monthly clinical examination of all herds by private vets (12/year);
- clinical examination every 3 months by official veterinarians (4/year);
- clinical examination at any occasion when pig identification is performed or when animals are moved;
- serological investigations to detect 10% antibody prevalence with 95% confidence;
- serological investigations of all animals 7 days prior to movement to slaughter or to other holding.

Result

The following 5 types of domestic pig holdings were identified:

1. **Industrial farms**: large farms with a high biosecurity level (61 farms with 444,341 pigs);
2. **Family farms type A**: smaller farms with a high biosecurity level (89 farms with 24,640 pigs);
3. **Family farms type B**: smaller farms with a low biosecurity level (1,728 farms with 41,279 pigs);
4. **Backyard farms**: holdings with a low biosecurity level, up to 5 pigs, no mother sows, kept for own consumption, not entering into the national trade cycle (58,673 holdings with 106.928 pigs);
5. Traditional outdoor holdings of East Balkan pigs: particular Bulgarian pig bread, kept outdoor in the Eastern parts of the country. All herds are under a strict supervision of the veterinary service (98 herds with 10,104 pigs).

The following results were obtained during the implementation of the CSF programme in 2009:

CATEGORIES	№ farms	№ pigs	№ tests serology	№ tests virology	№ clinical examinations
Industrial	61	444341	11959	167	5775
Type A	89	24640	3222	37	2145
Type B	1728	41297	17596	202	13118
Backyards	58673	106928	649	5	74244
East-Balkan pigs	98	10104	4570	4	878

Discussion

From a legal point of view all pig holdings, notwithstanding if they are professional or non-professional, are equal and have to fulfil the same requirements related to control and eradication of CSF. As such, all measures laid down in Council Directive 2001/89/EC concerning the control of classical swine fever [2] and in Commission Decision 2002/106/EC on the diagnostic manual for CSF [3] should be enforced by the veterinary authorities in case of a suspicion or an outbreak, regardless of the number and type of pigs reared in the holding. However, uniform and standardized disease control requirements are difficult to be followed in countries with a heterogeneous

omestic pig sector, as for example in Bulgaria, which is characterized by different farming types, biosecurity levels, trade patterns or social and traditional backgrounds. In particular non-professional pig holdings are often not fully under the control of the authorities and foreseen measures are therefore not as effective as desired. Surveillance and control measures are difficult to implement and the veterinary authorities may be faced with major problems in managing CSF outbreaks. It will supposedly take a lot of effort controlling and eradicating the disease within a reasonable time period, especially if a considerable amount of virus is circulating.

Since the level of biosecurity of a pig holding influences the risk of virus perpetuation directly and may play a key role in facilitating a CSF epidemic, we decided, as a first step, to categorize all pig holdings in Bulgaria based on biosecurity grounds. Basically three categories where identified: (i) holdings with high or appropriate biosecurity measures comprising large industrial farms or smaller family farms involved in trade, (ii) holdings with low or no biosecurity measures comprising smaller family farms involved in trade and back yard farms keeping pigs for own consumption only and (iii) traditional outdoor pig herds (East Balkan Pigs) which are under a permanent control of the veterinary service but under particular risk due to their potential contact with wild boar.

From a CSF risk point of view we considered the family farms with low biosecurity level (family farms type B) the most dangerous one, since pigs, pigmeat and meat products from such farms may enter the national market and may contribute to the spread of CSF virus. This is proven by most of the CSF outbreaks in domestic pigs during the past years which occurred in family farms type B. In contrast, back yard holdings which keep pigs only for family consumption do not pose such a high risk in promoting epidemics, but may play a role as a hidden virus reservoir.

Once the holdings were categorized, adequate CSF surveillance programmes manageable by the local and central veterinary service, could be designed for the different types of holdings. Additional tools including the electronic identification system for holdings and pigs and a check-list for clinical examinations were introduced to facilitate the surveillance activities.

The surveillance results from 2009 mirror the activities of the veterinary service in relation to CSF. They show also where emphasis has to be put to amend the activities in the following years. Laboratory examinations, in particular virological testing should be increased in combination with clinical examinations, where suspect animals are found.

Since family farms type B have been identified to carry the highest CSF risk, it is aimed to continuously reduce the number of these holdings as much as possible, either by upgrading the biosecurity level (become family farms type A) or by excluding them from local trade (converting them into backyard farms). As a result of the new strategy the number of type B farms could be reduced from 4981 to 1728 during the last 3 years.

We can conclude that the systematic approach of categorizing pig holdings based on their biosecurity level is an effective tool to plan and conduct surveillance programmes for CSF in domestic pigs.

By the new categorization of holdings, the check lists for active surveillance and the outcome of the implementation of the programme, Bulgaria could present to the European Commission and to the other Member States a strong and reliable CSF control and surveillance strategy.

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Use of a Predictive Epidemiological Simulation Model and Economic Analysis in the Disposition of Tuberculosis Affected Cattle Herds in the United States

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Abstract

Whole herd depopulation has been the primary tool for managing tuberculosis (TB) affected herds in the United States; however, this is becoming less tenable for animal health officials due to cost as well as public concern with mass depopulation of herds. For instances when depopulation is not feasible or cost effective, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS), Veterinary Services (VS) has been reexamining its policy and seeking alternative herd management options. One option is to manage TB-affected herds under a test-and-removal policy in conjunction with quarantines and restricted movement of animals to limit disease spread.

To determine what management approach to use for a given herd, VS has to weigh several factors (such as the prevalence of infection in a herd, disease transmission risks, the effectiveness of other possible management practices, and the cost effectiveness of depopulation). VS developed a predictive epidemiological simulation model to help make these decisions about managing TB-affected herds.

Keywords: modeling, epidemiology, bovine TB, cost efficiency, decision support.

Introduction

USDA-APHIS-VS-CEAH-National Surveillance Unit (NSU) has developed a predictive epidemiological (simulation) model, which along with cost analysis, can assist decision makers in assessing whole herd depopulation versus a test-and-removal protocol (TRP) for TB affected herds. Predictive modeling analysis is performed on a platform of Microsoft Excel and Palisade @Risk simulation modeling software.

We use the available data and knowledge about diagnostic tests, the herd, and the disease to describe probable outcomes of a test-and-removal protocol. Key outputs from the simulation model include prediction of probability a herd is free from disease after a series of herd tests, and informing the economic analysis by predictions of the number of reactors to be purchased, the number of test rounds required, and the number of supplemental tests to be performed in a TRP.

Use of this model is part of a risk-based, herd-specific approach to disease management. This approach allows for the release of quarantine as quickly as possible and provides a high level of confidence that a herd is free of disease. In short, this approach establishes performance-based conditions for quarantine release rather than inflexible standards.

VS uses the model with each affected herd on a case-by-case basis. We can use the model's predictions to help animal health officials develop a testing protocol for each herd. The model estimates how many rounds of testing and what type of tests are needed to be highly confident that a herd is TB free. We use this information to ***estimate*** when a herd can be released from quarantine. While the model guides disease management decisions, herd plans that require certain management and biosecurity practices are still essential to prevent the reintroduction of infection in a herd.

After each round of herd testing, we refine the model's predictions using actual test results. When test results provide greater than 95 percent confidence that a herd is TB free, we conduct another herd test. If we find no infected animals, and confidence the herd is free of TB remains $\geq$ 95%, the quarantine will be lifted. We conduct additional herd tests after the quarantine is released to provide assurance that no undetected infected animals are spreading disease and the herd remains free of TB.

Model Inputs and Assumptions

We incorporate the uncertainty we have about input parameters (i.e., test sensitivity, specificity, prevalence, *etc.*) used to predict simulation model outcomes.

One such parameter is caudal fold test (CFT) sensitivity (Se). From literature we know that the Se of CFT averages 82 percent but can vary widely, depending on the person conducting the test, the region, the cattle, and other factors [1, 2, 3, 4, 5, 6, 7]. Simulations use different values of Se and other parameters for each iteration that the computer runs through but chooses the values according to how likely they are based on prior studies and data. We can think of the iterations as testing similar herds many times; in this case, 10,000 times.

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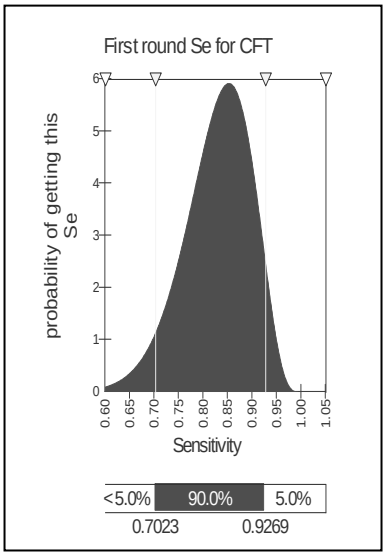
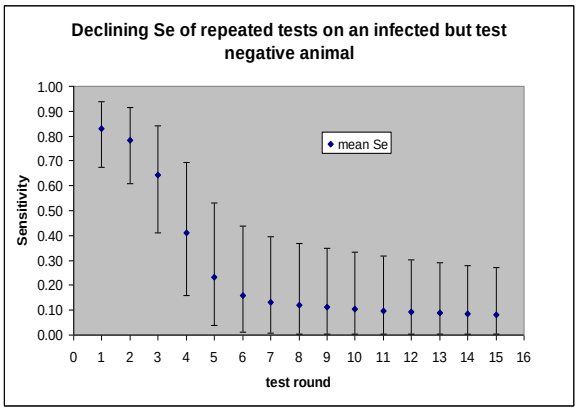
The figure to the left shows the distribution of the sensitivity of CFT. On average, it is 82 percent; sometimes it is much less (i.e., 5 percent of the time < 70.02 percent of the infected cattle) and sometimes more (i.e., 5 percent of the time > 92.69 percent of the infected cattle).



CFT Se is the probability that an infected animal will test positive. The initial Se of the test is described above and entered into the model as a Beta [2, 4, 5] distribution (median 82 percent with high uncertainty).

However, test Se does not remain constant for animals that are tested repeatedly. Conditional test Se is the value you might expect when you retest an infected animal that has already tested negative one or more times (i.e., probability of a test positive given that it is infected and previously tested negative). We don't have data to predict the decline in sensitivity but assisted by our experts, believe a reasonable assumption is that it follows a curve similar to the one



As with Se, the specificity (Sp) is assumed to change with successive tests. Initial test Sp (the initial probability that a test negative is uninfected) in the analysis of TB affected herds is assumed to be equal to the outcome of the first round of testing. The Sp is calculated as Beta(s+1, n-s+1) where n equals the number tested, and s equals the number of successes (number of test negative animals). Conditional Se is assumed to increase with each subsequent test (similar to the manner that Se decreases over repeated testing).

Initial apparent herd prevalence in the simulation model is based upon a diagnostic herd test. For each model iteration, an estimate of true prevalence with attendant uncertainty is derived.

After each round of testing estimated prevalence is updated in the simulation model by removing the true test positives and any infected animals that would be culled without detection through standard herd management practices. We incorporate herd specific culling and replacement data for use as model inputs. The new prevalence (post-test) is equal to the number of false negatives remaining in the herd plus any infected animals entering the herd as replacements and any newly infected cattle.

Literature on within-herd spread of TB is limited and from outside the U.S. [8, 9, 10]. For our model, contact rate resulting in newly infected animals was estimated from datasets of TB herds that VS has depopulated in previous years. Most herds appear to have 0 or 1 new case resulting from an initial infection, while a few may have several new cases. The data were fitted to a geometric distribution and that used to describe the probability of new cases. The outcome of this distribution allows for some infected animals to successfully spread TB to several susceptible cattle in a year. Most of the time however, the contact will only result in one, two, or no newly infected cattle within the first year.

The model maintains an inventory of the original herd as well as new additions to the herd (replacement herd) for each test round. We do so to apply appropriate estimates of Se and Sp for tested groups of animals by round. For example, a member of the original herd that is being testing for the fourth time will have a lower Se and higher Sp, than a replacement or newly infected animal that is being tested for the first time.

The model accounts for the number of animals culled from, and replaced in the herd by test round based on herd specific management practices. We assume replacements should come from Accredited Free States or certified herds. We modeled the prevalence of these replacements to be 0.005 percent based upon the probability of an animal being TB infected [11]. As an alternative, we looked at the prevalence in replacements being ten-fold higher at 0.05 percent. In this scenario, there was high confidence that the herd had no infected animals but after the second or third year, prevalence began to increase gradually. Because of this, it is imperative that infection is not reintroduced in replacement cattle.

Confirmatory tests may be either by comparative cervical test (CCT) or interferon gamma and the results are conditional on the result of the first test. Based on studies from Michigan herds [3], we assume that the Se is approximately 88.4 percent and Sp is approximately 98.5 percent for the CCT. For generation of simulation model outputs we assume no difference in Se and Sp regardless of which test is used as a supplemental test.

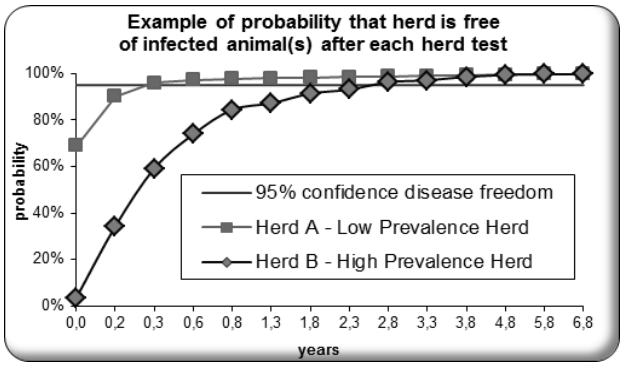
The prevalence of TB in free ranging cervids associated with TB-affected cattle herds in certain areas of the U.S. has been established through wildlife surveillance. For modeling herds in such areas, a wildlife exposure risk has been estimated. Exposure risk is estimated as the likelihood of a successful exposure resulting in infection of 1, 2, or 3 animals at a time. The rate of these events occurring is estimated from data in areas where transmission from wildlife has occurred. An "events per unit time" probability distribution (poisson) was used to describe this happening and is multiplied by the number of animals infected per event in each model iteration, and the product used to estimate new cases.

Results (Model Outputs)

The model provides a prediction of the probability that a herd is free from TB following a series of herd tests. A model simulation for a TB affected herd consists of 10,000 iterations or greater if convergence is not achieved. The interpretation of a model simulation of probability of disease freedom after a series of tests can be thought of as follows: a 95% prediction of disease freedom following a test indicates that if 10,000 similar herds were tested in a similar manner, 9,500 would have zero infected animals in the herd; while the remaining 500 herds would be expected to have one or more infected animals remaining in the herd.

The model output is used to assist in the development of a proposed TRP testing protocol (*e.g.*, how many TRP tests would be required). Various combinations of tests, as determined by federal epidemiologists in consultation with state counterparts, can be proposed and incorporated as model inputs.

The following graph illustrates, depending upon initial within-herd prevalence, two different probabilities that the herd is free from infection following a series of tests.



Outputs from the model inform the economic analysis of comparing a test-and-removal protocol (TRP) to whole herd depopulation. The number of animals predicted to be removed (purchased for diagnostic necropsy) during a test-and-removal protocol, the number of supplemental tests needed, and the total number of herd tests required are used in the economic analysis. This information is combined with herd specific on-farm testing costs, laboratory and materials costs, and any necropsy, disposal, and transportation costs to estimate total government costs for a TRP. These costs are compared with the net indemnity costs for whole herd depopulation to assist decision-makers in determining the most effective way to manage the affected herd.

Discussion

VS determines the disposition for each TB-affected domestic livestock herd on a case-by-case basis. The evaluation considers, but is not limited to, the following factors:

a. The apparent prevalence of infection in the herd;
b. The risk of disease transmission while under a test-and-removal plan;
c. Effectiveness of other management practices to mitigate disease spread;
d. The cost effectiveness of depopulation.

Use of predictive epidemiological modeling and cost analysis is part of a risk-based, herd-specific approach to disease management. Under a test-and-removal program, it allows for the release of quarantine as quickly as possible, while providing a high level of confidence that a herd is free of disease, and optimal use of federal dollars. In short, this approach establishes performance-based conditions for quarantine release rather than inflexible standards.

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Differences in distribution of the atypical scrapie in Italy can be in part explained by the level of surveillance applied

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Abstract
Atypical scrapie is an ubiquitous but rare disease that seems not to be contagious under natural conditions. Currently only genetic susceptibility is recognised as an important risk factor.

However the application of different diagnostic tests and the different intensity of the surveillance applied may influence the occurrence of the disease.

The objective of this study is to evaluate if the differences in the intensity levels of surveillance can partially explain the differences in the occurrence of the disease.

We described the distribution by province of the incidence rate observed by means of active surveillance, the percentage of farms tested by a diagnostic test able to identify atypical scrapie and the percentage of animals with the allele AHQ used as a proxy of high susceptibility. All these three variables are characterized by a geographical heterogeneity. In some cases, provinces with a high disease incidence rate coincided with provinces where the highest number of farms was tested with the most sensitive test.

The sensitivity of the surveillance system in detecting the disease played an important role to explain the geographical distribution of the Italian atypical scrapie. This study indicates that the evaluation of the surveillance distribution and of the diagnostic detection capability is necessary to describe the real presence of the atypical forms and the knowledge of their descriptive epidemiology deserves some re-assessment.

Keywords: atypical scrapie, geographical distribution, genotyping programme.

Introduction
The current European Transmissible Spongiform Encephalopathies (TSE) surveillance programme in small ruminants has led to the discovery of atypical scrapie, a new TSE, ubiquitous in many countries but rare. An European study showed that the frequency of disease tends to be quite similar in all countries [1].

How much atypical scrapie epidemiologically differs from classical scrapie is still unclear, however the epidemiological and pathological features support the hypothesis of the disease not being contagious under natural conditions or only at a very low level [2, 3]. Currently, only genetic susceptibility is recognised as an important risk factor.

Therefore, without other confirmed risk factors, the heterogeneity in the disease distribution could be attributed mainly to the geographical distribution of the resistant genotypes.

However another element that could influence the apparent distribution of disease is the diagnostic detection capability in terms of both the application of different diagnostic tests and the different intensity of the surveillance applied.

The objective of this study is to evaluate if differences in the occurrence of the atypical scrapie between Italian geographical areas can be partially explained by means of differences in the level of surveillance applied.

Materials and methods
We considered data from 3 different datasets: (a) data relative to the atypical scrapie outbreaks: 65 outbreaks (55 in sheep farms, 10 in goat farms), identified by active surveillance in Italy between 2005 and 2008 (in 23 outbreaks an AHQ index was identified); (b) data relative to the number of animals tested using a diagnostic test (Biorad TESeE rapid test) known as able to identify atypical scrapie (128,386 sheep and 48,383 goats); (c) data from the National breeding programme for scrapie resistance and in particular the geographical distribution of the sheep carrying the AHQ haplotype (14,334 AHQ animals out of 169,610 genotyped sheep) used as a proxy of high susceptibility (unfortunately, no data is available on the distribution of the AFRQ haplotype which is associated with the highest susceptibility).

Therefore data collection and analysis have been restricted to the geographical area where the Biorad TESeE rapid test has been used: as a consequence only data regarding a subset of Italian Regions during the 2005-2008 period have been considered. For each Italian province we calculated the observed annual flock-level incidence rate (outbreaks/1,000 registered flocks). In order to estimate the incidence rate we used as denominator the number of farms registered in the National Animal Registry Office (n=94,238 sheep flocks; n=44,483 goat herds, i.e.), which is consistent with the average number of farms opened from 2004 to 2009.

For the evaluation of the diagnostic test distribution, for each province we estimated the percentage of farms in which they tested at least “n” animals with the diagnostic test able to identify atypical scrapie on the registered farms. We considered “n” the median of the distribution of tested animals in each farm. Finally, we calculated the percentage of animals AHQ out of the total number of genotyped animals.

Afterwards, we produced descriptive choropleth maps to illustrate, by province, the distribution of: I) the

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incidence rate observed by active surveillance; II) the percentage of farms tested by the diagnostic test able to identify atypical scrapie; III) the percentage of animals with the allele AHQ.

Result

Atypical scrapie incidence is characterized by a geographical heterogeneity in sheep and goats (Figures 1 and 2). The provinces with the highest incidence values were mostly located respectively in the case of sheep in Sardinia and in central Italy, and in southern regions for goats. We identified an heterogeneity in the distribution of the farms tested with the diagnostic test able to identify the disease. In 4 Italian regions (Lombardy, Emilia Romagna, Abruzzo, Sicily) the diagnostic test was only sporadically used (Figure 3). In some cases, provinces with a high disease incidence rate coincided with provinces where the highest number of farms was tested with the most sensitive test.

Figure 1: Atypical scrapie incidence distribution (sheep)

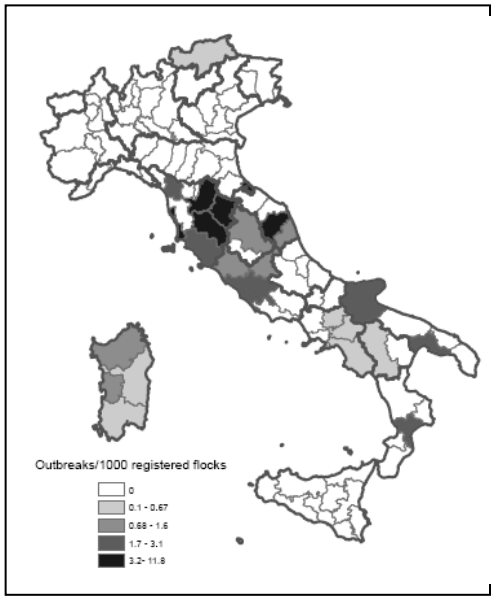
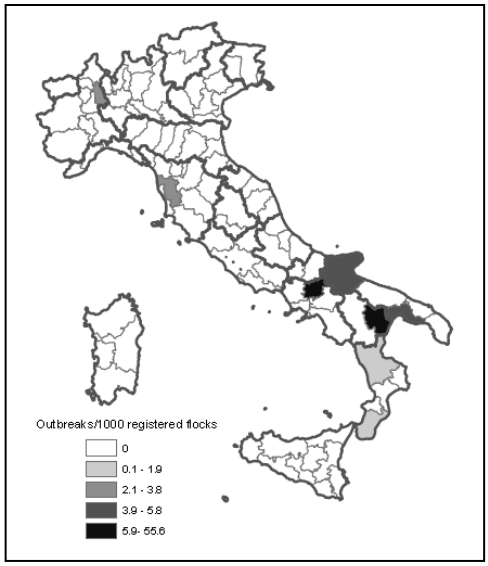


Figure 2: Atypical scrapie incidence distribution (goats)



The distribution of the percentage of AHQ sheep is characterized by heterogeneity, among the different Italian regions and the provinces, too (Figure 4).

Figure 3: Percentage of farms tested by the diagnostic test able to identify atypical scrapie out of the total number of registered flocks (sheep)

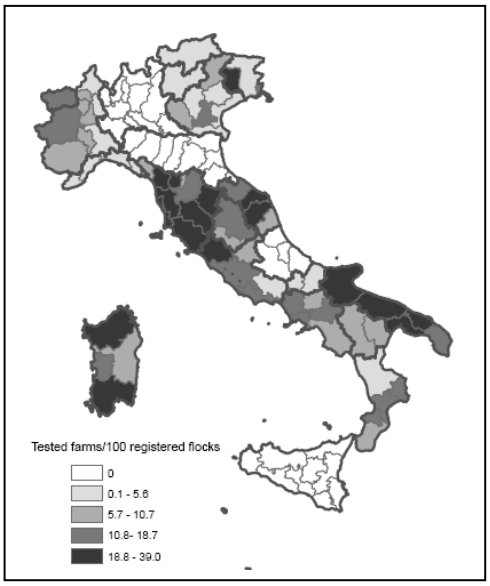
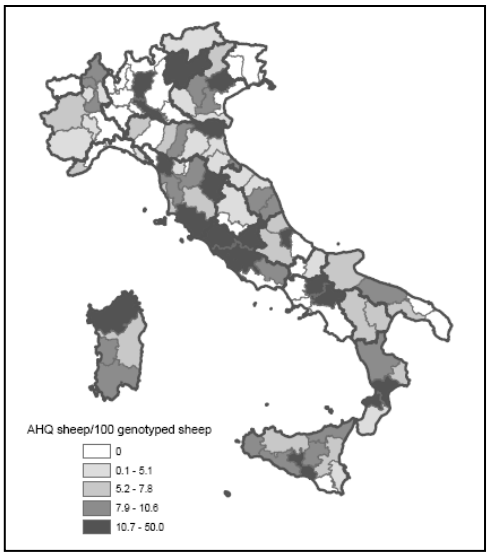


Figure 4: Percentage of AHQ sheep out of the total number of genotyped sheep



Discussion

This study indicates that to describe effectively the real presence of a disease, it is necessary to account for the detection capability both in terms of the diagnostic test applied and the intensity of surveillance.

Atypical scrapie was found in a limited number of Italian provinces. Provinces of the same regions evidenced heterogeneous distribution. To try to explain these differences, we decided to use the distribution of AHQ animals because it is one of the allele associated with increased disease risk [4] and it is immediately available from the National database of the genotyping programme for scrapie resistance.

However, even if the heterogeneity of the geographical distribution of the Italian atypical scrapie could be partially explained by the distribution of AHQ animals, we showed that the sensitivity of the surveillance system in detecting the disease played a main role.

Moreover the genetics data come only from the subset of farms joining the breeding programme for scrapie resistance and therefore they may not be a representative sample of the entire population.

In the case of scrapie, the diagnostic laboratories involved in the surveillance can use only rapid tests authorized and listed in the European regulation. In the past, the selection of rapid tests has often been based exclusively on commercial and practical reasons: as recently the European legislation has been amended to take in account the tests' sensitivity in detecting the atypical forms, their descriptive epidemiology deserves some further re-assessment.

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The passive surveillance for classical scrapie in Italy is not able to provide additional and complementary information compared to active surveillance

S. Bertolini[1*], C. Bona, P. Barzanti and G. Ru

Abstract
Prevalence and incidence data obtained from active and passive surveillance for scrapie are the relevant information used to describe the real distribution of the disease in a country.

The objective of this study is to compare the ovine classical scrapie incidence data at flock-level obtained by the two surveillance systems in Italy, in order to assess their relative efficacy.

We considered the outbreaks of classical sheep scrapie identified in Italy by passive surveillance (1995-2008) and active surveillance (2002-2008) for each province and we calculated the relative annual incidence rate.

Furthermore, we produced descriptive maps to illustrate, at province level, the distribution of the results.

Data obtained by passive surveillance are characterized by lower values and a minor number of provinces involved in the distribution of the disease.

Passive surveillance was able to capture the disease only partially and wasn't able to provide additional information in comparison with the active system. Moreover where the passive approach performs better this is likely attributable to a positive effect of a concomitant active surveillance.

This study indicates that the active surveillance plays a key role in effectively detecting and describing the distribution of the disease. In order to asses control measures for scrapie, it is important to maintain the efficiency of this system and to interpret correctly (*e.g.* using standardized data) its results.

Keywords: scrapie**,** surveillance, incidence rates, maps.

Introduction

Since 2002 in the European countries the passive surveillance system for scrapie has been integrated with an active surveillance programme targeting samples of the sheep and goat populations older than 18 months of age. Prevalence and incidence data obtained from these activities are the relevant information used to describe the disease occurrence in our country. In passive surveillance the decision on the inclusion or exclusion of the individual animal is done by the owners or practitioners and it is not under the direct control of the veterinary authorities [1]. Therefore attempts of hiding the disease or the ignorance in recognising clinical symptoms can result in the under-reporting of the disease occurrence. However passive surveillance might represent a valuable source of information to integrate active surveillance data and improve our knowledge of the real distribution of the disease.

The objective of this study was to compare incidence data obtained respectively by active and passive surveillance and to assess the relative efficacy of the two approaches. In particular, we wanted to evaluate if passive surveillance is able to provide additional and complementary information compared to active surveillance. Finally, we investigated if the local application of active surveillance improves the performance of passive surveillance.

Materials and methods

We considered 192 Italian outbreaks of ovine classical scrapie identified by means of active surveillance from 2002 to 2008 and 160 outbreaks identified by means of passive surveillance from 1995 to 2008 (64 outbreaks during 1995-2001; 96 outbreaks during 2002-2008).

We calculated annual incidence rates (outbreaks/1000 registered farms) due respectively to either active or passive surveillance. In order to estimate the incidence rates we used as denominators the number of farms registered in the National Animal Registry Office (n=94,238 farms, i.e. the average number of farms opened during the period 2004-2009).

In the case of active surveillance data, we estimated directly standardized rates adjusted for both surveillance intensity (in term of number of animals tested per farm) and risk category (fallen stock vs. regularly slaughtered animals). In the case of passive surveillance, since the adjustment was not applicable, we estimated crude rates separately for the two periods (1995-2001 and 2002-2008). Furthermore, we produced descriptive choropleth maps to illustrate the distribution by province of: 1) the standardized incidence rates from active surveillance; 2) the incidence rates from passive surveillance, separately for the two 7-year periods (1995-2001 and 2002-2008 i.e. before and after the launch of the active surveillance); 3) the ratio of active to passive incidence rates; 4) the percentage of farms involved in active surveillance out of the total registered farms.

Result

Even after adjusting for surveillance intensity, in Italy scrapie shows an heterogeneous geographical distribution. In general the incidence rates based on passive surveillance data are lower than those from active surveillance (Figures 1 and 2): the latter enabled the detection of higher numbers of provinces affected by scrapie outbreaks whereas there were no provinces where scrapie had been detected by passive surveillance only.

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Provinces showing the highest incidence rates from passive surveillance partially overlap with provinces where a more intensive active surveillance was practised. Finally the number of scrapie outbreaks detected by passive surveillance after December 2001 is remarkably higher than the number identified before.

Figure 1: Classical ovine scrapie incidence distribution based on active surveillance data. Standardized rates to surveillance intensity and risk category.

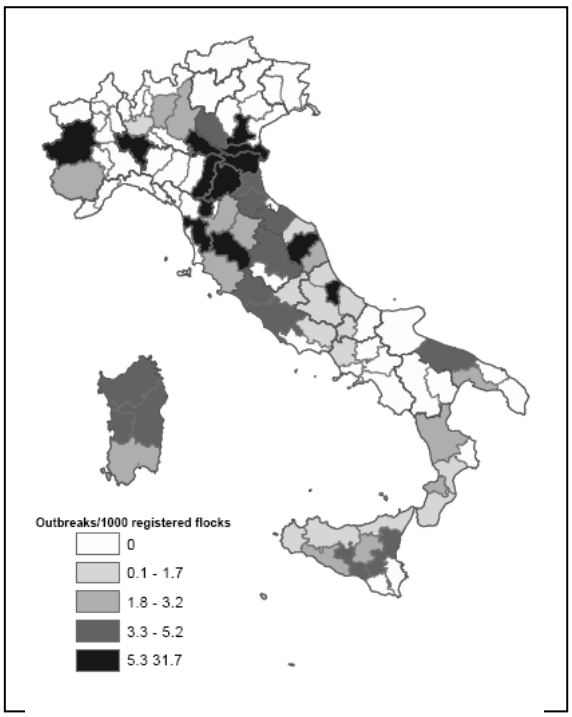
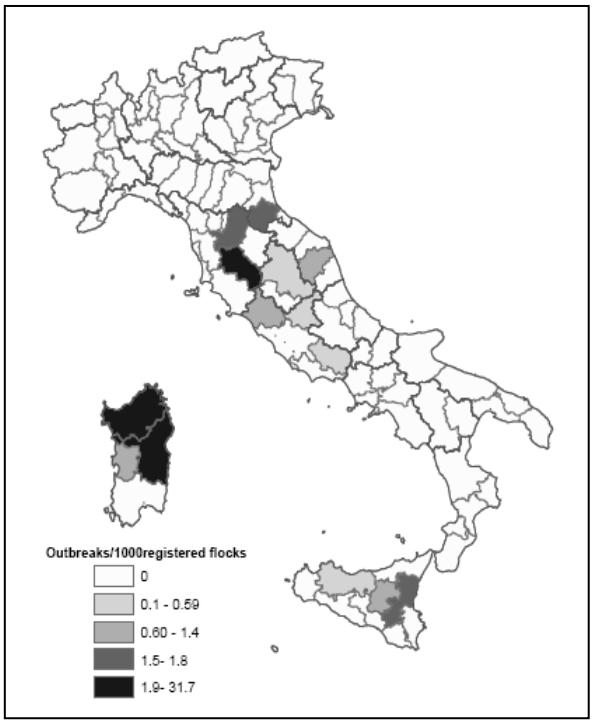


Figure 2: Classical ovine scrapie incidence distribution based on passive surveillance data.



Discussion

Our study (a) confirms the improving of the overall surveillance due to the implementation of the active approach, (b) shows that due to its intrinsic limitations the passive surveillance did not provide any additional informative contribution, (c) suggests that where the passive approach performs better this is likely attributable to a positive effect of a concomitant active surveillance. Even after adjustment, data from active surveillance indicate that scrapie has a really heterogeneous spatial distribution and, in particular, that it had spread much more than it was suggested by passively obtained data.

After 2002, when the passive surveillance paralleled the active one, it was not able to provide any further information *e.g.* identifying gaps in the distribution of the disease.

Moreover our data suggest that where the passive approach performs better this is likely attributable to a positive effect of a concomitant active surveillance as it has been shown at the end of the '90s in the case of BSE in Switzerland after the introduction of the active surveillance [2]. A more intensive active surveillance may improve the passive surveillance efficacy through a better awareness of the clinical disease by the owners and the veterinarians involved in the active surveillance. That explains why the number of outbreaks identified by passive surveillance before and after the introduction of active surveillance increased.

This study confirms that the active surveillance plays a key role in effectively detecting and in describing the real distribution of the disease in our country. In order to asses control measures for scrapie, it is important to maintain the efficiency of this system and to interpret correctly (*e.g.* using standardized data) its results.

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Within-flock incidence in sarda sheep scrapie outbreaks

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Abstract
Scrapie is a rare, fatal neurological disease of sheep and goats, belonging to the group of Animal Transmissible Spongiform Encephalopathies (TSEs). In Italy the disease has been reported since 1976 and in 1991 the scrapie mandatory reporting was enforced.

The aim of this work is to study the incidence of scrapie within all the outbreaks come out after clinical reporting and affecting sarda sheep between 1995 and 2009.

All farmers were interviewed, using a standardised questionnaire, including the number of sheep, classified into three age classes, and the number of animals showing scrapie-like symptoms observed during the last 12 months prior to confirmation of the disease in the flock. The association between scrapie and age class was quantified in terms of relative risk (RR). Moreover the year-specific age adjusted incidence was calculated to describe the secular trend.

The mean crude within-flock incidence was 2.06 cases per 100 animal-years (CI 95% 1.95-2.18). As expected, in general most farms show low rates with the highest incidence affecting the 2 to 4 years old animals. The decreasing trend in the incidence deserves further studies: it may indicate a real phenomenon or rather may be a collateral effect of the improved awareness of farmers, leading to an anticipation of the recognition of the disease when the incidence is still very low. However the validity of our data, collected through oral interviews, may have been negatively influenced by the farmer's inability of recognising the disease and recalling the cases.

Keywords: Scrapie, Incidence, Standardized questionnaire.

Introduction

Scrapie is a neurological disease in sheep and goats, which belongs to the group of Animal Transmissible Spongiform Encephalopathies (TSEs) including Bovine Spongiform Encephalopathy (BSE) in cattle and Chronic Wasting Disease (CWD) in wild deer. For more than 250 years, this infectious disease has been observed in European sheep flocks and was spread to many other countries of the world [1]. The incidence of infection is more difficult to determine because the disease is difficult to diagnose in live animals and can only be confirmed by post-mortem pathological investigations because of the absence of any diagnostic tests to detect preclinical infection.

Farmer-based surveys have been used to estimate the occurrence of scrapie in UK [1, 2, 3, 4], in the Netherlands [5], in Ireland [6] and in the Shetland Isles [7]. A study carried out in the Netherlands [5] showed that approximately 6% of farmers had seen a case of scrapie in their flock in the past. An anonymous postal survey carried out in 1999 in UK [1] among more than 6,000 sheep farmers, reported that 5.3% of farmers had had at least one case of scrapie in their herd in the previous six years, and 2.4% in the last year.

In Italy the disease has been reported since 1976 [8] and in 1991 the scrapie mandatory reporting was enforced. Since 1995 most of the Italian scrapie outbreaks has been involving sarda sheep, the main ovine Italian breed.

The aim of this work was to study the incidence of scrapie within the outbreaks detected through clinical reporting i.e. passive surveillance and affecting sarda sheep between 1995 and 2009.

Materials and methods

All farmers were interviewed, using a standardised questionnaire, including the number of sheep (classified into three age classes: < 2 years old, from 2 to 4 years old, >4 years old) and the number of animals showing scrapie-like symptoms observed during the last 12 months prior to confirmation of the disease in the flock. The incidence of scrapie was calculated as crude and age-specific rates both in the whole sarda sheep scrapie affected population and within each scrapie-affected flock. The association between scrapie and age class was quantified in terms of relative risk (RR) using the >4 years old age-class as reference. Moreover the year-specific age adjusted incidence was calculated to describe the secular trend.

Between 1995, year of the first scrapie confirmed case after the enforcement of the mandatory reporting system, and 2009, 495 scrapie outbreaks have been identified in Italy; 146 outbreaks were detected through passive surveillance and 131 farms completed the questionnaire (89.7%). Based on interviews, 96 per cent of farmers thought that they had had at least one case of scrapie in the 12 months before the survey.

Statistical analysis was carried out using the package Stata 10.1.

Results

The mean crude within-flock incidence was 2.06 cases per 100 animal-years (CI 95% 1.95-2.18); the data show a very skewed distribution (Figure 1) with high incidence affecting a small proportion of outbreaks.

The highest risk of developing scrapie (Table 1) was observed in the middle age class (2-4 years old) compared to the older animals.

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Figure 1: Within flock incidence density

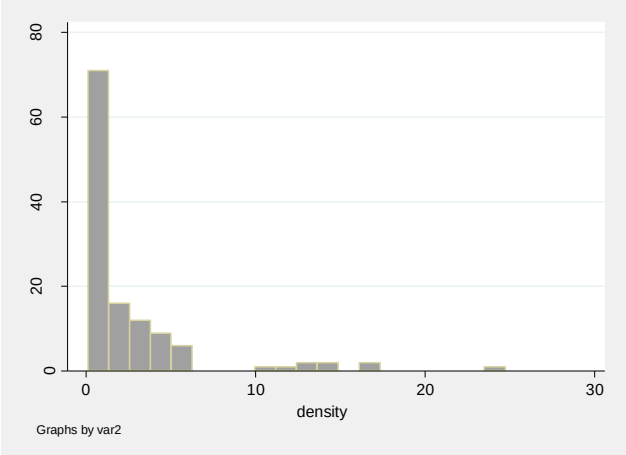
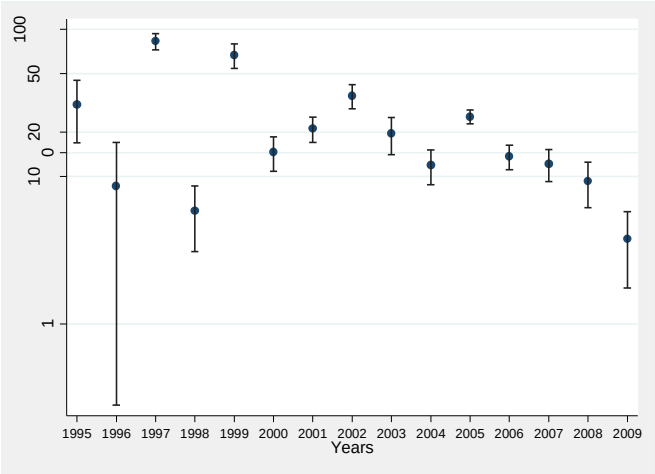


Table 1: Incidence Risk Ratio density by age

Age class	*RR*	*CI 95%*
< 2 years old	1.64	1.30 - 2.07
from 2 to 4 years old	7.23	5.93 - 8.83
>4 years old	1	

The age-adjusted incidence rates show a decreasing trend after 2002, the year of enforcing on the active surveillance paralleling the passive one; moreover during this period the incidence tend to be lower than the rates observed in the previous period (Figure 2).

Figure 2: Age-adjusted annual incidence



Discussion

As expected, in general most farms show low rates with the highest incidence affecting the 2 to 4 years old animals.

The higher risk in the younger animals could be a real risk of disease but might also reflect a major care of the farmers for this category.

The decreasing trend in the incidence deserves further studies: it may indicate a real phenomenon or rather may be a collateral effect of the improved awareness of farmers, leading to an anticipation of the recognition of the disease when the incidence is still very low. Moreover it may be a first signal of the effectiveness of the breeding program running in Italy. However the validity of our data, collected through oral interviews, may have been negatively influenced by the farmer's inability of recognising the disease and recalling the cases. Hopefully, the development of *intra vitam* tests will be helpful to better monitor the dynamics of the scrapie epidemics.

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A new concept: the animal epidemiology of defence

B. Davoust[1]*, N. Faure[2] and J.L. Marié[1]

Abstract
The animal epidemiology of defence is a new concept developed by French military veterinarians in order to prevent the transmission of zoonoses to humans and the dissemination of highly contagious animal diseases. This operational concept relies on a network coordinated by a special working group, bringing together laboratories capabilities and international experts. Data are provided by the traditional tools of epidemiology: monitoring, awareness and surveys, applied on all the animal populations (wild and domestic) living in the environment of French soldiers in continental France, overseas and abroad. Databases on animal diseases have been built and a bank of biological samples has been created. Since 2,000, this organization enabled us to collect and analyse more than 10,000 biological samples providing relevant information for the benefit of all.

Keywords: epidemiology, animals, army, emergences, zoonoses.

Introduction

Within an international context still marked by armed conflicts and the emergence of epidemics, the French military health service found useful to propose a new concept: the animal epidemiology of defence (AED). The purpose of this paper is to lay the foundations of this concept. We will first provide a definition before discussing the tools developed. Finally, some recent examples of actions led by French military veterinarians will illustrate the expertise they efficiently implement, usually on the field.

The animal epidemiology of defence is the scientific field referring to the descriptive epidemiology applied on animal populations. AED also includes the field of analytic epidemiology with the study of risk factors and etiological research. The animal populations involved are various: animals of the armed forces (dogs, horses, falcons), pets, synanthropic and wild animals living in the environment of the armed forces in France, overseas or abroad. The final aim of AED is to prevent the transmission of zoonoses and the dissemination of highly contagious animal diseases. Sometimes AED is also useful to preserve the national interests.

The strategic importance of EAD is demonstrated by the sanitary, economical, social and political repercussions of animal diseases or human diseases emerging from an animal reservoir. It is only a part of national capacities needed to assess the threats and to ensure the safety and preservation of French interests.

The AED is an area of increasing importance including the control of the biological environment of the battlefield, zoonotic emerging and re-emerging diseases, bioterrorism and epizootics. Armies have a major role to play when they are involved in animal health and public health in countries disrupted by war or disasters, where health and veterinary services can't play their role. Indeed, only one country with serious gaps in animal health is a major threat for the rest of the world.

Materials and methods

Network organization: A group working on animal epidemiology (AEWG) was created in 2006 by the Central Director of the French military health service; its aim was to fight against animal and human diseases through sanitary support of the armed forces. It consists of nine veterinarians, from various regions of the country, supervised by the veterinary service of Marseille. It brings together experts who have technical relationships with the other French military veterinarians (about 70). Their mission is to coordinate actions in animal epidemiology upstream of human or animal diseases. This preventive approach is based on the identification of animal reservoirs of pathogens and monitoring of vectors.

The AEWG advises the central military administration (veterinary office) on health risk assessment for troops, civilian population (public health) and military or civilian animals.

The activities include epidemiological surveillance of known infections as well as medical intelligence, to recognize emerging diseases. Epidemiological investigations are conducted in the field. The aim of these actions is to set up and implement measures to control known or potential zoonotic risks.

The military personnel is based in continental France or overseas (Guyana, West Indies, Reunion, New Caledonia, Polynesia) and abroad (Senegal, Gabon, Djibouti, Balkans, Ivory Coast, Chad, Lebanon and Afghanistan). Scientific collaborations exist with many experts in various fields, working in military and civilian laboratories. A network of reference laboratories has been established. Relationships are also maintained with French and foreign experts, working in different organizations, mostly in research centres, schools and universities. Information is also shared with infectiologists and epidemiologists and contributes to build bridges between scientific areas, according to the concept of one world, one health. I

Data collection: The methods used in the AED are those of epidemiology. A scanning device helps in identifying abnormal events that are transmitted to AEWG for evaluation. These exchanges must be

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very fast and are led by phone and confirmed by email. Within the AEWG, the evaluation of the transmitted signal is most often performed collectively, but some members with special expertise on pathogens can be consulted.

A monitoring of all diseases of military animals exists, based on an exhaustive computer record. These data are currently examined monthly but in the future, a server will allow a real-time monitoring. In addition, military animals are submitted to a yearly serological surveillance of canine monocytic ehrlichiosis, canine leishmaniasis and equine West Nile fever. However, early data collection is mainly based on epidemiological surveys, most often transversal. Prospective cohort studies are sometimes performed for a better health risk assessment.

Each action in the field of AED is the result of a work carried out according to different steps from the identification of the hazards to the implementation of preventive measures ("hazard analysis critical control point" method [HACCP]). In addition to the main analysis that motivated the sampling, other analyses can be realized to provide new information. Indeed, the reservoir species and the species susceptible to a given pathogen are not always well known and can change during the time.

For AEWG, a good sampling practice is essential: "good sample, at the right time, well stored". Analyses are made in field laboratories and more often in reference laboratories in France. In this context, biosecurity is of a major concern and under the responsibility of the different actors from sampling to analysis. The AEWG helps the other veterinarians and physicians in mission abroad, disseminating information, sending sampling equipment and organizing the reception of the samples.

Creation of databases: Since 2004, a unit of the French military health service based in the Epidemiology and Public Health Department of Marseille, works on health risk assessment. Health risk assessment is the collection, analysis and dissemination of health information about countries where armed forces are present and those where they could be sent in case of international crises, in order to identify and to prevent the potential medical risks. A veterinarian has joined the medical team to develop 2 databases, as it was done for humans: one documented database on the major animal diseases; the second on the zoosanitary situation of countries where the forces are present or could be sent. These data are available to all defence officers as well as practitioners and contribute to the dissemination of information. The results of the investigations led by the AEWG are included in these databases. The veterinarian of this unit also realises a daily event-scanning focusing on animal diseases and zoonoses, that is complementary to that performed by the medical team; it can eventually lead to an alert-triggering.

Expertise in case of emergency: In the case of an outbreak due to a zoonotic agent or in case of an epizootic, the French army can decide to use its veterinarian cells. These reactive structures are adapted to deal with animals (wild or domestic, living or dead), even if they are suspected of zoonotic or unknown infections, or likely to be asymptomatic carriers of pathogens (reservoirs). These structures can be deployed in war or disaster situations; they are equipped to collect (anytime, anywhere) and receive samples (blood, faeces, biopsy, parasites, *etc.*) of animal origin. They are mobile and can carry out epidemiological investigations in the field, concerning domestic animals in contact with ill animals, capture of rodents in the environment of a patient suffering from an emerging disease, sampling of animals slaughtered in an area of epidemiological interest, check-up of stray animals *etc.* Animals can also be used as sentinels of infectious agents circulating in the environment of a patient.

Management of a bank of biological samples: Recent experiences have shown the importance of providing retrospective information on health risks for military personnel (*e.g.* those submitted to atomic radiations). A veteran's health observatory was established to study the long-term effects of military activities on human health. In addition to longitudinal epidemiological surveys (cohort studies), retrospective serological detection in humans always provide interesting information. Meanwhile, the AEWG collects sera from animals and realizes retrospective sero-epidemiological research on these samples. For twenty years, a sera bank containing more than 10,000 sera has been built. Detection of antibodies in animals living close to militaries (dogs, horses, *etc.*) can show the circulation of pathogens. Bloodsucking parasites are also kept in the bank of biological samples. This practice could be extended to other types of animal samples (muscles, organs, *etc.*) to highlight radiobiological or chemical contaminations.

Civil-Military Cooperation: Civil-military cooperation (CIMIC NATO) is a new operational function of the armed forces. It means the integration of the force in the human environment in order to facilitate the mission and to help the reestablishment of a safe situation. This cooperation can deal with the gaps of the home country capabilities. In the area of surveillance and fight against animal or zoonotic diseases, military veterinarians can build a partnership with local institutions and act for the benefit of local populations (vaccination, deworming, epidemiological surveys, slaughtering of contagious animal, *etc.*). Nevertheless CIMIC should not be confused with humanitarian action.

Results

Plans for preventing the introduction of biological agents: The AEWG conducted a hazard analysis on the possible introduction in France of infectious agents, when troops are returning home. The AEWG proposed to the command the implementation of corrective measures (control plan) and preventive measures (risk management). They regularly assess the effectiveness of these measures and the appropriateness of the

guidelines (disinfection, disinfestation, rodent control, sanitary education). Procedures and instructions are also available within the framework of quality management applied to veterinary actions. The disease control operational plans are submitted to the Ministry of Agriculture, in charge of sanitary control of imports of living animals and animal products. A memorandum of understanding was signed between the veterinary services of the State and those of the army, to set rules for their collaboration and formalize the exchange of information.

Field Investigations: Since 2,000 a lot of epidemiological investigations were undertaken. The statistical report shows that over 10,000 biological samples (blood, faeces, organs, muscles, ectoparasites, *etc.*) were collected and resulted in over 20,000 screening tests for nearly 70 viral, bacterial or fungal infections and parasitic diseases. The samples were collected on about 50 vertebrate species (1/3 domestic and 2/3 wild) in 25 regions of the world. The main analytical techniques implemented are culture, serology, molecular biology, microscopic observation and morphological or genetic diagnosis of ectoparasite species.

Some examples of field studies are now reported. In Mitrovica, Kosovo, in collaboration with a local veterinarian, we were able to demonstrate the presence of *Trichinella britovi* in stray dogs, *Bartonella schoenbuchensis* and Crimean-Congo hemorrhagic fever antibodies in cattle, toxoplasmosis in sheep and *Anaplasma phagocytophilum* in dogs. In French Guiana, where severe cases of neotropical toxoplasmosis occurred among military training in deep forest, an investigation was carried out on bushmeat. *Toxoplasma gondii* seroprevalence estimated from muscle fluids was high (30%) particularly in peccaries. Prevention was implemented recommending sufficient cooking of meat and water filtration. In this French overseas territory, we detected *Trypanosoma cruzi* infection (agent of Chagas disease) in dogs. It encouraged us to continue monitoring dogs, considered as good sentinels of this serious human disease. Rift Valley Fever is a major zoonotic risk in sub-Saharan Africa. We conducted seroprevalence surveys in Chad where we demonstrated a recent circulation of the virus enabling the local veterinary authorities to report the disease to the World Organization for Animal Health (OIE). In Ivory Coast (Abidjan), cases of canine trypanosomiasis (*Trypanosoma congolense*) have been observed and studied by molecular biology. Our study has alerted physicians, because the tsetse fly is the same vector as that of human trypanosomiasis. In Afghanistan, in addition to the CIMIC realized when the security allows it, we conducted epidemiological surveys that have shown a circulation of zoonotic agents borne by rodent fleas, including *Bartonella quintana*, responsible for trench's fever. In Lebanon, we participated to the first molecular detection of *Bartonella henselae* (cat scratch disease) and *Rickettsia felis* (flea borne spotted fever) in fleas collected on stray cats.

Discussion

The animal epidemiology of defence is a concept that gradually became established and structured. The information gathered relates to situations that can be directly or indirectly of military interest and useful in the conception and the implementation of the military strategy [1]. The military veterinarian must trigger the alert when he thinks that a sanitary situation in the military environment could represent a threat. Within his area of competence, he advises the command and provides measures to be taken to protect the forces and more generally preserve human and animal health. The production of epidemiological information requires active screening, sometimes carried out without the cooperation of the local players. It is based on the detection, without any *a priori*, of already known or emerging pathogens in animals but also in bloodsucking arthropods [2]. Specific studies are part of the response to the needs identified by the command or health experts. They are always based on risk assessment; for example the probability of emergence and spread of a zoonotic disease with an international impact (simian retroviruses, SARS, West Nile, H5N1avian influenza *etc.*) [3, 4]. As military dogs are also often sent to missions abroad, they are likely to introduce potentially infectious agents from one country to another [5].

In the future, the risk of human exposure to zoonotic pathogens is expected to grow as human-animal interactions are more frequent, especially in countries whose population is rising sharply. The approach of the French military veterinarians already offers, at a small scale, a new tool of prevention and fight against animal diseases and zoonoses. Our suggestions have recently been studied by a NATO group. It would also be desirable if military veterinarians worked with military medical epidemiologists in the new NATO health surveillance centre, located in Munich. At an even more global scale, the International Committee of Military Medicine (veterinary section) has signed an agreement with the World Organization for Animal Health (OIE) to facilitate the exchange of information, particularly epidemiological, between military and civilian veterinary services. The importance of AED will grow in the future and the missions will provide sustainability of military veterinarians positions, especially if they know how to deal with today's challenges.

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Comparison of different strategies for the surveillance and control of classical swine fever – results of a simulation model

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Abstract

Up-to-date knowledge on epidemiological aspects of infectious diseases is needed in order to make a comprehensive policy decision about surveillance and control strategies. In the presented study we used a spatial, stochastic simulation model based on contacts between premises to estimate the effect of several parameters on an outbreak of classical swine fever (CSF) in Switzerland. Based on the model results, the size of an outbreak depends mainly on the type of index premises and a rapid detection of the disease, and less on vaccination or pre-emptive culling, in addition to basic control strategies (rapid culling of infected herds and contact herds and establishing of movement restriction zones). The knowledge gained in this project provides a basis for the choice of surveillance and control strategies for CSF and serves to make animal disease control more efficient in Switzerland.

Keywords: classical swine fever, epidemiological model, decision-making policy, Switzerland.

Introduction

Classical swine fever (CSF) caused by the *pestivirus* classical swine fever virus (CSFV) has a high morbidity and mortality in infected herds depending on virus strain, immune status of the herd and age of the pigs [1]. The disease occurs worldwide but has been eradicated in most countries in Europe. However, recent outbreaks were detected in Eastern Europe and in Western Europe the disease was found in wild boar in France until 2007 and in Germany until 2009. In Switzerland, the last outbreaks were detected and successfully managed in 1993 in domestic pigs [2] and in 1998-2000 in wild boar [3].

CSF is one of the most highly contagious animal diseases and declared as such in the Swiss legislation on animal epidemics. In case of an outbreak, the prescribed control strategy by Swiss law consists of culling of all animals of infected herds and movement restrictions for animals, material and persons of premises within a distance of the infected herd. The same policy is applied by the European Union legislation (EU legislation 2001/89/EC). However, in high pig-density areas disease eradication may require several months as described for the CSF outbreak in the Netherlands (NL) in 1997-1998 [4]. Due to movement restrictions, additional animals need to be slaughtered and destroyed for welfare reasons as their finishing weight is reached and space gets too small to keep them longer. Additional measures can be applied on top of these basic control measures, such as pre-emptive culling of animals in the vicinity of an infected premises or emergency vaccination. However, pre-emptive culling may enhance ethical problems of mass culling of healthy animals and vaccination impedes the determination of virus free pig populations after the outbreak. A marker vaccine and diagnostic test differing between vaccinated and infected animals (DIVA) are under development but so far not yet available on the market.

CSF outbreaks can cause enormous losses in a naive population. To control the outbreak in 1997 in the NL, which lasted for 15 months, a total of 11 million pigs were culled or slaughtered and destroyed and an economical loss of US$ 2.3 billion was reported [5, 6]. Pig farm and animal density in the affected area was very high, with 2500-3000 pigs per km^2 with about 1000 pigs per farm [5]. This is not comparable with Switzerland where, even in the high production regions, we have a ten-fold lower pig density, and substantially smaller farms. A Swiss-specific analysis of the epidemiological situation in case of a CSF outbreak is thus needed to provide a basis for decision-making for the surveillance and control of the disease. In the present study a spatial, stochastic disease transmission model was implemented to explore the circumstances causing an above average number of CSF outbreaks during a potential epidemic and to compare different strategies controlling CSF in Switzerland.

Materials and methods

The DADS (Davis Animal Disease Simulation) model was developed and published in 2001 for the simulation of food-and-mouth disease (FMD) outbreaks in California, USA [7,8]. The model is programmed in R (http://www.r-project.org/) and is based on direct and indirect contact probabilities between different types of premises within a given area. Generally, it can be adapted to any highly contagious disease and region as long as data on the susceptible units (*e.g.* exact location, size, susceptibility of herds), the agent (*e.g.* incubation time, infectiousness) and surveillance and control measurements (*e.g.* time to detect the disease, tracing parameters, movement restriction efficacy) are available. To populate the model data from all officially recorded Swiss pig premises from 2010 (n=9759), including their coordinates and herd sizes were used. Four different premises types were defined: breeding premises (n=1231), premises with only weaning piglets (n=168), fattening premises (n=6042) and mixed premises containing fattening pigs and sows (n=2318). Daily shipment rates and sizes were allocated to each premises based on animal shipment data from the Swiss Pig Health Service. This dataset contains origin, destination, and shipment size of all shipments

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that were sent over the time course of four years from a subset (approx. 20-35%) of all Swiss pig premises. The DADS model used actual shipment sizes and frequencies for premises that were included in this data set and sampled values from premises of the same size and herd type for all other premises. The probabilities of direct contacts (shipments) between the four herd types and their distances were calculated from the same data set. Values of indirect contacts between the premises (*e.g.* by veterinarians, lorries, visitors) were taken from former models on FMD in Switzerland [9]. Virus characteristics were mainly taken from several publications of the CSF outbreak in the Netherlands in 1997 [10-14]. Seven control strategies were defined (Table 1). Most of the parameters in the model are implemented as distributions.

Besides the control strategy, three parameters varied between simulations: type of the index premises (4 levels, see above), detection delay for each new infected herd after detection of CSF in Switzerland (2 levels, 6 and 16 days) and a network parameter, which reflects the probability of recording direct contacts and applying measurement to all premises of the network built by these contacts (2 levels, 0.6 and 0.9). Each of the resulting 112 different scenarios was run for 300 iterations with a time frame of 200 days.

Table 1: Definition of the 7 control strategies

Abbr.	Strategy	description
BS	Baseline strategy	Culling of all pigs in infected and detected contact premises; restriction of direct and indirect contacts within a surveillance zone of 10km and an infected area of 3km
C1	culling 1km	In addition to the BS measurements, culling of all pigs in premises within 1km of the infected premises
V1	Vaccination 1km	In addition to the BS measurements, vaccination of all pigs in premises within 1km of the infected premises
V2	Vaccination 2km	In addition to the BS measurements, vaccination of all pigs in premises within 2km of the infected premises
V5	Vaccination 5km	In addition to the BS measurements, vaccination of all pigs in premises within 5km of the infected premises
C1V2	Culling 1km and vaccination 2km	In addition to the BS measurements, culling of all pigs in premises within 1km and vaccination of those within a 2km of the infected premises
C1V5	Culling 1km and vaccination 5km	In addition to the BS measurements, culling of all pigs in premises within 1km and vaccination of those within 5km of the infected premises

We compared the alternative scenarios based on whether each single simulation resulted in the following: an outbreak with more infected or culled premises than the 75 percentile of all scenarios; an abortive outbreak (no other infected premises than the index herd); an outbreak of less than 40 days, and; an outbreak with a longer duration than the 75 percentile of all scenarios. The association of the four independent variables (control strategy, type of index premises, length of detection delay and network probability) on the outcomes were tested by a logistic regression analysis examining up to 2-way interactions. The level of significance was set to 0.05.

Result

A significant influence of the control strategies was detected only for the number of infected or culled premises (Table 2). This can be explained by the additional pre-emptive culling and was thus expected. A longer detection delay of 16 days (compared to 6 days) resulted in larger (more infected or culled premises) and more long-lasting outbreaks. If the outbreak started in a premises with weaning piglets or fattening pigs the outbreaks were longer and resulted in fewer abortive outbreaks; when they started in weaning piglet premises they in addition were bigger in losses. Mixed premises as index herd resulted in outbreaks with smaller losses. A higher network probability of 0.9 (compared to 0.6) led to more short outbreaks (less than 40 days). Several interactions of the examined parameters were detected (Table 2).

Table 2: P-value and odds ratio (OR) of the significant parameter main effects per outcome; baseline of the strategy was the basic strategy (BS), of the detection delay 6 days, of the network probability 0.6, and of the index premises type breeding herds; *Wald probability level

Outcome	p value*	OR
outbreaks with more infected or culled premises than the 75 percentile of all scenarios		
strategy		
C1	<0.0001	35.85
V2C1	<0.0001	31.93
V5C1	<0.0001	33.11
detection delay 16	<0.0001	3.89
type of index premises		
mixed premises	0.0481	0.57
weaning piglets	<0.0001	8.58
Interactions		
C1 * detection delay 16	<0.0001	0.30
V2C1 * detection delay 16	<0.0001	0.29
V5 * detection delay 16	0.0039	0.59
V5C1 * detection delay 16	<0.0001	0.28
C1 * index premises mixed	0.0241	1.91
V2C1 * index premises mixed	0.0099	2.09
V5C1 * index premises mixed	0.0121	2.05
abortive outbreak (no secondary infected premises)		
type of index premises		
fattening pigs	<0.0001	0.60
weaning piglets	<0.0001	0.09
Interactions		
V2 * detection delay 16	0.0004	1.40
outbreaks shorter than 40 days		
network parameter 0.9	0.0297	1.19
type of index premises		
fattening pigs	<0.0001	0.56
weaning piglets	<0.0001	0.08
Interactions		
V2 * detection delay 16	0.0026	1.33
detection delay 16 * index premises weaning piglets	0.0422	0.79
outbreaks longer than 75 percentile of all iterations		
detection delay 16	<0.0001	5.46
type of index premises		
weaning piglets	<0.0001	3.39
Interactions		
detection delay 16 * index premises weaning piglets	<0.0001	1.63

Discussion

Based on the simulations of CSF outbreaks in Switzerland, four main results can be pointed out from this study. First, the size (number of infected or culled premises) and the duration of the outbreaks depend on the type of index premises. When the index case was in either a premises with weaning or fattening pigs, outbreaks were of longer duration and larger in losses. Outbreaks starting in these two premises types also resulted in more effective (i.e. transmission of the disease) indirect contacts and although the direct contacts were rarer in those outbreaks the total sum of effective contacts was significantly larger. The relatively high within herd spread of CSF in premises with weaning or fattening pigs might explain this effect of high effectiveness of indirect contacts. Second, delayed detection of secondary outbreaks resulted in longer and larger outbreaks. Third, an effective tracing network was only associated with more short-lasting outbreaks but not to extreme outbreaks in either the duration or size. Fourth, vaccination or pre-emptive culling strategies applied in addition to the baseline strategy (BS in Table 1) was of no additional benefit under the model assumptions and strategies examined here.

The knowledge gained in this project provides a basis for the choice of the surveillance and control strategy for CSF and, therefore, serves to improve the efficacy of animal disease control in Switzerland. Specifically, regarding surveillance, early detection of outbreaks and thus good disease awareness are of crucial importance, particularly in herds with weaning piglets or fattening pigs.

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Evaluation of Bluetongue surveillance in Germany

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Abstract
In August 2006, Bluetongue disease (BT) occurred for the first time in Western and Central Europe. To comply with regulation (EC) No 1266/2007, Germany established a sentinel program in cattle and carried out a cross-sectional study in winter 2007. We analyzed the suitability of both the sentinel program and the cross-sectional study and its benefit for BT control, taking the officially reported BT outbreaks into consideration. The evaluation shows that particularly the sentinel program was time-consuming and expensive.

Keywords: bluetongue disease, Germany, surveillance system.

Introduction
In August 2006, Bluetongue disease (BT) occurred for the first time in Western and Central Europe. To improve the understanding of the epidemiological situation and to establish proportionate measures, a working document was developed by the European Commission, DG-SANCO, with guidelines for a harmonized approach to monitor BT in restricted zones and for surveillance in non-restricted zones adjacent to restricted zones (SANCO/10581/2006 Rev 4). This working document was the basis for Commission regulation (EC) No 1266/2007, which has been amended several times. The aims of the monitoring and surveillance systems were to detect the introduction of new BT serotypes and to demonstrate the absence of certain serotypes. Other objectives may include the demonstration of the absence of BT virus circulation. Furthermore, they are necessary tools for exempting certain animals of susceptible species from the exit ban.

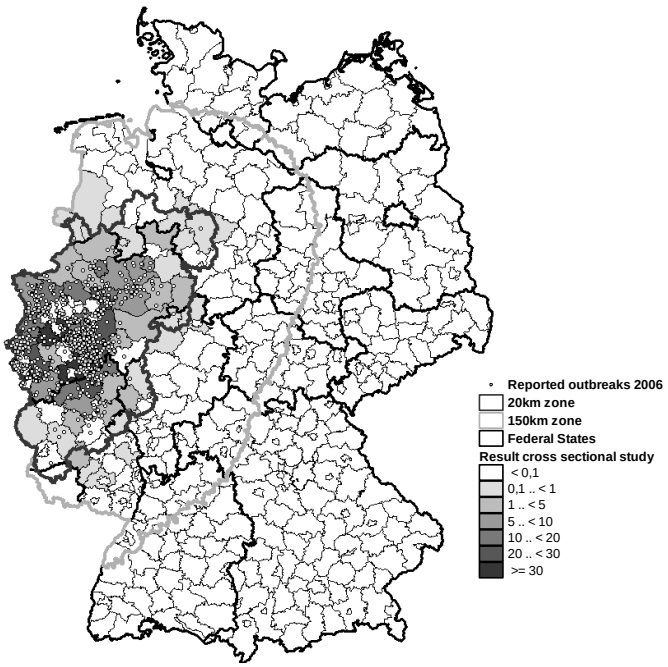
To comply with the regulation, the following activities were carried out in cattle in Germany:

1. Cross-sectional study in winter 2007 in all districts in the 150 km zone and in the surrounding districts to get information about the spread of the disease in 2006,
2. Sentinel program to detect the re-occurrence of BT. About 150 animals in 10 to 15 farms were tested monthly serologically in each federal state. After the occurrence of the first positive test result in May 2007, the sentinel program was stopped.
3. Vector monitoring in the restricted areas.

For disease control, a 20 km and a 150 km zone around the outbreaks were established. All affected premises had to treat their animals with insecticides. Before animals could be moved from the 20 km zone into the 150 km zone or from the 150 km zone to free zones, they had to be tested with a negative result for BT and treated with insecticides.

Materials and methods
For the analysis of the cross-sectional study and the sentinel program, we used data provided by the federal states, the German animal disease notification system (TSN, TierSeuchenNachrichten) to estimate the time point when the desired detection level of the sentinel program was reached. The surveillance system was analyzed and the detection level of the sentinel program in Germany was calculated for a two staged design.

Results
1. The cross-sectional study conducted in winter 2007 showed that the prevalence on district level had been up to 66% at the end of the transmission season 2006. It also showed that only a small fraction of the infected farms and animals had been reported. Only in a few districts, cattle with positive test results were found outside the 20 km zone [1].



2. It seems that the sentinel program was sensitive as it detected the first new BT case in 2007. However, implementing the program was very time-consuming and expensive. For several reasons animals could not be sampled randomly and in the selected herds the number of tested animals was between 10 and 15. This two staged design affected the detection level of the program. Instead of the desired 2%, the detection level rose to 2.0-6.7%. Furthermore, many farmers were reluctant to participate in the sentinel program because they were not allowed to sell sentinel animals and suspected that the monthly testing might affect the performance of the animals.

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Discussion

The cross-sectional study conducted in winter 2007 provided comprehensive information on the distribution of BT in cattle at the end of the transmission season of the year 2006 in the affected region in Western Germany. In winter 2008, no cross-sectional study was carried out to get information about the spread of bluetongue disease in 2007 but reports from the mainly affected federal states indicated that the prevalence at the end of 2007 had been nearly 100% in the core region of the epidemic.

Since the program had to be adapted for the benefit of practicability, it failed to achieve the desired detection level but succeeded to detected the first fresh BT case in Germany in 2007. The overall cost-benefit ratio of the sentinel program was poor.

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We thank the German federal states for providing the data of the cross-sectional study.

A sero-surveillance programme for Early detection of Low Pathogenic Avian Influenza outbreaks in layer chickens

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Abstract
We developed a model for the design and evaluation of serological-surveillance programmes aiming at early detection of Low Pathogenic Avian Influenza infections in layer chickens. This model takes into account the within and between flock infection dynamics and investigates the required sample size and sampling frequency for early detection. The use of eggs instead of sera as samples is also evaluated.

Keywords: early detection, Avian Influenza, transmission, surveillance.

Introduction

Low pathogenic avian influenza viruses (LPAIv) of the H5 and H7 subtypes are able to mutate to highly pathogenic avian influenza virus (HPAI), with increased virulence [1, 4] and transmissibility [5] in poultry. Hence, they are notifiable to the OIE and Member States (MS) of the European Union (EU) have implemented serological surveillance programmes. These programmes are carried out (in most MS) yearly and in the event of detection of infected – H5 or H7 – farms, eradication measures are implemented [6]. However, despite this programme and the apparent low prevalence of seropositive farms in the EU [7], mutations from LPAI to HPAI virus did occur [4]. To date, it is not possible to predict this mutation rate. They could already take place in the first infected farm [4, 8] or after transmission of the LPAI virus to several other farms [3, 9]. Therefore, implementing surveillance programmes that target early detection of LPAI outbreaks, which probably won't be detected by passive surveillance, will reduce the probabilities of virulence mutations and consequent animal health, welfare, and economic consequences.

In the Netherlands, layer chickens – in particular free-range layers – are expected to have the highest risk of becoming infected with AIv [10, 11]. Hence, aiming at early detection, all of these farms are serum-sampled every 90 days [12]. Given this scenario of frequent sampling, there is the opportunity to use eggs instead of sera as a sample commodity for serological testing. The objective of this study was to develop a model for the design and evaluation of surveillance programmes for early detection of LPAI infections in layer chicken farms. The model takes into account the within-flock infection dynamics, which determines both the prevalence of infected animals in the flock and the performance of the diagnostic test used for surveillance. We compare sampling eggs to sampling sera for serological testing.

Materials and methods

First we analyse the within flock infection dynamics using a deterministic SEIR (susceptible – exposed – infectious – recovered) model [13]. The prevalence of infectious birds ($I(t)$), from the start of the epidemic to its end, is used to estimate the expected infection pressure of an infectious flock to other flocks. This expected infectiousness times a constant "c" [14] defines the expected number of secondary infected farms, a primary infectious farm makes during its whole infectious period (the whole duration of the within-flock epidemic) in a susceptible population. This expectation is the between-farm reproduction ratio.

The prevalence of recovered (sero-converting against LPAI) birds ($R(t)$) in the SEIR dynamics is used to estimate the probability of escaping detection. This probability depends on: (i) the prevalence of seroconverting chickens at the time of sampling, (ii) the sensitivity of the test at the animal level , (iii) the number of chickens sampled (sample size) and (iv) the sampling frequency (in days). This probability is used to estimate the expected infection pressure of an infectious flock up to the time of detection and consequent culling (end of high risk period, HRP). Next, we use this expected infectiousness to estimate the between farm reproduction radio when surveillance is performed. Since the objective of this model is early detection, we aim at detecting an outbreak (single infected farm) before it infects on average more than one other farm (between farm reproduction ratio < 1). In the model we work with a typical farm as one with 20000 layer chickens and assume a similar transmission pattern for other (layer) farms. We applied the model to evaluate the current Dutch sero-surveillance programme. The parameters' values used in this model were obtained from chicken to chicken transmission experiments [Gonzales *et al.* in preparation] and literature [15].

Results

The overall infection pressure of a flock affected with a LPAI virus in the absence of surveillance was considerable. Despite the infection being low pathogenic, another 2 or 3 farms would get infected before the end of the HRP. If surveillance would be performed and 10 serum samples/flock would be sampled every 30 days, the between farm reproduction ratio would be lower than 1. If sample size is increased to 30 samples, every flock needs to be sampled every 90 days, which is the current surveillance programme

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in the Netherlands. If eggs would be sampled instead of serum, only sampling 30 eggs/per flock every 30 days would keep the between farm reproduction ratio below 1.

Discussion

The results of this study show, given the parameters' values here used, that the current surveillance programme in The Netherlands can sufficiently reduce the probability of between farm transmission to a restricted size LPAI epidemic. The use of egg-samples instead of sera may be a good alternative. An egg based programme would require a higher frequency of sampling than serum-samples. Sampling with this higher frequency would not be a problem, since eggs can be collected from packaging units or directly sent to the lab by the farmers. Hence, the use of egg samples not only enhances animal welfare but also reduces the costs in the logistics and biosecurity risks involved in individual animal sampling. Whether one should sample eggs or sera in a surveillance programme could be better decided by a cost benefit analysis comparing these two options.

In conclusion, this model, which takes into account the infection dynamics, can be used to define the required sample size and frequency of sampling when designing a sero-surveillance programme for early detection of LPAI or evaluate surveillance programmes already in place.

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Surveillance data to compare rapid tests for atypical Scrapie

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Abstract
In Italy in the frame of active surveillance of Scrapie six different rapid tests have been used leading to the identification of both classical and atypical Scrapie cases. Despite all the 69 Italian atypical cases (found mostly in sheep) were identified by one rapid test (Biorad TESeE), the assumption that the different diagnostic kits used in surveillance of TSEs were equally able to identify the atypical form was investigated. In particular, we wanted to check whether the number of animals tested by some of the tests could have been so small as to be consistent with the lack of identified cases of the disease, assuming equal diagnostic sensitivity. Using regression models to compare each test with Biorad TESeE, only the small number of IDEXX tests carried out is consistent with the identification of zero AS cases.

Keywords: atypical Scrapie, rapid test, multivariate logistic models

Introduction
The official diagnostic laboratories belonging to the Scrapie surveillance network, can use rapid tests approved and listed in the European legislation. The list is consistent with the results of studies conducted by EFSA which examined the diagnostic effectiveness to detect Scrapie in general and the atypical form in particular [1, 2, 3]. Then, the choice of the rapid test was mainly based on commercial criteria or related to the test's practicabilities. A certain degree of turnover in the type of test used was recorded in European labs, leading to the employment of test able to identify atypical Scrapie [4].

From 1st January 2005, year of the first atypical Scrapie case (hereinafter abbreviated with AS) identified in Italy, to 31th December 2008, 69 AS cases were found out of more than 314,000 rapid tests (Table 1): all AS cases were detected by the Biorad TESeE rapid test, (hereinafter abbreviated with TESEE) while sheep and goats were also tested by other five different tests: the Prionics Check Western (hereinafter abbreviated with PRIO), the ENFER, the Prionics Check LIA (hereinafter abbreviated with LIA), the IDEXX and the Prionics sheep and goat Western Blot (hereinafter abbreviated with WBPRION).

Aim of this study was to verify the assumption that the different rapid diagnostic tests used in active surveillance of TSEs were equally able to identify AS cases. In particular, we wanted to check whether, with a predetermined statistical confidence level, the number of animals examined for some of the kits used could have been so small as to be consistent with the lack of AS cases identified with the same diagnostic sensitivity.

Materials and methods
The statistical analysis was conducted using the database provided by the active surveillance of Scrapie performed between 2005 and 2008.

Table 1: Number of TSE rapid tests performed and AS cases carried out during 01/01/2005-31/12/2008.

Half-year	PRIO	ENFER	TESEE	LIA	IDEXX	WB PRION	AS Cases
2005h1	1,929	5,743	14,633	1,614			4
2005h2	71	8,167	20,371	2,800			7
2006h1		5,422	19,687	3,773			9
2006h2		15,187	38,933	6,192			13
2007h1		16,698	49,395	7,495			17
2007h2		9,571	32,672	2,897	2,070		9
2008h1		2,657	19,025	1,073	1,999	4,866	5
2008h2		36	4,897	1,218		13,722	5
Total	2,000	63,481	199,622	27,062	4,069	18,588	69

The basic idea of the study is: if TESEE had the same ability to identify AS cases of the other rapid tests then the absence of infected animals where other rapid tests have been used, should correspond to the absence of AS cases. Estimating the number of AS based on the expected number of tests conducted by type of test, the same diagnostic sensitivity should lead to consistent estimates with 0 AS cases identified, and then with confidence intervals including zero.

Five multivariate logistic models were fit to estimate the expected number of AS cases that would have been detected with the different rapid tests used in Italy during the period of study. The first step was to fit the models only to the sheep and goats tested with TESEE; then, the coefficient obtained from the models was used to estimate the expected number of AS cases for each of the other rapid tests on the basis of the number of examination performed.

In order to control for confounders, we included in the models the following variables:

- Category (hereinafter abbreviated with categ): healthy slaughtered, fallen stock, culled for reason not related with TSE.
- In the model animals culled within the outbreaks were not included: these animals might not present the same risk of AS. Therefore, we excluded 31,608 tested animals, including 6 AS cases.

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- age class (hereinafter referred to as classeta): <18m, 18-35m, 36-59m , 60-95m, ≥ 96m.
- year of testing (year).
- Region of the farm (Region).

The dependent variable was the presence or absence of AS. In order to take into account unmeasurable factors related to the geographical distribution of tested animals, the Region was considered as a clustering variable. Then random-effect models were fit.

Therefore the we fit the following models:

M1) it includes all the Regions: ordinary least squares (OLS) robust variance , with Region as cluster-variable and categ, classeta, and year as covariates;

M2) it includes all the Regions: two-levels hierarchical model (animal, Region) random effect model, with categ, classeta, and year as covariates;

M3) it includes only the Regions in which at least one case of AS was identified: OLS robust variance, with Region as cluster-variable and categ, classeta, and year as covariates;

M4) it includes only the Regions in which at least one case of AS was identified: two-levels hierarchical model (animal, Region) random effect model, with categ, classeta, and year as covariates;

M5) it includes only the Sardinia Region (in order to maximize the control of confounding variables): OLS robust variance with categ, classeta and year as covariates.

Since our interest was mainly focused on the homogeneity of the effect of the variable Region rather than on the direct action of Regions about the estimate of the AS expected cases, neither fixed effects nor OLS robust variance estimation regression models were fit.

Finally, in order to increase the level of confidence of the results, we calculated both 95% and 99% confidence intervals (CIs) of AS cases (expected).

Results

The estimate of expected AS cases, with 95% and 99% lower (lb) and upper bounds (ub), are reported in Table 2: all the rapid tests showed both point estimates and lower bounds of the CI for the expected AS cases greater than zero. Only the IDEXX test showed zero estimates obtained from models 3 and 4. Excluding TESEE, in the first and second model, ENFER presented the largest number of expected AS , followed by LIA and WBPRION. The ranks changed in models 3 and 4 (WBPRION first, LIA second) ; ENFER didn't appeared because it was used only in Sicily, where none AS cases were identified. Also the model 5, fit with animals tested only in Sardinia, led to both the point estimate and the lower limits greater than zero.

Also results with the confidence level set to 99% (thus allowing only 1% probability of making a mistake) were consistent: no AS cases detected by tests other than TESEE.

Table 2: Summary of the estimates obtained by the fitted models

Model	Rapid test	AS expected cases				
		value	95%lb	95%ub	99%lb	99%ub
M1	PRIO	0.7	0.3	1.7	0.3	2.4
	ENFER	17.8	9.9	34.3	8.3	42.8
	TESEE	63.0	33.9	127.3	28.2	162.5
	LIA	10.3	5.6	20.9	4.7	26.9
	IDEXX	1.2	0.6	2.6	0.5	3.3
	WBPRION	6.4	3.2	13.5	2.6	17.4
	Total	99.4	53.5	200.3	44.6	255.3
M2	PRIO	0.7	0.3	1.5	0.3	1.9
	ENFER	17.5	9.7	32.6	8.1	39.9
	TESEE	61.9	34.0	115.6	28.3	141.5
	LIA	10.1	5.6	18.8	4.6	23.0
	IDEXX	1.2	0.6	2.3	0.5	2.9
	WBPRION	6.1	3.0	12.6	2.4	16.0
	Total	97.5	53.2	183.4	44.2	225.2
M3	PRIO	0.1	0.1	0.4	0.0	0.5
	ENFER					
	TESEE	62.0	32.8	128.6	27.3	165.8
	LIA	1.6	0.9	2.8	0.8	3.4
	IDEXX	0.0	0.0	0.1	0.0	0.1
	WBPRION	3.9	1.9	8.7	1.5	11.3
	Total	67.6	35.7	140.6	29.6	181.1
M4	PRIO	0.1	0.1	0.3	0.1	0.4
	ENFER					
	TESEE	61.9	34.0	115.8	28.3	141.8
	LIA	1.6	0.9	2.7	0.8	3.2
	IDEXX	0.0	0.0	0.1	0.0	0.1
	WBPRION	3.8	1.8	8.0	1.5	10.2
	Total	67.4	36.8	126.9	30.7	155.7
M5	PRIO					
	ENFER					
	TESEE	11.0	4.0	36.9	3.0	57.1
	LIA					
	IDEXX					
	WBPRION	1.0	0.2	5.6	0.1	9.9
	Total	12.0	4.2	42.5	3.1	67.0

Discussion

To summarize:

a) problems in identification of AS cases emerge for rapid tests other than TESEE. The small number of test performed could justifies the lack of AS cases only for IDEXX, which showed both 95% and 99% lb of CIs equal to zero;

b) the results cannot be accounted by the effect of confounders as they were included in models as covariates (categ, classeta, year and Region) or a restricted design (as in M5) was applied;

c) the 95% and 99% confidence levels assure that difference between the expected and observed number of AS cases by rapid tests other than TESEE is not due to the chance only;

d) removing from the study the animals culled within the outbreaks prevents from a possible selection bias and however the 6 AS cases reported in this stream of the population were all identified by TESEE.

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Space-time clustering of mortality notifications in Pacific oysters of Charente sluices, France, 2008-2010

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Abstract

Current monitoring of exceptional mortality events of oysters in France consists in passive surveillance, leading to delayed investigation of causation by scientists and management by policy makers. Early detection of clusters of mortality events in time and space would increase possibilities for their investigation and management.

A space-time cluster analysis of existing mortality notifications in Pacific oysters was conducted in France, Charente sluices, from 2008 to 2010. As an example of potential demographic confounder of clustering, the analyses were adjusted on oyster age. Several clusters were identified in space and time each studied year. Patterns of mortality notifications were consistent with the introduction and spread of a contagious disease in 2008, which may have become endemic since 2009. Adjustment on oyster age did not change patterns of clusters, suggesting that other variables should be considered in risk factor analyses for mass mortality, either at animal or at environmental level.

The routine space-time cluster analysis would be a useful tool to early detect unusual mortality events, with both objectives to improve the epidemiological understanding of the event and to implement timely and efficient control measures. The evolution of the French surveillance strategy from a passive to such a cluster-based surveillance system may be conceivable, if full collaboration of all stakeholders could be achieved.

Keywords: notification, oyster, mortality, cluster analysis, spatiotemporal.

Introduction

According to current EU regulation [1], current monitoring of exceptional mortality events of molluscs in France consists mainly in passive surveillance, based on the observation of increased mortality by shellfish farmers and its notification to the local competent authority. The national surveillance network of molluscs diseases (REPAMO) is then involved when mortality occurs, consisting in anamnesis and biological samples for laboratory diagnosis [2].

Since three years, mass mortality has occurred among Pacific oysters (*Crassostrea gigas*) in France. An atypical pattern in space (from north to south) and time was previously described for the mortality cases which were notified in 2008 [3]. In addition, the REPAMO detected unusual pathogenic agents in samples collected during mortality events since 2008. An infectious implication in the mass mortality of oysters was thus suspected. Policy measures to control the spread of a potential disease have included restriction to oyster movements in 2009 [4] and 2010 [5].

Assessing the space-time distribution of a health event and its association with epidemiological or environmental factors is one of the uses of surveillance data. To identify unusual occurrences of health events that happen close together in either time and/or space, formalized methods can be applied such as space-time cluster analysis [6]. If a disease process is infectious, proximity of cases in both space and time is likely to occur. Space-time cluster analysis has become a standard tool in human chronic disease surveillance in France [7] such as cancer and its use is increasing in animal infectious disease surveillance such as raccoon rabies in the United States [8] among others.

Implementing routine space-time cluster analysis would help all animal health stakeholders, either to identify causes of disease or to suggest effective methods of control. From a scientific standpoint, routine space-time cluster analysis of mortality notifications would help scientists to test hypotheses regarding disease causation and a large of putative causal factors as confounders for clusters. Here the mass mortality mainly affected the spat and juvenile oysters [3]) and the age distribution of the oyster population is defined by the spatial arrangement of the leasing grounds. Therefore, cluster analysis would account for the demographic structure of oyster populations. In addition to improve the understanding of the disease pattern, cluster analysis may help to focus complementary survey efforts or to enhance the surveillance on identified clusters. From a policy standpoint, routine identification of space-time clusters may help to target control measures and to manage the policy resource allocation on identified clusters.

Mortality notification data of molluscs are not analyzed on a routinely basis in France. A retrospective epidemiological analysis of the mortality notifications was thus conducted to describe the mortality notifications of one of the main French oyster production area (Charente Maritime) since 2008, using a space-time cluster investigation.

Materials and methods

Data origin: Data used for the analysis were obtained from the oyster farmer notifications of mortality to the Departmental direction for territories and sea of Charente-Maritime (local competent authority), from 2008 to 2010. Oyster farmers have to complete a paper form, providing details of the mortality event:

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- the identification number of the leasing ground concerned;
- the technical characteristics of the animals, *i.e.* oysters ploidy, year of birth, size, origin details (hatchery or spat collection site), date of placement on the leasing ground concerned, type of rearing conditions;
- the sanitary characteristics of the oysters: percentage of mortality, date of observed mortality;
- the date of notification.

Data processing: The notification date reflected the date when the farmer has made the declaration to the local competent authority. However, true dates of mortality were unknown as most of leasing grounds can be reached only in low tide. Therefore, mortality may have occurred earlier. To account for this potential delay, notification dates were aggregated into blocks of 14 days of extension, representing a high-low tide cycle.

The geographical coordinates (latitude, longitude) of the leasing grounds were obtained from the official landscape.

As oyster age could represent a demographic confounder of clustering [3], this variable was defined as categorical, consisting in spat (<1 year), juvenile (1-2 years) and adult (>2 years).

A mortality case was defined as the notification of mortality in an epidemiological unit, during a whole tide cycle. The epidemiological unit was defined as a group of animals of the same class of age (spat, juvenile or adult) that shared similar rearing conditions, *i.e.* located on the same leasing ground. Only complete mortality cases, *i.e.* with no missing notification date, location and age, were considered for cluster analysis.

Statistical analysis: Variables were described in terms of frequency distribution (qualitative data) or mean and standard deviation (SD) (quantitative data).

The space-time permutation scan statistic [5] was used to search, test for significance and characterize the spatiotemporal clusters of mortality cases. The search was performed using cylindrical windows of variable size that move in space and time across the study area. These windows had a circular geographic base centered at leasing grounds centroids and a height corresponding to time. The window size varied from zero up to a maximum of 50% of the total number of mortality cases and 50% of the study period. The time aggregation was set to a whole tide cycle (14 days). Clustering was assessed by comparing the number of mortality cases within a scanning window to what would be expected under the null hypothesis of spatial and temporal randomness of mortality cases. The significance of identified clusters was tested using a maximum likelihood function based on Poisson distribution. P-value of the test was obtained from 999 Monte-Carlo simulations. The most likely cluster and any secondary cluster not overlapping with another reported cluster with a higher likelihood, having an associated P-value <0.05 were reported.

The spatiotemporal cluster analyses were conducted separately for each year. Cluster analyses were conducted both unadjusting and adjusting for oyster age.

Statistical analyses were conducted using SAS 9.2 and SaTScan 9.0. Spatial results were displayed using ArcGIS 9.3.1.

Result

Descriptive analysis: The yearly description of the mortality notifications is presented in Table 1. The number of notifications decreased over time. The average number of notifications per shellfish farm was 5.2 (SD=4.3) in 2008, 4.5 (SD=3.9) in 2009 and 4.5 (SD=1.4) in 2010.

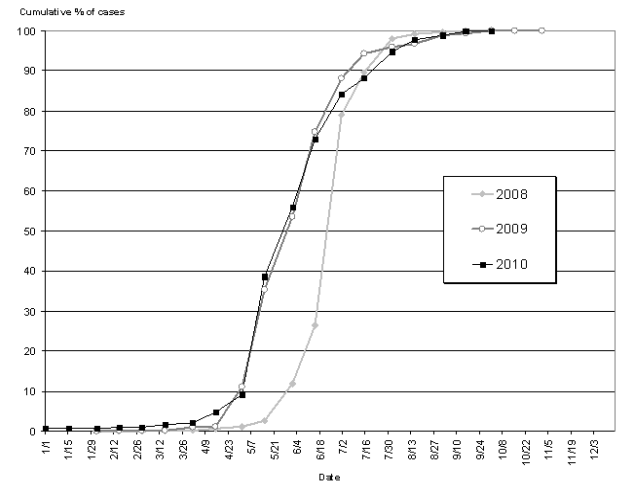
Table 1: Number of observations per item in the mortality notifications, Charente-Maritime, 2008-2010

Year	2008	2009	2010
Notifications	3,717	1,655	892
Farms	667	368	199
Leasing ground ID	3,417	1,640	887
Geographical coordinates	3,417	1,316	885
Notification date	2,918	1,520	845
Oyster age	3,037	1,705	870
Spat	1,581 (52%)	1,344 (79%)	701 (81%)
Juvenile	782 (26%)	273 (16%)	145 (17%)
Adult	674 (22%)	88 (5%)	24 (3%)
Raw* cases	2,918	1,322	718
Complete** cases	2,693	1,300	702

*No missing data for location and date
**No missing data for location, age and date

Pattern of cumulative time distribution of mortality notifications varied between 2008 and the other years (Figure 1). In 2008, notifications started about one month later (2nd tide cycle of May) than in 2009 and 2010 (2nd tide cycle of April).

Figure 1: Yearly time diffusion curves of mortality notifications



Spatiotemporal cluster analysis: Significant space-time clusters of mortality cases were detected with 3 clusters during the year 2008, 6 during 2009 and 4 during 2010. Table 2 describes the characteristics of the most likely cluster identified each year.

Table 2: Most likely cluster identified per year

Year	Nb. cases	O/E*	P-value	Geographical coordinates	Radius (km)	Date
2008	295	2.25	0.001	46.133025N 1.241461W	13.85	3/14 to 6/19
2009	89	2.62	<0.001	45.544846N 1.111820W	1.35	6/21 to 7/18
2010	14	6.61	<0.001	45.520246N 1.091561W	0.81	7/22 to 8/18

* Observed/expected counts of mortality cases

The adjustment of the space-time cluster analysis on oyster age did not show any major change of the clusters identified every year.

Discussion

To our knowledge, this is the first study analysing space-time patterns in the mortality notifications in oysters, based on surveillance data, with a formalized method.

The pattern of the mortality notifications in 2008 seemed to differ from the next ones, with a higher number of notifications occurring later in the spring. Mortality cases were clustered in space and time every studied year. Clusters were detected in close areas in 2009 and 2010. These results were consistent with a contagious process in mass mortality events, such as the spread of an infectious disease. A previous study has described an epidemic process in 2008 [3]. The present study goes further. The particular pattern of the mortality cases distribution in 2008 was consistent with the introduction of a disease and its spread. The similarity between the mortality cases patterns in 2009 and 2010 may suggest that this disease has then become endemic. Moreover, the high proportion of spat, *i.e.* animals with a naïve immune system, affected on the last 2 years strengthened the endemic aspect of the disease. Adjustment on oyster age did not change patterns of clusters, suggesting that oyster age was not a major risk factor for mass mortality. More than animal level variables should be considered in risk factor analyses for mass mortality.

As the distribution of the susceptible oyster population is unknown, the space-time permutation scan statistic method which requires only case data [9] was well-adapted here. However, this method is biased by spatially heterogeneous changes in population density over time [10]. As French oyster farming involves many movements of animals, called transfers, to ensure optimal growth conditions for each part of the rearing cycle, it would be necessary to get census population data over time in order to adjust cluster analysis.

The method is also sensitive to missing data or incomplete data [9]. The number of official notifications was found to decrease over years whereas their completion increased. This may reflect either shellfish farmer discouragement to notify mortalities (reporting bias) with fewer farmers who notified better, or a decrease in mortality events.

Current surveillance system led to effective implementation of policy measures only since 2009. But early detection is fundamental in aquatic population surveillance as only sanitary measures can be implemented (no treatment, no vaccine) to prevent the continued spread of contagious disease among oysters and other potential animal reservoirs. Performing prospective time-periodic (*e.g.* tide cycle) surveillance of oysters mortality notifications is crucial for the timely identification of areas in need of further diagnostic scrutiny involving the REPAMO. This would also facilitate to identify key areas, in relation with the hydrodynamics of the basin, where targeted control measures should be applied, in hopes to prevent the continued spread of this disease among oysters and other potential reservoirs. Lastly, such analysis would enable to adapt the control measures to the epidemiological situation of the disease.

Such a time-periodic analysis requires rapid transmission of data among all animal health stakeholders (farmers, scientists and policy makers). In the current French monitoring system of exceptional mortality events of molluscs, it is crucial to maintain the motivation of shellfish farmers to notify the observed mortalities. Regular information campaigns or formations to "good notification" may be implemented to encourage these data providers to contribute to such a surveillance system.

This study has shown that mortality cases were not equally distributed throughout Charente-Maritime and their pattern has changed since 2009. These results could form a basis for further investigations of risk factors aiming to design rapid and effective control strategies. They may also support a conceivable evolution of the French surveillance strategy from a passive to a cluster-based surveillance system, if full collaboration of all stakeholders could be achieved.

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Using Infrared Thermal Imaging for Mass Screening of Production Animals for Early Detection of Febrile Diseases

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Abstract
The use of infrared thermal imaging (IRT) in human populations as a tool for pre- and early-clinical fever detection has been demonstrated. However, infrared thermal imaging has yet to be successfully applied to animal mass screening and surveillance efforts. By using a *population-based* infrared camera screening protocol, whole groups of animals can be examined for pre-clinical or early febrile illness detection. To demonstrate the "proof-of-concept", we conducted a field pilot study simultaneously comparing the performance of two infrared cameras to a gold standard mercury thermometer and two additional digital thermometer models in a herd of 91 cows. Results of this pilot study showed statistically significant correlations between both IRT cameras and the gold standard thermometer. Our study demonstrates that infrared thermal imaging holds promise as a mass screening tool to detect fever earlier than traditional thermometry, resulting in shorter time-to-intervention and more effective animal isolation.

Keywords: Infrared thermal imaging, food animals, febrile illness, anal and nasal temperatures.

Introduction

The use of infrared thermal imaging (IRT) as a tool for early detection of fever has been demonstrated in several studies. IRT has been utilized for a wide range of infectious disease surveillance applications. Following the SARS outbreak of 2003, several airports installed IRT systems to conduct mass screening of patients for early detection of febrile individuals. More recently, the H1N1 outbreak in 2009 spurred renewed interest in these systems, resulting in numerous airports and hospitals installing IRT scanners for mass screening.

A recent study conducted by Nguyen and colleagues [2010] and published in Emerging Infectious Diseases (November 2010 issue) reported that when compared with oral temperatures in human patients, two temperature detection systems (OptoTherm and FLIR) were reasonably accurate for detecting fever; the authors indicated that these systems predicted fever better than self-reports. Despite their use in human fever detection, infrared thermal imaging has yet to be successfully applied to food animal surveillance and production medicine purposes. Current detection of febrile illnesses, such as "Shipping Fever", Rift Valley Fever (RVF), Foot and Mouth Disease (FMD), *etc*., depends on labor- and time-intensive methods involving individual-based interaction, typically after the disease has progressed to clinical manifestations. This focus on the individual demonstrating clinical signs of illness inherently misses the opportunity to proactively prevent transmission of infectious cases that results in reduction of morbidity and mortality across a population.

When properly used, the infrared thermography (IRT) technology can detect illness prior to clinical signs. By using a *population-based* infrared camera screening protocol, whole groups of people/animals can be examined for febrile illness, efficiently and cost effectively, providing caretakers opportunities to intervene much earlier during the course of infection than is currently available. The economic impact, both domestically and internationally, of lost production animals due to delays in detecting illness and banning of international trade due to sanitary and phytosanitary issues is staggering. Some examples include "Shipping Fever", which is a primary loss of revenue to the beef industry in the U.S. and Rift Valley Fever which has a great impact on export market of animal products in eastern and southern Africa. Therefore in this pilot project, we propose to utilize infrared thermal imaging to successfully screen a population of animals for the purpose of early detection of highly febrile diseases.

Materials and methods

To demonstrate the "proof-of-concept", we conducted a field pilot study comparing the performance of two infrared cameras (FLIR Thermacam E65 and "Wahl") to a gold standard mercury thermometer and two additional digital thermometer models. We obtained anal temperature readings from 91 cows and conducted simultaneous infrared imaging of both anal and nose areas (see Figures 1a, 1b).

Results

The preliminary results indicate statistically significant correlation between both the FLIR and the "Wahl" cameras and the gold standard thermometer we used in the pilot study. The FLIR camera used in this study resulted in lower overall temperature values, but was highly predictive of gold standard temperature when using a regression model. When data (nose temperature) from the FLIR E65 is entered into the model, it correctly predicted the actual gold standard anal temperature.

*Gold Standard Temperature = 93.592 + (0.082 * FLIR Temperature)*

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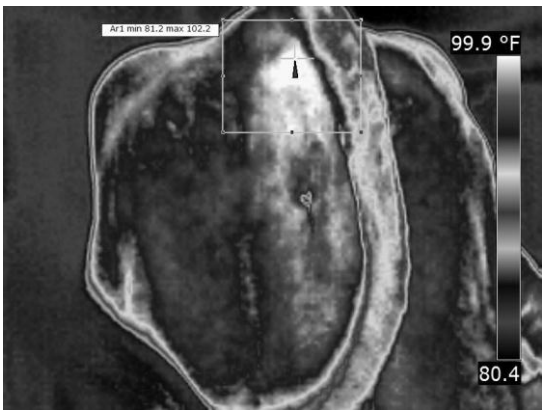
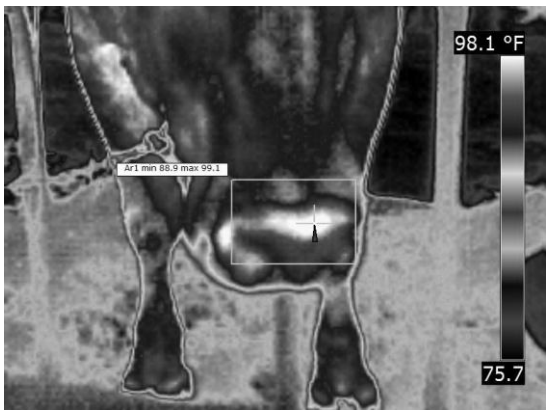
Figure 1a,b: FLIR E65 Corrected Anal (p=0.087) and Corrected Nose (p=0.01)

1a



1b



It was also shown that the area distal to the anal region was generally had the highest temperature reading as compared to other parts in the animal body. We also found that eyes were often warmer than the nose, but that data were not included in this analysis.

The temperatures captured through the FLIR IRT system were consistently lower than the Wahl IRT system and the three traditional thermometers. However, the data correlated well when nose temperature was compared to anal thermometer temperature. Findings of the measurement for individual associations are summarized as follows:

Gold Standard Thermometer 1: (Strongest → Weakest): Thermo 2 (0.644) → Thermo 3 (0.608) → FLIR Nose (0.398) → Wahl (0.256) → FLIR Anal (0.187)[4]

Table 1a: Thermometer 1 (Gold Standard) v. FLIR Nose

		Gold Standard Thermometer
FLIR Nose	Pearson Correlation	. 398*
	Significance	.010
	N	41

* Correlation is significant at the 0.01 level (2-tailed)

Table 1b: Thermometer 1 (Gold Standard) v. "Wahl"

		Gold Standard Thermometer
WAHL	Pearson Correlation	.256*
	Significance	.014
	N	91

* Correlation is significant at the 0.05 level (2-tailed).

Discussion

Results of the pilot study indicated there is a statistically significant correlation between both the FLIR and the "Wahl" cameras and the gold standard thermometer (Tables 1a, 1b), suggesting the need for further detailed studies involving increased and expanded herd populations.

The infrared thermal imaging tested in this pilot study could easily be applied to field surveillance programs in various production units including: dairy, beef, and swine in feed lots, slaughter facilities, meat processing plants, and milking parlors.

In order to validate further the IRT systems, we are planning additional herd-level field surveillance comparisons between the FLIR A-Series IRT, Wahl IRT, and traditional thermometers typical of current national (US) and international illness surveillance programs.

We believe that application of IRT technology to herd-level food animal surveillance programs has the potential to effectively detect highly febrile illnesses and thereby reduce infectious diseases incidence through early (pre-clinical) disease detection, intervention, and transmission interruption.

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Feasibility of applying syndrome surveillance algorithms to animal health and production data to improve emerging animal disease surveillance

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Abstract

This paper presents the results of a feasibility study on applying syndrome surveillance algorithms to animal health and production data to enhance early detection of emerging animal diseases. The case of the introduction of bluetongue virus serotype 8 in Northern Europe in 2006 was investigated while looking at historical mortality data that collected on a daily bases by the rendering plant. Several candidate algorithms were identified from literature and applied to the data.

This study shows that it is technically feasible to apply existing syndrome surveillance algorithms to animal health and production data. The application of syndrome surveillance methodology on animal disease and production data needs further investigation to clearly assess their sensitivity and specificity.

Keywords: syndrome surveillance, emerging diseases, early detection, stat.

Introduction

Emerging diseases are those that appear in a population for the first time, or that may have existed previously but are rapidly increasing in incidence or geographic range. Outbreaks of emerging animal diseases, particularly those harmful to humans (zoonoses) can cause considerable economic and social upheaval. Early detection of an emerging animal disease incursion is crucial for an efficient eradication and will at least reduce the economical and social losses. For example, the time to detection of the classical swine fever introduction in 1996-97 in the Netherlands and Belgian differed considerably, i.e. resp. 3 and 1 month after introduction and this contributed to much higher losses in the Netherlands compared to Belgium, i.e. € 1.8 billion versus € 11 million respectively [1-3].

The primary purpose of animal disease surveillance systems is to provide cost-effective information for assessing and managing risks associated with trade in animals and animal products, animal production efficiency, animal welfare and public health [4]. Competent authorities wish to improve surveillance systems in order to better prevent introductions and enhance early detection of emerging diseases to reduce the risk for future epidemics and related economic deficits. The ability of the surveillance system to detect new and emergent diseases has been recognized as a key feature of modern veterinary surveillance systems [5]. But emerging diseases usually do not have suitable diagnostic tests and are not in the sphere of experience of clinicians.

In this context we see an opportunity in applying syndrome surveillance to enhance early detection. A recent definition of syndrome (or syndromic) surveillance is the continual statistical monitoring of population health data to identify change that may be due to disease [6] and is currently applied in human disease surveillance to improve the timeliness of disease detection [7]. It uses statistical process control (SPC) methods to detect disease outbreaks which can be of value if a disease is not in the realm of experience of clinicians [8]. The potential to apply SPC methods to a wide range of generic animal disease and production data must result in an improvement to the overall surveillance system in terms of sensitivity, specificity, and timeliness, although the capacity to add it to surveillance systems in not fully clarified [8-12]. The aim of this study was to assess the feasibility of applying syndrome surveillance algorithms to animal health and production data to improve overall emerging disease surveillance systems.

We investigated the introduction of bluetongue virus serotype 8 in Northern Europe in 2006, which was first confirmed by laboratory testing in the Netherlands in a sheep flock in the southern province of Limburg on 16 August 2006 [13]. The origin of the infection was localized near the tripoint where Belgium, the Netherlands, and Germany share borders [14]. As early as late June, Belgian veterinary practitioners were seeing bovine cases that were attributed at that time to photosensitization or exposure to mycotoxins, but retrospectively might have been caused by BTV-8 [13].

Materials and methods

The study database consisted of the registry of the carcasses of deceased animals that were collect by the Belgian rendering plant 'Rendac' between 1 January 2002 and 31 December 2006. Records were added to the registry at the moment a collection was ordered through an automated telephone query by the animal owner. The registry contains data on cattle and sheep/goats and included following variables:

- The address from which the carcasses were collected;
- The species, age group, weight;
- The number of carcasses collected;
- The ordering and collection date.

The data were investigated using different syndrome surveillance algorithms. A first, most basic, procedure was the application if statistical process control methods to the data. In this method, an upper control

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limit (UCL) and lower control limit (LCL) is constructed for a univariate time series under investigation. If the counts remain between these limits, then the process is said to be in control, if not the process is said to be out of control. In the case of biosurveillance, it is of interest to study possible outbreaks, therefore we will focus on count numbers higher than expected. Suppose that $X_1, ..., X_n$ represent the available historical data on the process being monitored. Assuming a normal distribution, the mean μ and standard deviation σ can be estimated from the historical data using the expressions

$$\hat{\mu} = \frac{1}{n}\sum_{i=1}^{n} X_i, \text{ and } \hat{\sigma} = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(X_i - \hat{\mu})^2}$$

The Z-statistic, constructed as $Z = \frac{X_{n+1} - \hat{\mu}}{\sigma}$ evaluates the departure of X_{n+1}, the 'current' observation, from the expected historical average $\hat{\mu}$. In addition, the moving average algorithm, being a special case of a quality control chart was used. It constructs a prediction interval for the next time step based on an average of values in a certain time window. Two possible approaches for electing the time window, were applied:

- considering a fixed number of previous A observations, or;
- comparing with observations from similar time periods of previous years.

As an extension of the moving average algorithm, the Exponentially Weighted Moving Average Algorithm was applied [6], in which autoregressive types of weights are assigned to current and prior observations: the further in the past, the smaller the weight and this weight is exponentially decreasing.

Next, a regression model as described by Farrington [15] were investigated. The model underlying this approach is a Poisson regression model allowing for overdispersion:

$X_i \sim Poisson(\mu_i)$, where

$$\log \mu_i = \alpha + \beta t_i, \quad E(X_i) = \mu_i, \quad \text{and } V(X_i) = \phi\mu_i$$

The dispersion parameter ϕ is estimated as:

$$\hat{\phi} = \max\left\{\frac{1}{n-p}\sum_{i=1}^{n}\omega_i\frac{(x_i - \hat{\mu}_i)^2}{\hat{\mu}_i}, 1\right\}$$

where ω_i is a weight determined to reduce the influence of past outbreaks. Now, let t_{n+1} denote time block 0 and x_{n+1} the last observed number of events, then the expected number of events at t_{n+1} is estimated by:

$$\hat{\mu}_{n+1} = \exp(\hat{\alpha} + \hat{\beta}t_{n+1}).$$

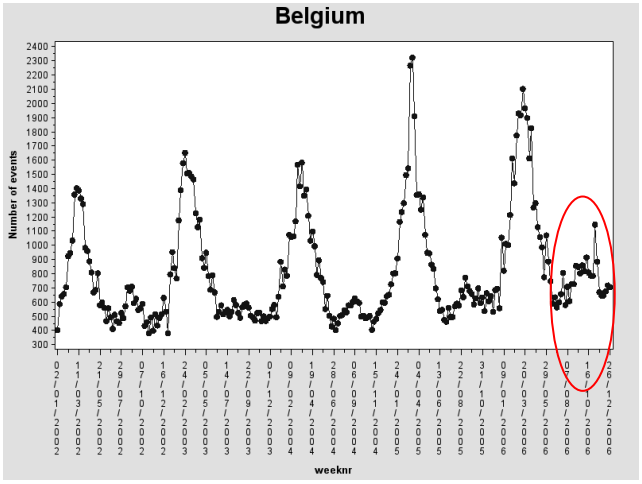
Finally, generalized linear mixed regression models were applied to account for spatial and temporal correlation which may exist in the data. The model consisted of the same Poisson model previously described, but incorporating cluster-specific random intercepts.

The different algorithms were evaluated for their capacity to identify 'unusual mortality' in Limburg province in Belgium during the weeks prior to the confirmation of Bluetongue serotype 8 on 19 August 2006.

Results

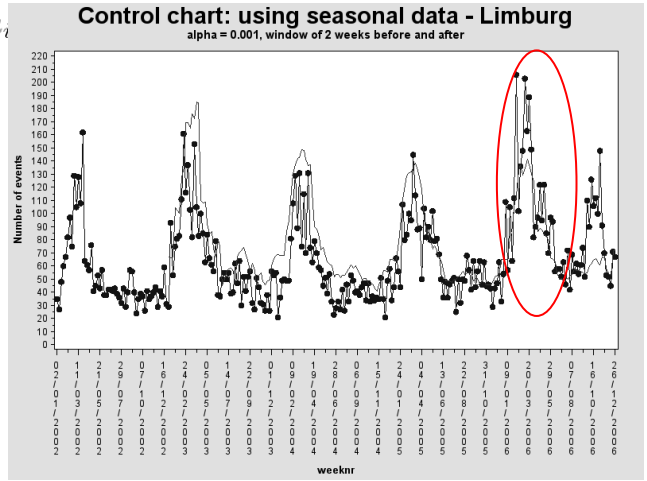
On average, 4175 cattle carcasses and 818 sheep and goat carcasses were collected on a weekly basis in Belgium between 02/01/2002 and 30/12/2006. A more detailed overview of the weekly collected number of carcasses for sheep and goat is shown in Figure 1. A clear seasonal trend can be observed.

Figure 1: Plot of the weekly number of collected sheep and goat carcasses in Belgium



The different algorithms managed to detect unusual mortality events during the weeks prior to confirmation but with different levels of sensitivity and specificity. One of the better predictions is predictions is presented in Figure 2. In this case the statistical process control procedure was used were mortality was compared with data from the same time window of the previous years

Figure 2: Control chart with moving average using seasonally comparable information for the number of collected carcasses in Limburg



Discussion

This study shows that it is technically feasible to apply existing syndrome surveillance algorithms to animal health and production data. The different algorithms

identified unusual mortality events with different levels of sensitivity and specificity and these characteristics would not be consistent for cattle or sheep/goat mortality data.

The application of syndrome surveillance methodology on animal disease and production data needs further investigation. The results of this study show the success of the application will be highly data dependent.

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West Nile Disease (WND) – the current epidemiological situation and future surveillance trends in the Veneto region (Northeastern Italy)

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Abstract

In Italy an outbreak of West Nile virus (WNV) infection involving humans, domestic animals and wild birds was documented from August 2008 in areas around the Po river delta. This paper presents the results of surveillance activity carried out in 2008-2010 in the Veneto Region on equines, wild birds and mosquitoes. In the same period, the infected area also had human cases reported. Surveillance data and environmental variables were used to produce preliminary risk maps for the distribution of WNV in the Veneto region. The surveillance activities set up in 2010 enabled a rapid identification of viral circulation in a new area (i.e. surveillance area) and in previously affected areas and the co-circulation of USUTU virus (USUV).

Keywords: WND, surveillance, remote sensing, Vector borne disease.

Introduction

West Nile virus (WNV) is a mosquito-borne Flavivirus belonging to the Japanese encephalitis antigenic complex of the *Flaviviridae* family. WNV infection is transmitted in natural cycles between birds and mosquitoes, particularly *Culex* spp. mosquitoes. Humans and horses are susceptible dead-end hosts. Firstly identified in tropical Africa [1] WNV infection was also detected in northern Africa, Israel, India and Australia and since 1999 [2] has progressively spread to the Americas. In Italy, the first cases of equine WNV infection were detected in 1998, among horses living in proximity of the Tuscan wetland area called Padule di Fucecchio. After this epidemic no further cases have been reported [3].

After about ten years, in 2008, a large outbreak affected three Italian Northern Regions, including part of Veneto's territory. In 2009 and 2010 WNV re-emerged in approximately the same area and also appeared in Central and Southern Italy. The results of three years of surveillance activities in the Veneto region are described herein. The resulting data served to compile WND risk distribution maps.

Materials and methods

Surveillance Data: From September 2008 to November 2010 three surveillance programs were implemented monitoring horses in the Veneto Region. In 2008 a representative sample (expected prevalence 10±3%, CI 90%) of horse stables was monitored in the provinces of Padua, Rovigo and Venice (study area). Only the equines that had not travelled outside the Veneto region and/or showed clinical signs compatible to WNV infection were serologically sampled. In 2009 according to national surveillance plan and ministerial law dated 15th of September 2009 the surveillance area was extended 20km northward and westward from the location of the most outlying stables that resulted positive. The new area was defined as the "Surveillance Area" (SA), whereas the area where WNV was previously identified was defined as the "Area with WNV Circulation" (AWC).

In 2009 and 2010, passive surveillance (i.e. based on the detection of clinical symptoms) on all neurological cases in equines was enhanced. In addition in 2010, in the SA, the horses that tested negative at previous controls were enrolled as sentinels. They were sampled for serological investigation according to the scheme provided by the national surveillance plan: 1st sampling in springtime; 2nd in late summer; 3rd in autumn.

Since January 2009, all the wild birds found dead in the AWC were tested for WNV. At the same time, healthy non migratory birds of the *Corvidae* family were virologically and serologically tested.

Entomological surveillance was conducted using CO_2 mosquito traps in 43 sites from May through November.

All the serum samples were submitted to the National Reference Laboratory to detect antibodies against WNV by means of sero-neutralization assays (SN) based on IgG virus neutralization and plaque reduction neutralization. Blood, organs and pools of mosquitoes were delivered to the same laboratory for virus detection by PCR assay.

Climate data: Environmental and climatic data were collected, through satellite imagery, for all the tested farm in 2008-2010. The data were available at intervals of 8-16 days using the following "channels": Middle Infrared, day and night-time Land Surface Temperature (LST), Normalised Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI). Other satellite and ground-collected data on precipitation and elevation were also available. Environmental data were temporal Fourier transformed to obtain their annual and seasonal variations. In this preliminary model, the data on arthropod vectors were not included.

Data were processed using ESRI ArcGIS and IDRISI software. Farms that tested positive and negative to WNV where respectively used to assess the "presence" and the "absence" of the disease on the territory.

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Risk maps were produced by establishing the relationship between the satellite and Mathematical algorithms based on non-linear discriminant analysis, developed by the EDEN Low Resolution Remote Sensing (LRRS) team of the Oxford University, and applied to define the relationship between the satellite and disease data. A hundred bootstrap models were performed and the variables were selected in a step-wise inclusion manner to minimise the corrected Akaike Information Criterion (AICc). In any one model the selected variables were ranked from 1 to 10, while variables not included in the model were given a rank of 11.

Result

A total of 368/2,075 (17%; 95%CI=16.09%-19.38%) horses in 194 (29% 95%CI=26.29%-33.31%) farms tested positive for WNV in 2008-2009 (detail in Table 1). In 2010 passive surveillance detected 3 and 1 horse practices that resulted positive in AWC and SA respectively. Serological and virological tests performed on the samples collected from sentinel horses gave negative results however, seroconversion for Usutu virus (USUV) was detected in 20 horses in 17 stables.

Table 1: Results of the active surveillance on equine practices in the Veneto region in 2008 and 2009

			Tested (N)	Positive (%)	95% CI	
2008	Study Area	Horses	1,257	22.0	19.7	24.3
		Farms	421	31.6	27.1	36.0
2009	AWC	Horses	162	16.0	10.4	21.7
		Farms	56	35.7	23.2	48.3
	SA	Horses	656	10.1	7.8	12.4
		Farms	174	23.6	17.3	29.9
2010*	SA	Horses	77	0.0	-	-
		Farms	45	0.0	-	-

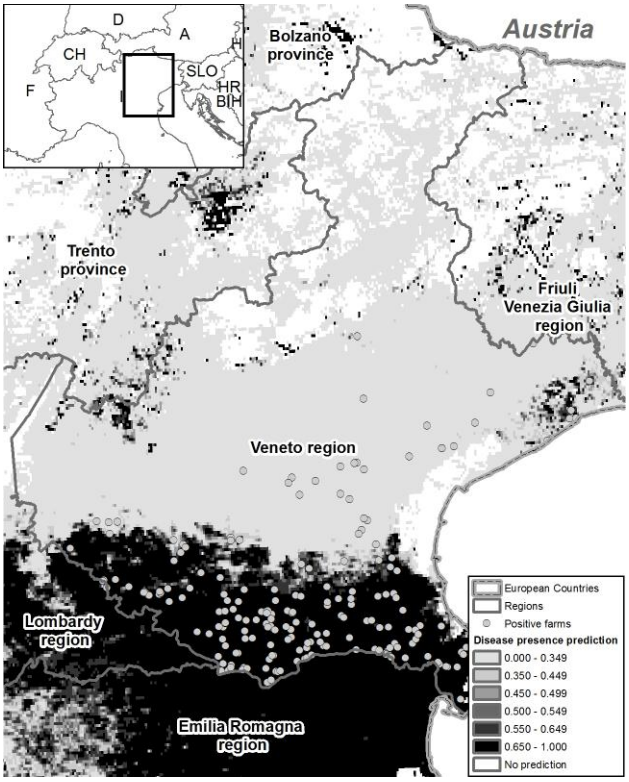
* Four horse practices resulted positive to passive surveillance activities (3 in AWC and 1 in SA) with a total of 23 positive out of 138 tested animals (16.7%).

In 2009, 621 wild birds of the *Corvidae* family, mainly hooded crows (*Corvus corone cornix*) and magpies (*Pica Pica*), were captured and tested. Eleven birds (1.8%; 95%CI=0.9%-3.1%) were positive; ten were positive by serology (two hooded crows and eight magpies in the provinces of Rovigo and in Padua) and one by PCR (a magpie in the province of Padua reported in February 2009) while in the province of Treviso a blackbird (*Turdus merula*) resulted positive to USUV. In 2010 one dead collared dove (*Streptopelia decaocto*) in Treviso resulted positive for WNV.

Between 2008 and 2009 the totality of the mosquito pools tested was negative for WNV, while 5 pools from the provinces of Rovigo, Venice, Padua and Verona were USUV positive. In 2010, 10 and 23 pools of *Culex pipiens* out of 2,652 pools resulted positive for WNV and USUV respectively. Positive pools of mosquitoes came from the AWC and were trapped in proximity to human cases or stables with the symptomatic horses.

The integration of the surveillance data on the horse premises and the environmental variables allowed to create preliminary risk maps for the distribution of the disease (Figure 1) and to identify the most important ecological variables related to the presence of the WNV. The mean accuracy indices (Cohen's *kappa*) calculated for each decade of bootstrap models exceeded 0.75, showing an overall good fitting of the models to the data [4].

Figure 1: Risk map based on surveillance data and environmental variables. Likelihood of WNV presence. High: black; low: gray.



In general rainfall variables resulted to be the most important in determining the distribution of WNV in the Veneto region, followed by thermal variables (both night-time and day-time LST), while no correlation was observed with vegetation variables (NDVI and EVI). The overall top ranked variables and their average ranks are reported in Table 2.

Discussion

The surveillance plan implemented in the Veneto region was aimed at monitoring WNV circulation in endemic areas and to early detect newly introduced WNV in disease-free areas.

Results illustrate that from 2008 WNV spread from the provinces of Rovigo and Venice, northward and westward to the provinces of Verona, Padova, Vicenza in 2009 and to Treviso in 2010.

Table 2: Average ranking of the key variables

Av. Rank	Variable
7.07	CMORPH 6-month precipitation amplitude
7.16	WORLDCLIM 12-month precipitation phase
7.35	WORLDCLIM overall precipitation mean
8.74	Nighttime LST 12-month mean
9.16	CMORPH overall precipitation maximum
9.19	Nighttime LST 4-month phase
9.20	Daytime LST 6-month amplitude
9.33	Nighttime LST 4-month amplitude
9.54	WORLDCLIM 4-month precipitation phase
9.59	Daytime LST 4-month amplitude

A monitoring surveillance system based on serological tests of dead-end host as horses is useful to provide information on the geographical distribution of the virus. Whereas it is not effective to predict the risk of human infection (as observed by Corrigan *et al*. [5]) and in the end, it should be applied in free areas at risk of WNV exposure. On the contrary, in endemic areas passive surveillance on horses appears to be an efficient tool for detection of virus reactivation.

The potential use of dogs as sentinel indicators for WNV circulation in urban environments was described [6] and investigated in a study conducted in the provinces of Padua and of Rovigo. A serological survey was carried out on stray dog captured by the local veterinary services [7].

Results of WNV surveillance in 2008-2010 and recent evidence of USUV co-circulation stress the need to develop specific tools to:

a) properly monitor the presence of mosquito borne diseases;
b) promptly detect the circulation and further spread of WNV and USUV and;
c) to identify the wild reservoirs correctly.

Management of a vector borne zoonose requires the veterinary authority to adopt a new approach to disease control. Other than the usual surveillance system specialized expertise and *ad hoc* modelling are required. To make the surveillance plan more effective, data analysis must be considered in a new way, combining surveillance data with ecological and climate variables.

Although the model applied was still preliminary, the results were accurate and encouraging. In particular, the ability to identify the key predictor variables related to the presence of the disease could be of great help as they could be monitored in the context of early detection programs. To improve the modeling and the validation phases, a collaborative approach between institution of different regions is needed along with more accurate basic information on such variables as humidity (atmospheric and land surface) and mosquitoes population and dynamics.

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Chemical food safety incidents in England and Wales

A. Murphy[1], A. Otter[2] and J.H. Payne[1]

Abstract
Potential chemical food safety incidents have been investigated by Veterinary Laboratories Agency (VLA) on behalf of Food Standards Agency (FSA) since 1990. This paper describes the criteria on which these incidents are selected and the type and numbers of incidents investigated over the last two decades.

Keywords: chemical food safety, surveillance, chemical toxicity.

Introduction

The VLA carries out the diagnosis and surveillance of food animal diseases in England and Wales. The Regional Laboratories of the VLA identify and investigate potential food safety incidents caused by exposure of food producing animals to chemicals on behalf of the FSA. The objective of the surveillance is to identify causes of contamination, investigate release and exposure and advise farmers and the Food Standards Agency on possible risk controls

Materials and methods

The identification of potential food safety issues arises from samples submitted by private veterinary practitioners and necropsy examinations. These are received throughout the network of regional laboratories in England and Wales. The Veterinary Investigation Officer responsible for the case reports their findings to the Chemical Food Safety Project staff within the VLA. They then compile the relevant information required by FSA to carry out a risk assessment for each individual case. This may require an advisory farm visit depending on the circumstances surrounding the incident. Input is provided from the VLA project staff to aid FSA in this assessment.

Potential food safety incidents have been defined by the following categories:

- Any incident suspected to be caused or contributed to, by a chemical agent or biological toxin;
- Any incident where food animals have been exposed to unusual or unusually high doses of chemicals, regardless of whether they are showing signs of disease;
- Any incident where a suspected adverse reaction to a medicine or other substances is involved;
- Any incident suspected to be caused by or related to any purchased feeds or feed supplements or a particular batch of home produced feed;
- Any incident affecting food animals that remains undiagnosed following preliminary investigations, where poisoning due to a chemical contaminant cannot be ruled out;
- Any incident where, as the investigation progresses, the possibility of chemical contamination becomes apparent.

Within this description of incidents there are those which are regarded as being more serious than others. In such cases there may be an immediate and widespread risk to the food chain and such incidents include:

- When ruminants producing milk for human consumption or poultry producing eggs are exposed.
- When animals close to slaughter have been exposed.
- When more than one farm is seemingly affected simultaneously.
- When feed suspected to be contaminated has been delivered to more than one farm.

Results

The Veterinary Laboratories Agency (Veterinary Investigation Service <1995) has investigated over 1500 potential food safety incidents since 1990 on behalf of the FSA. Lead accounts for over 50% of the cases and these show a significant seasonal variation relating to turnout to grazing. Cattle represent the majority of these incidents although there have also been several involving sheep and poultry.

The table below outlines the top five incidents for the primary UK food producing species.

PRINCIPAL FOOD SAFETY INCIDENTS 1990-2010

Cattle	Lead	835
	Botulism	188
	Copper	31
	Metaldehyde	16
	Mycotoxins	10
Sheep	Copper	112
	Lead	58
	Botulism	22
	Ionophores	11
	Selenium	6
Pigs	Mycotoxins	3
	Asphalt	2
	Lead	1
	Cadmium	1
	Metaldehyde	1
Avian	Lead	31
	Ionophore	26
	Rodenticide	2
	Mercury	1
	Ethylene glycol	1

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Discussion

The aim of this area of work is protect the food chain. In such cases it is critical however to ensure the stock owner receives balanced and proportionate advice. This encourages cooperation whilst investigating an incident and minimises the risk of the owners not reporting any future, unrelated potential cases. The advice provided is specific to each individual situation and voluntary restrictions are necessary in many cases. The vast majority of producers realise the importance of producing safe food and acknowledge that consumer confidence is essential for their industry to exist.

Additionally, new EU food hygiene Regulations came into force on the 1st of January 2006 which introduced hygiene requirements for livestock farmers for the first time. Livestock for food must be raised in compliance with Annex 1 of Regulation (EC) 852/2004. The aim of these regulations was to simplify and consolidate all the various pieces of EU food hygiene legislation, through proportionate controls to protect the food chain, from the primary producer stage through to the supply of the final consumer. The "farm to fork" approach.

Under the farm to fork approach livestock producers have been required to provide food chain information (FCI) when they submit their animals for slaughter. This has been introduced progressively, since the creation of the Regulations and since the 1st of January 2010 FCI must accompany all species of animal.

As outlined in the data provided there have been a considerable number of potential food safety incidents involving chemicals over the years. The VLA plays a critical role in food safety by means of its surveillance activities which safeguard animal health, animal welfare, food production and public health.

Acknowledgements

The authors wish to thank VLA colleagues who collected the information and conducted farm visits relating to these cases.

How to deal with an emerging vector-borne disease when it broke into a free area: the experience of Bluetongue (BT) surveillance in Piedmont region

M.C. Radaelli[1*], L. Chiavacci[1], P. Vignetta[2], G. Moda[2] and N. Vitale[1]

Abstract

Control of emerging animal diseases depends on their early detection. However, designing surveillance programs for exotic and emerging diseases is very challenging because of knowledge gaps on the probability of incursion and mechanisms of spread. Bluetongue virus serotype 8 (BTV8) was detected in Northern Italy for the first time in January 2008 following the detection of BTV8 in Central Europe (Belgium, The Netherlands, Luxembourg, Germany and France).

In this work are reported the measures adopted in Piedmont region (North-western Italy) in order to assess the risk of BTV8 introduction by commercial trades as Piedmont borders France and has an intense livestock import activity with France. Measures in this step included serological survey and risk map based on entomological data. After the BT detection in the region to control BT spread .a massive vaccination plan was settled and a survey on wildlife susceptible ruminants was performed. Measures undertaken after BT introduction were satisfactory as between 2008 and 2009 only 25 outbreaks were detected on 16.000 herds and in 2010 just one purchase head in January.

Keywords: Bluetongue, Piedmont region, Targeted surveillance, Control measures.

Introduction

Bluetongue (BT) is an insect-transmitted viral disease of wild and domestic ruminants. It is caused by Bluetongue virus (BTV), a RNA virus of the genus *Orbivirus* in the *Reoviridae* family, and exists as 24 distinct serotypes; all of them are capable of causing severe clinical disease at least in sheep. It is transmitted between its hosts by biting midges belonging to *Culicoides* species [1]. As a vector-borne infection, the presence and abundance of the vector determine the distribution and spreading of the virus in the temperate and tropical regions of the world. Although impact varies between strains, outbreaks of all bluetongue serotypes can have significant economic impacts in terms of farm losses and movement restrictions. Bluetongue does not affect humans so there are no human or public health implications.

In August 2000 BTV2 was confirmed for the first time ever on Italian territory. Sardinia was affected first but the virus spread rapidly to Sicily and southern mainland Italy [1]. After this onset, five different BTV serotypes (BTV1, BTV2, BTV4, BTV9, and BTV16) have been detected from 2000 to 2007.

In order to detect BTV circulation, a structured entomological and serological surveillance system has been place in Italy since May 2001 [2]. Serological surveillance is based on the periodical testing of unvaccinated sentinel cattle that are uniformly scattered throughout Italy in a grid of 400 km^2 cells. Sentinel animals are serologically monitored twice at month during the period of vector activity, and their minimum number per geographical unit (58-60 heads shared out among 8-10 farms) is representative and sufficient in order to detect a monthly incidence of seroconversion of 2% with a 95% of confidence interval (CI) in each geographical unit. Entomological monitoring consists of an active program of vector catching with traps located in each 1600 km^2 geographical unit. Traps were located outside near livestock and operated during the night; the midges collected in the traps are sent to CESME (the Italian Reference Centre for BT) for counting and identifying Culicoides species on a routine basis.

In August 2006, BTV8, a serotype which was previously reported from the sub-Saharan region, Asia and South America, unexpectedly broke into Central Europe and outbreaks were reported for the first time in several animal holdings in The Netherlands, Belgium, Germany, France, Luxembourg and then many other countries [3, 4]. BTV8 represents a serious hazard to the European livestock population as it is able to cause severe disease in cattle which have never been previously exposed to this serotype.

Following the spread of BTV8 epidemic in France during 2007, a targeted monitoring program has been implemented in Piedmont (a region of North-Western Italy) [5, 6] by Piedmont Regional Animal's Health Authority, to assess the risk of BTV8 introduction, as Piedmont is located next to France and every month more than 20.000 bovines are imported mainly to fattening units.

At the beginning of 2008, BTV8 was detected for the first time in North-Eastern Italy [7]. In late autumn, the infection was also reported in cattle herds located in an area bordering the Alps of Piedmont. Positivities arose from sentinel network cattle farms.

The aim of this work is to describe control measures adopted by Piedmont region to deal with BT before and after BTV8 detection in the region.

Materials and methods

Piedmont is one of the most important Italian regions for cattle farming. It is located next to France and Switzerland and it is bordered and protected by the Alps Mountain.

Starting to 2007 BTV8 European epidemics the following group of measures was undertaken in order

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to avoid the spread of the infection in the region: a protocol to trace imported heads from BTV8 infected countries, a serological survey to control the imported susceptible animals, a risk map for BTV8 occurrence.

To enforce the monitoring system in place through sentinel herds, a **special serological survey** involving more than 20.000 cattle imported from high risk areas of France (mainly from restriction areas) was settled. A random sampling was conducted in order to identify the risk of BTV8 introduction by commercial trades. Additionally a serological surveillance was conducted in sampled farms on a radius of 4 km from positive herds.

A **risk map** was developed to evaluate the potential distribution of the BTV8 in the region: the detection of the risk area for vectors abundance was based on data of entomological surveillance. The analysis of BTV vectors occurrence was based on: temperature (minimum and maximum), seasonality and environmental data of all trap sites such as land cover activities (according to CORINE Land Cover 2000 database), geographical coordinates (latitudes and longitudes) and mean altitude above sea level. The study area included 22 traps sites one for each grid cells (1600 km^2). Information on date of capture, presence/absence and abundance of *Culicoides* spp. from August 2001 to December 2008 were gathered by CESME database. A Generalized Estimating Equations (GEE) using SAS® was performed to correct for repeated measures. The output of GEE was employed to generate risk maps in ArcGIS, underlining risk areas for vector abundance and outbreaks occurrence.

After BTV8 infection was confirmed in Piedmont:
Two years (2009 and 2010) of **massive vaccination plan** was performed with BTV8 inactivated vaccines. Vaccination was monitoring to assess efficacy. The study unit was a head that had received a primary vaccination by double injection. A stratify randomized field trial design was followed. Stratification was carried out by quadrant. According to BT Italian rules, for each quadrant of 400 km^2, a random sample of 9 vaccinated animals per susceptible species (cattle, sheep, goat) [8] were selected from BT vaccination database by using proc surveyselect of SAS® systems. Sera samples from selected animals were collected by official veterinarians at least 30 days following vaccination starting from May, and being completed in December 2009 in those herds which went to the alpine summer pasture. To monitor the vaccination response, sera were tested for the presence of BTV8 antibodies by using a competitive ELISA (ELISA) ("ID Screen® Bluetongue competition", ID VET, Montpellier, France) and serumneutralisation assay (SN) performed according to the method described by Savini and others (2004) [9].

A survey on wildlife susceptible ruminants was conducted in the area favorable to vectors. The survey was carried out during the hunting season and within the Wildlife Regional Surveillance Plan. Samples were collected by hunters and park keepers trained by Regional Veterinary Services on specimen collection prior to the hunting seasons. The study covered an area of approximately 16.000 km^2.

Result

Special serological survey: From 2007 summer to the onset of the vector-free period, serum and EDTA blood samples of 23461 cattle imported from restriction areas were collected. Serological analysis showed low rate of antibodies against BTV8 by competitive ELISA (17 positive heads: 0,07%; CI95%: 0.04%-0.12%), and few real time RT-PCR were detected (10 positive heads: 0,04%; CI95%:0.02%-0.08%). The virus isolation attempted on the RT-PCR positive blood samples was unsuccessful.

When positive cattle were detected during period of vectors activity, additional serological survey was performed in the involved herd and in 4 km radius from positive farm, providing the absence of seroconversion.

Risk map: From August 2001 to December 2008, a total of 7139 collection were taken from 22 traps distributed along the 8 Piedmont provinces. The best model for the BTV risk area prediction (Figure 1), included three factors related with vector abundance: mean altitude (>500 m OR=1.13; CI 95%: 1.03-1.27), land cover activities (natural grasslands vs. others OR=1.14; CI 95%: 1.10-1.17) and minimum temperature (OR=1.01; CI: 95% 1.006-1.018.) The model localised the highly risky area between the province of Cuneo and Turin where in 2009 were registered 16 BTV8 outbreaks.

Effectiveness of mass vaccination plan: From May to December 2009 sera samples from 1.783 vaccinated animals were analyzed: 890 from cattle, 470 from sheep and 423 from goats. The overall seroprevalence was 77,83% (CI95%: 75,9-79,8) by ELISA (Table 1) and 31,72% (CI95%: 29,6-34,0) by SN (Table 2).

Wildlife survey: From January 2009 to April 2010, a survey on the presence of BTV in wild ruminants was conducted in several parks, game areas and municipality's territories of the region. Sera and EDTA blood samples were obtained from 964 animals hunted among different game areas (Table 3). A total of 929 sera were tested by competitive ELISA and 126 whole blood samples were tested by real time RT-PCR; 3 deer sera out of 929 (0.3%; 95% C.I.: 0.7-9.4%) wild ruminants samples showed antibodies against BTV8, two had neutralising antibodies titres of 1:20 and one 1:10 at the SN assay The real time RT-PCR confirmed the presence of BTV RNA in the EDTA blood sample of the deer with 1:20 BTV8 neutralising antibodies, while the type specific RT-PCR revealed that the RNA segment detected in the blood sample was of BTV8. The virus isolation attempted on the RT-PCR positive blood sample was unsuccessful. All positive samples were from red deer culled in the Regional Park “La Mandria”, sited close by a cattle farm in which BTV8 positivity was detected in April 2009. The proportion of red deer infected with BTV (6.25%; 95% CI: 1.3-17.2%) was higher ($p<0.001$) than that of roe deer (0%; CI95%: 95.6%-100%)) hunted at “La Mandria” Park [10].

Table 1: Results BTV8 vaccination monitoring by ELISA

ELISA				Heads
	Cattle	Goat	Sheep	
Negative	211 23,73%	128 30,26%	56 11,91%	395
Positive	678 76,27%	295 69,74%	414 88,09%	1387
Tot Missing=1	889	423	470	1782

Figure 1: BTV risk area

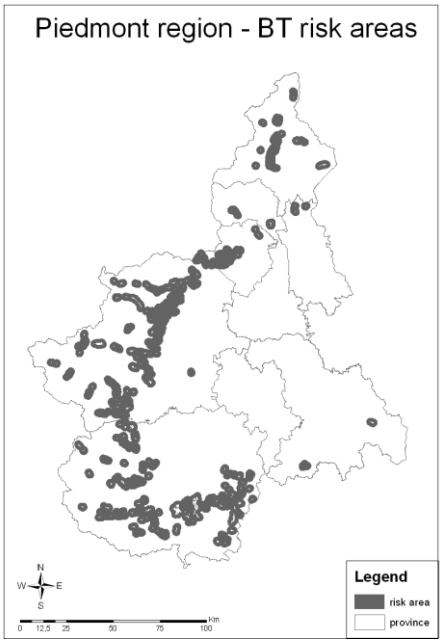

Table 2: Results BTV8vaccination monitoring by SN

SN				Heads
	Cattle	Goat	Sheep	
Negative	667 74,94%	288 68,25%	261 55,65%	1216
Positive	223 25,06%	134 31,75%	208 44,35%	565
Tot Missing=2	890	422	469	1781

Table. 3: Distribution of wildlife species by game area

Study area	Roe deer	Chamois	Red deer	Fallow deer	Muflons Bison	Tot
Alpine	247	184	31	10	11	483
Hill	392	0	0	1	0	393
Mandria	27	2	48	2	0	79
Rural	0	0	8	0	1	9
Total	666	186	87	13	12	964

Discussion

The aim of the work was to describe control measures implemented by Piedmont region to deal with BT before and after BTV8 detection in the region in addiction to BT national surveillance plan.

There is no certainty on how BTV8 entered Piedmont. However the intense livestock import activity between France and the region support the hypothesis of a French origin. The findings from the 2007 targeted serological survey involving cattle imported from restriction areas of France was able to demonstrate the import of BTV8 infected heads, and in 2009 first positivities in the region occurred in cattle farms located in the neighborhoods of the main commercial routes connecting France to Piedmont.

Indeed the risk map on BT vectors abundance was validated by data as the 16 outbreaks registered in 2009 occurred in the area underlining by the model.

Aiming to improve disease surveillance and control methods, risk maps might give precious help by providing a basis for targeting of financial and monitoring efforts to pre-defined areas. Vector surveillance has particular relevance to assess potential areas of infection spread and is important the continuous refinement of the distribution map of *Culicoides* together with the detection and elucidation of the prevalence of new suspected vectors and in order to identify zones that are seasonally vector free.

The Piedmont mass vaccination plan was implemented in 2009 soon after the detection of BTV8 in several cattle farms of a small area of the south western part of the region, in order to prevent further spreading of the virus and eradicate the infection from the region.

The matter of the fact that the vaccination campaign in Piedmont was efficient as only 9 new BTV8 positive herds of the sentinel network were reported in the latter 2009. No infected cattle herds arise in 2010.

The survey conducted on wildlife susceptible ruminants in the area favorable to vectors, demonstrated that the BTV can also affect wildlife, underlining that susceptible wild ruminants should be included in surveillance programs also in relation to eradication purposes.

The results of this work can help in the design of a targeted surveillance program, for optimal utilization of human and financial resources for instance in the time period of risk for BTV outbreaks. This could dramatically minimize economic losses by early detection and rapid response [11].

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Antimicrobial resistance surveillance: bacterial prevalence estimates are not enough

H.M. Scott[1*], B. Norby[2] and G.H. Loneragan[3]

Abstract

Surveillance programs for monitoring antimicrobial resistance in foodborne (enteric) bacteria largely focus on estimating and comparing phenotypic prevalence among different livestock species, their farm environments and food products, and assessing trends over many years. Often, these monitoring systems have a high threshold for detection of rare resistance geno- and pheno-types owing to: 1) relatively small sample sizes that are further diluted by multiple species/strain/antimicrobial combination endpoints, and 2) the use of non-selective media that, while assuring unbiased resistance prevalence estimates at the bacterial population level, provide little confidence in freedom from the presence of bacterial resistance at the sample / animal / pen / farm / industry / country level, particularly for commensal (indicator) organisms. Much of the latter problem lies with the enormous size of the bacterial population in a single gram of feces, and therefore the relatively small probability of culturing a strain unless it is present in relatively high numbers.

Very few surveillance programs specifically target early detection and characterization of emergent strains of resistant bacteria, particularly in the years immediately following the introduction of new classes, variants or formulations of antimicrobial. This has serious implications for the utility of these surveillance programs in ensuring food safety and protecting public health. Using the relatively recently introduced 3rd generation cephalosporin class of antimicrobial and the more longstanding tetracycline class as contrasting examples, we illustrate an alternative approach to monitoring and surveillance that blends elements of more traditional resistance prevalence estimation with targeted detection and strain characterization; this, in order to better understand novel threats to food safety and potentially mitigate emerging problems before it is too late.

Keywords: antimicrobial resistance, surveillance, emergence, detection.

Introduction

The emergence, dissemination, propagation and persistence of bacteria resistant to a variety of antimicrobials have plagued human and veterinary medicine for almost as long as the antimicrobials themselves have been in existence. Indeed, the relative prevalence of resistance (measured as a proportion of total bacteria of a certain bacterial family, genus, species, or strain) often correlates very well with the number of years since an antimicrobial was first marketed, as well as the number of formulations sold, and doses used in the intervening time from introduction to the present. Of particular concern to animal agriculture are those zoonotic bacteria such as the enteric pathogens found in livestock that sometimes find their way to the consumer via the food supply [1].

Worldwide, there exist a number of surveillance systems for monitoring antibiotic resistance in enteric bacteria of human and animal origin (*e.g.*, 2, 3). These systems have proven very useful for characterization of bacterial populations over long periods of time, and have given rise to some very important and sometimes novel findings. Most of these systems are designed to estimate the bacteria-level prevalence of specific resistance phenotypes within sub-populations of pathogenic and commensal bacteria. The detection limits of these systems tend to be relatively high, often due to modest sample sizes. This means that for bacterial populations exhibiting high levels of resistance (*e.g.*, to tetracyclines) prevalence estimates will be quite stable and relatively precise. However, when resistance levels appear to be very low (or, even zero) there exists uncertainty as to the actual prevalence or the presence or absence of resistance in the population and when it truly might have emerged (see Figure 1).

For resistant commensal bacteria to be estimated at a very low equilibrium prevalence of say, 1%, and based on a total population size of 10^8 *E. coli* per gram of feces, 10^6 (i.e., fully 1,000,000) resistant bacteria must exist in a single gram of feces to provide a minimally stable estimate. Achieving this quantity in a population of bacteria can require decades of fitness improvement among emergent resistant bacteria. During the years following the introduction of a new antimicrobial into the market, resistant bacteria can falsely appear to be absent. Surveillance goals should include as goals the determination of when resistance first emerged in a population of bacteria, and an understanding of the features contributing to its rise before it becomes firmly established and irreversible.

While existing approaches are invaluable for characterizing medium and long-term trends in resistance, especially for human clinical pathogens, we contend that the US National Antimicrobial Resistance Monitoring System (NARMS: Figure 1) [3], and very similar programs like the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) [2], could benefit by including in their animal and retail meat surveillance a sample-level (be it farm, pen, animal, carcass, or meat) estimate of resistance prevalence. Expressed another way, it behooves the

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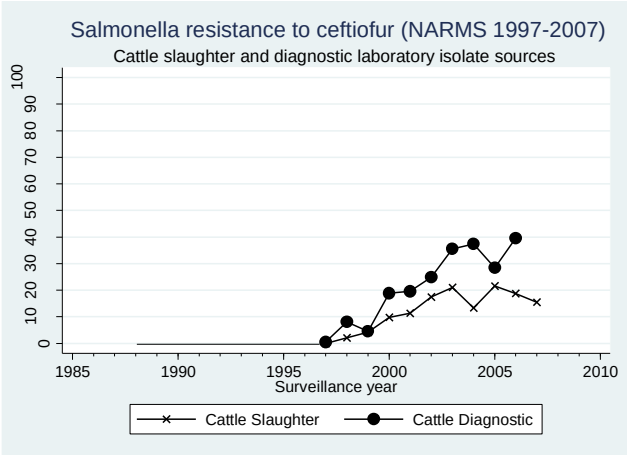
epidemiologist to correctly classify the sample (or, aggregate) as to the presence or absence of resistant organisms or traits. This is especially important for recently introduced antimicrobials and those with apparently low or else zero (i.e., undetectable) levels of resistance.

Figure 1: Ceftiofur prevalence in *Salmonella* from two cattle sources in the USDA-NARMS animal sampling arm from 1997-2007. Note the apparent zero prevalence of resistance from 1988 (when ceftiofur was introduced; not shown) to 1998 [3].



Approaches

In our approach, based on a model of enteric microbial ecology supported by empirical data from several of our research groups' field trials and two national surveillance systems, we consider North American beef feeder and dairy cattle in their respective production settings as our model host systems. We further refine our focus to two representative antimicrobial classes and resistance endpoints; that is, resistance expressed by bacteria to tetracyclines and to 3rd generation cephalosporins. The tetracyclines represent a very long-standing, broad-spectrum, and widely used class of antibiotic in cattle with therapeutic individual- and group-level, and growth-promotion formulations available in the U.S. and Canada. Ceftiofur is a relatively recently introduced 3rd generation cephalosporin, typically used at the individual-animal level; however, increasingly longer-acting formulations have lead to expanded group-level use in cattle. The World Health Organization (WHO) has proclaimed 3rd and 4th generation cephalosporins (along with fluoroquinolones and macrolides) [4] to be critically important for human medicine and have called for restrictions on their use in animal agriculture [5].

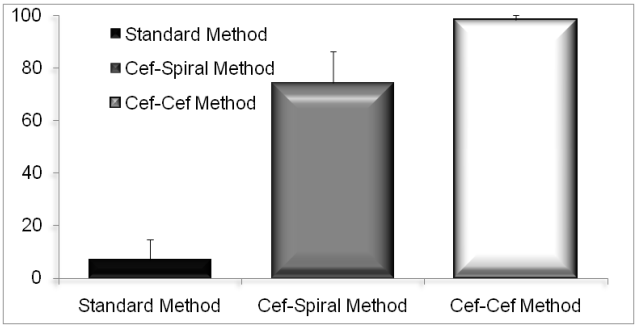
Importantly for our approach, among Gram-negative enteric bacteria there are relatively few known resistance mechanisms against ceftiofur and these are believed at present to be largely plasmid-borne. On the other hand, there exist several tetracycline resistance mechanisms, coded on multiple genes arising from a variety of classes, and housed in both genomic and plasmid DNA. In our surveillance system, host resistance prevalence would be presented and analyzed both as absolute quantity and relative carriage of: 1) ceftiofur-and tetracycline-resistant phenotypes, and 2) ceftiofur-resistance, tetracycline-resistance and total bacteria reference genes (*e.g.*, $bla_{\text{cmy-2}}$, *tet*(A) and *tet*(B), and 16SrRNA, respectively) in the feces of cattle. In this paper, we restrict our consideration of resistance phenotype to *E. coli* for a number of reasons; chief among them that the mobile resistance elements of concern are readily transferred via plasmids to those foodborne pathogens (*e.g.*, *Salmonella*) for which antibiotics remain important in human medicine and *E. coli* are readily and consistently cultivated from almost all fecal samples.

Example

As a proof-of-concept pilot study, in the autumn of 2009 fecal samples were collected from 70 U.S. feeder steers. Feces were inoculated onto MacConkey (Mac) or Mac supplemented with ceftiofur (8 µg/mL; Mac-Cef) agar plates. Feces were also inoculated in Mac-Cef broth. Agar and broth were incubated for 24 hrs at 37C. Approximately 1 month later, fecal samples were collected from another 20 steers. Ten g feces were inoculated into 90 mL Tryptic Soy Broth then streaked onto Mac agar and spiral-plated onto Mac-Cef agar. Additionally, 1 g of feces was inoculated into 9 mL Mac-Cef broth. Agar and broth were incubated for 24 hrs at 37C. Up to 9 distinct colonies from a Mac agar was inoculated onto a grid-layout Mac-Cef plate. Broth was streaked for isolation on a Mac-Cef plate. Plates were incubated as previously described.

From the initial 70 samples, 22.9% (95% CI, 13.0 to 32.7) were positive from direct plating onto Mac-Cef whereas 77.1% (67.3 to 87.0%) were positive when Mac-Cef broth and then Mac-Cef agar were used. The former estimate (22.9%) suggests lower sensitivity for detecting ceftiofur resistance presence in the fecal sample, whereas the latter estimate (broth step) of 77.1% is likely a better estimate of 'sample-level' prevalence. Of the 20 samples directly inoculated onto Mac agar then onto Mac-Cef, the estimate of prevalence of steers harboring ceftiofur-resistant E. coli was 10% (0.0 to 23.1%). The sample/animal-level estimate of prevalence in the second study for direct spiral-plating onto Mac-Cef plates was 90% (76.9 to 100%). Prevalence using both Mac-Cef broth and agar was 100% (83.9 to 100%) (Figure 2).

Figure 2: Sample-level prevalence of ceftiofur-resistant *E. coli* by isolation method. Standard method [7] is random selection of isolates from non-selective MacConkey agar, Cef-spiral is spiral-plating directly to MacConkey agar infused with 8 µg/ml of ceftiofur sodium. Cef-Cef involves broth enrichment prior to plating on infused agar.

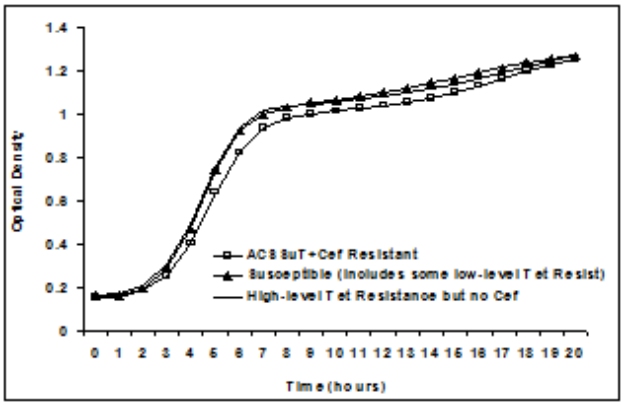


Discussion

Our example illustrates that sample-level prevalences are grossly underestimated when using non-selective approaches, and even selective approaches without broth enrichment steps. If we had conducted our study in the year 1999 rather than in 2009 it is highly probable that we would have estimated the sample-level prevalence of ceftiofur resistance at a very low level unless we incorporated both an enrichment and selective step. We speculate this is what likely has happened in the U.S. over the past two decades and as a result we may have missed an opportunity to track, understand, and act against the emergence, spread, and propagation of emerging resistant strains.

Relatively low levels of resistance to ceftiofur among commensal enteric bacteria like *E. coli* characterized much of the late 1990s. The rise in levels of resistance to ceftiofur in the US cattle population has been quite marked since 2000 [3], while somewhat less so in Canada [2]. Interestingly, in almost all isolates of *E. coli* and *Salmonella* from cattle, ceftiofur resistance (encoded by the bla_{cmy-2} gene) occurs in combination with a penta-resistant backbone (T-type) containing genes coding for resistance to: Ampicillin (A), chloramphenicol (C), streptomycin (S), sulfasoxisole (Su), and tetracycline (T): ACSSuT [8]. Ceftiofur resistance has thus far been restricted to plasmids making it readily shared among gram-negative enteric bacteria. Its association with other commonly used antimicrobials makes co-selection a threat, but also provides a relative fitness disadvantage when compared to other strains of *E. coli*, except when under selection pressures. We suggest that a combination of selective media with broth enrichment, combined with molecular and phenotypic characterization of features of relevance to modeling microbial ecology (*e.g.*, fitness via growth curves – Figure 3) be used to enhance surveillance to provide tools to understand resistance emergence and potentially take action while emerging strains remain relatively less fit than established strains.

Figure 3: Mean growth curves for three different resistance phenotypes (n=133 total isolates) estimated using a standardized optic density technique



The curves in Figure 3 suggest a disadvantage in terms of reproductive rate (in non-competitive MacConkey broth) for those *E. coli* bacteria exhibiting the ceftiofur + ACSSuT R-types versus those either largely susceptible to all antimicrobials or else to tetracycline only. Such disadvantages could play a role in mitigating emerging resistance strains in the future if surveillance programs grow beyond simply estimating prevalence.

Summary

We propose enhancing antimicrobial resistance monitoring and surveillance efforts to include enrichment and selective media that favor relatively less fit but emerging strains of bacteria; particularly during the years following the introduction of new antimicrobial products. We provide evidence that such approaches yield much higher sample (or, animal) level estimates of prevalence than current surveillance schemes estimate. The latter are (rightly) designed to estimate bacteria-level resistance prevalence for more longstanding and established resistance types. However, there is an urgent need to also focus on emerging threats to animal and public health.

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Studies on Modifications of the Sampling Interval and Size in the Framework of CSF Surveillance of Wild Boar

J. Sonnenburg[1*] and C. Staubach[1]

Abstract
Classical swine fever is a serious viral animal disease affecting pigs and wild boar. In wildlife population surveys data are often reported as small-area counts in time and space. Resources are tremendous to detect low virological prevalence for CSF in wild boar. Nevertheless up to 1.2 / 5.1 samples per sqkm were taken per month / year in some regions of Germany.

A methodology was applied to show, that modifications of the sampling interval and / or the sample size will save resources. The probability of the detection of the infection can only be guaranteed until a certain limit.

Keywords: wild boar, surveillance, Classical Swine Fever.

Introduction

Classical swine fever is a serious viral animal disease affecting pigs and wild boar. It has caused major socio-economic damages in the EU during the last decades [1, 2].

Disease monitoring and disease surveillance systems (MOSS) are of increasing importance to veterinary authorities and policy makers because they allow the detection of changes in the prevalence of infectious diseases at an early stage and can lead to the fast implementation of control measures.

The present work was developed in the framework of the CSF_goDIVA project of the EU. It is part of the work package: early warning systems of CSF and surveillance in domestic pigs and wild boar.

Materials and methods

As study area we used 65 hunting grounds (about 230 sqkm) in the district Euskirchen in the Federal State North Rhine-Westphalia within the municipalities Euskirchen and Bad Münstereifel. We considered a time period of 72 month from 1st January 2004 until 13th December 2009 (state: 15th December 2009). During this time period 4652 virological investigations with 72 virological positive results were recorded.

We conducted a simulation study. We merged the monthly virological investigations into several sampling periods of 2, 3, 4 and 6 month length. Out of these merged investigations we sampled randomly 45 or 90 samples and tested if we found at least one positive sample. We repeated this sampling 100 times to estimate the probability of detection of the infection.

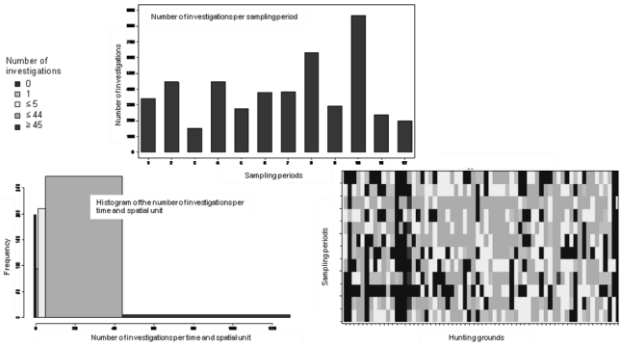
Result

The histogram of the number of investigations per hunting ground shows that 36 of the 65 spatial units sampled more than 45 animals that are necessary to detect 5% prevalence in a population of 100 animals with a confidence level over 95%. Separated for time and space, the overall numbers of investigations seem to be sufficient and detecting at least one case. But the distribution of the investigations in time and space show only 4 of 4680 fields contain more than 45 samples. Most of the combinations between time and space are empty or poorly investigated (without figures).

To check, if the number of fields with an adequate number of samples taken would increase and still detecting at least one case, we merged in the first step the time periods into several sampling periods of 2, 3, 4 and 6 month length.

Figure 1 shows the results for the 6-months sampling period and consists of 3 plots. The first two plots show the distribution of investigations over time and spatiotemporal as histogram. Furthermore, the number of investigations in time and space were plotted. The grid shows that also for the 6-months period there is a lack of data. Only 6 combinations of time and space reach the required sample size of 45 and higher. Nevertheless, the combinations with more than 5 samples increased significantly.

Figure 1: Distributions of investigations in time and combined in time and space for the 6-months sampling period



In the next step of our study, we simulated the sampling of 45 samples out of the merged investigations. We repeated the sampling 100 times and count the cases with at least one positive result within the sample. During the 2-months sampling period there were only 7 sampling periods with virological positive results. In the sampling simulations we found 3 out of these periods with a probability over 95%. Table 1 shows the results for all merged sampling periods. Only 25.0 to 42.8% of the virological positive sampling periods could be found with a sample size of 45 samples. Therefore, we enlarged the sample size to 90 samples per sampling interval.

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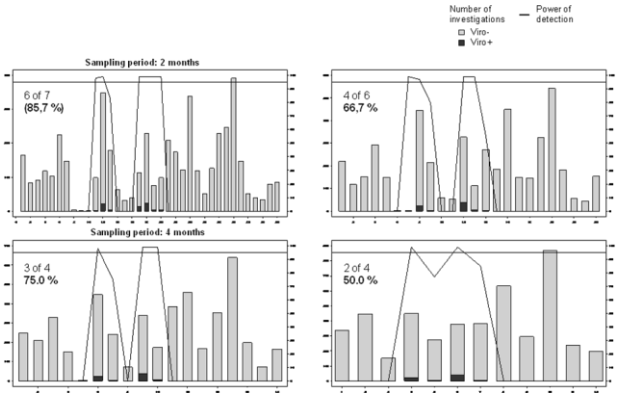
Table 1: Simulation results with a sample size of 45

Sampling period	*Probability of detection > 95%*
2 months	3 of 7 viro+ sampling periods **(42.8%)**
3 months	2 of 6 viro+ sampling periods **(33.3%)**
4 months	1 of 4 viro+ sampling periods **(25.0%)**
6 months	1 of 4 viro+ sampling periods **(25.0%)**

Figure 2 shows the results for this study. With 90 samples per sampling interval we were able to find 50.0 to 85.7% of the positive sampling periods.

Figure 2: Results simulation study concerning the surveillance in the merged sampling periods with a sample size of 90



Discussion

Modifications of the sampling interval and / or size save resources. Nevertheless, the probability of detection of the infection can only be guaranteed until a certain limit.

In the study several sampling intervals and sizes were tested. An optimal scheme could be developed based on assumptions regarding population density and design prevalence. Furthermore, targeted sampling of certain age classes, passive sampling and ecological driven time periods will increase the probability of detection of the disease. In CSF free areas same methods could be applied for targeting serological positive animals.

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The authors wish to thank all the veterinarians and hunters of the district Euskirchen, North Rhine-Westphalia for the successful co-operation. We would like to thank Doris Kämer and Kathrin Teske for the record of supplemental data and Detlef Klöß for technical support.

Syndromic surveillance in animal shelters: Practicality, feasibility, and validity of its application to prevent and control infectious/zoonotic disease

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Abstract

This paper proposes the application of syndromic surveillance as a method of disease surveillance to improve detection, prevention and control of diseases in animal shelter populations. Syndromic surveillance has been used for bioterrorism detection, pandemic flu, emerging diseases, nosocomial infections and in veterinary referral hospitals, but has been underutilized in general in veterinary medicine. In animal shelter populations where funding is limited, staff difficult to keep trained and infectious and zoonotic disease levels high, syndromic surveillance could be a practical approach to disease surveillance. Syndromic surveillance's simplicity and ease of use make it a feasible approach to disease prevention and control in animal shelters. Research is needed to determine the validity of a syndromic surveillance system for companion animals in shelters. Animal shelter policy makers have limited experience with the potential for this type of approach for disease control in their animal population. A sound policy will require building the awareness among policy makers with a scientific and practical method of syndromic surveillance in animal shelters.

Keywords: syndromic surveillance, animal shelter, companion animals

Background

Animal shelters vary tremendously in their size, facility, organization, budgets, numbers of workers and level of staff and volunteer training. The American Humane Association estimated in 1990 that there were between 3,000-5,000 animal shelters in the United States with 16.3 to 27.1 million dogs and cats entering these facilities each year [3]. International numbers are unknown Shelters worldwide range from rescue groups that house a few animals a year to major city facilities where thousands of animals pass through yearly. Some provide no other services than impounding of strays and/or relinquished animals, some provide full spay and neuter services for the shelter as well as surrounding community.

Most shelters have high turnover of staff and volunteers so that even those shelters with training programs may not have a well informed staff at all times. The age and education level of shelter employees and volunteers varies greatly from high school students looking for animal experience to college educated managers, directors and veterinarians and retired persons volunteering their time. Shelters may also have foster care programs that have participating families with small children and/or older relatives in the home. This variety in age, education level and level of involvement of those in animal shelter work is an added challenge to the prompt recognition of animal disease and in delivery of animal health care.

Sheltered animal in developed and developing countries suffer from high levels of infectious disease [1]. The challenges to disease prevention and control in animal shelters are great and disease surveillance in veterinary medicine in general is an underused and uncommon practice [2]. An unknown number of animals worldwide suffer morbidity and/or mortality due to infectious disease while housed in animal shelters. Most animal shelters do not have an infection control plan [4] and given the volume and density of animals, control of disease spread is a major problem. Fighting the spread of disease is one of the most frustrating, time consuming and costly jobs in animal shelters [5]. The impact and consequences of zoonotic disease in animal shelters remains unknown. Because of the volume of animals with unknown medical and behavioral histories encountered on a daily basis, shelter workers and volunteers may experience greater exposure to zoonotic diseases. Many of these diseases, such as rabies, leptospirosis, anthrax, and ringworm, have been around for hundreds of years, where others such as SARS, MRSA, leptospirosis and monkey pox are new and emerging. Animal health authorities in several countries have direct or indirect involvement in animal shelter populations mainly due to the zoonotic disease control programs. Funding for animal shelters varies considerably but is usually very limited. Limited funding often precludes diagnostic testing to determine etiologies and may also preclude administration of medicines and vaccines for prevention and control of disease.

Syndromic surveillance is a method whereby disease trends are tracked using health-related data to signal a "case" or "outbreak" before definitive diagnosis is made [6]. Clinical signs of disease are grouped into "syndromes" and when a critical threshold is reached in a population, investigation is triggered [7]. Syndromic surveillance has often been used to identify outbreaks in complex populations not easily monitored by other methods [2]. Data collected by this method do not detect a specific disease but is used as an early warning system. Syndromic surveillance is easier to implement because it does not require lab diagnosis or detailed clinical interpretation [2]. Syndromic surveillance has been used in veterinary hospitals, particularly large referral centers, to quickly and easily identify the indicators of infectious disease [2].

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Syndromic surveillance could be part of animal health policy to prevent and control infectious and zoonotic diseases in animal shelters. This paper suggested syndromic surveillance could be used in animal shelters to decrease the time to response/intervention thus decreasing morbidity and mortality in 4 ways - as a simple, straightforward method of disease detection and triggering of heightened infection control practices in individual animals; as a method of outbreak detection in the shelter population; and as a disease prevention activity by identifying animals on arrival, before entering the shelter and before they can potentially spread infectious or zoonotic diseases to the rest of the shelter population. In animal shelters with computerized record keeping systems, syndromic surveillance data could also be linked to national databases and serve as part of an early warning system for emerging diseases.

Training shelter workers on disease terminology, etiologies, and specific clinical signs associated with particular diseases and the various modes of transmission require a level of training and education on the part of the trainers as well as the trainees that may or may not exist in this group. The information can be confusing and a volume of information necessary to act in the face of disease may be overwhelming. Identification of syndromes (5 or 6 syndromes) – with associated infection control action responses - may be a method more quickly and easily taught, adopted and retained by shelter workers, volunteers and foster families.

Application of syndromic surveillance may help reduce the burden of disease in animal shelters globally. This policy paper suggests the investigation of the application of syndromic surveillance in animal shelters for the purpose of increased animal welfare, prevention and control of infectious and zoonotic disease, to alleviate suffering and improve the wellbeing of sheltered animals worldwide. The authors seek input and advice from the international surveillance community to move forward with these goals.

Areas in need of further investigation

In order to determine the feasibility and validity of applying syndromic surveillance in animal shelters some work needs to be done.

Defined disease syndromes: Well defined syndromes are necessary particularly syndromes focused on companion animals in an animal sheltering setting. Suggested syndromes might include: **Intake** (a general disease sign based on change in the volume or type of food intake); **Output** (a general disease sign based on change in volume or consistency of feces, urine or vomit – use of fecal scoring sheets; **Activity/temperament** (a general disease sign, rabies, *etc*. based on change in activity, behavior, depression, lethargy); **Respiratory system** (indication of upper or lower respiratory disease: discharge from eyes, nose, sneezing, coughing); **Coat/skin** (indication of ringworm, parasites, MRSA: presence of fleas, skin lesions); **Fever** (a general disease sign) and **Human illness** (any human health conditions on that day/week/month).

Methods of data collection: How will data be collected, by whom, what is the most user friendly form? Would handheld devices be practical in some situations? Where will data be stored – shelter computer if available or central location? How will data be analyzed and by whom and what is the best reporting system for getting information back to the shelter?

Baseline syndrome rates: Determination of baseline rates of defined disease syndromes must be determined in order to set thresholds over which action will be taken [7]. This may have to be shelter or shelter-type specific because disease varies considerably between shelters and regions and would require collection of data for a period of time in order to determine “normal” levels of syndromes

Trigger and response: For individual cases, any change in parameter for the individual animal would be a signaling event and would trigger taking it to a higher level such as infection control officer, shelter manager, and veterinarians. Actions would include increased infection control activities including isolation, hand hygiene, barrier precautions, elevated cleaning and disinfection and others. For disease outbreaks a change in reporting over threshold instigates action which might include shelter wide precautions, Inter-shelter communication, national reporting.

It is anticipated that application of a syndromic surveillance system in animal shelters and the ongoing collection, analysis and interpretation of the data generated would signal individual disease events, trigger infection control and personal protection responses, detect disease earlier, prevent illness, decrease costs, decrease antimicrobial drug use and improve animal wellbeing.

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The Belgian MoSS: A Monitoring and Surveillance System for the early detection and identification of (re-)emerging animal diseases

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Abstract

To ease the early detection of (re)emerging animal diseases, the Belgian sanitary Authorities promoted the study on and the implementation of a web based application designed to manage the monitoring and surveillance of atypical syndromes. This "Monitoring and Surveillance System" (MoSS) website is an epidemiological information system which allows the detection of unusual syndromes in domesticated and wild animals, via an online recording system to be used by veterinary field practitioners and (veterinary experts). A hierarchical ascending clustering process automatically compares new records with all records in the database and creates clusters of cases based on homology in clinical description, animal typology and spatial-temporal proximity. The onset of a new cluster is accompanied by the invitation of an expert to lead the identification process via a dedicated forum page, where information about the evolution of cases, treatments and lab results can be shared, to facilitate the diagnostic process. In the MoSS, essential data on atypical syndromes is centralized and analyzed to shorten the detection time of any possible emerging disease in animals. This PhP-MySQL application runs now in English, French and Dutch and manages with cross-border reporting.

Keywords: emerging diseases, MoSS, early detection, cluster analysis.

Introduction

The analysis of the process that led to the identification Bluetongue in Belgium (2006) identified a lack of structured communication between field veterinary practitioners, confronted with an emerging disease, and experts scattered over several institutions. Also, it was agreed that a system was needed to reduce the delay between the occurrence of first clinical signs - of a possible emerging disease – and the identification of the causative agent, to restrict animal discomfort and other socio-economic consequences related to outbreaks of epidemic diseases. As a response to this understanding, Belgian Veterinary Authorities promoted the development of a focal point based on the online reporting and of atypical syndromes by veterinary field practitioners and experts in different fields of expertise. This was meant to detect and follow-up clinically suspicious cases; a crucial element for the early detection of (re-)merging diseases by means of passive surveillance [1]. The project led to the development of the online web application 'MoSS' (Monitoring and Surveillance System). The MoSS is an epidemiological information system which aims at i) the facilitation of early detection and identification of emerging animal diseases and ii) the development of a strong epidemiovigilance network of (veterinary) practitioners and experts, collaborating in the diagnostic process and identification of the causative agent of possible emerging syndromes. In the past 2 years, a feasibility study, functional analysis and software development resulted in a first implemented version of the MoSS website [2]. Recent developments and improvements with regard to communication and feedback tools have led to a second version, to be found at http://www.moss.be.

Materials and methods

To detect new emergences, the MoSS project is based on active reporting of atypical syndromes by veterinary field practitioners and veterinary experts. An overview of the process of web-based recording and analysis in the MoSS is shown in Figure 1. Registered web users are encouraged to record three categories of atypical syndromes into the system: i) unknown, emerging syndromes/diseases, ii) known diseases with an unusual clinical expression and/or non-responding to the usual treatment and iii) rare or inadequately documented sporadic disease and re-emerging diseases. After entering the secured website, the user has access to an enquiry form which collects information about cases with regard to date of onset, geographical location, clinical signs, epidemiology and possible factors related to the occurrence of the observed case. Subsequently, records are clustered based on the real-time automatic comparison between new records and all previous records. Similar cases with regard to clinical signs, affected animal categories and spatio-temporal proximity are automatically grouped using a fully definable hierarchical ascending clustering process. This method creates a dissimilarity matrix based on pair wise comparison of all records in the database, for which differences and similarities are defined using logical rules. The dissimilarity matrix is subsequently used as input for the ascending clustering process, which keeps on combining records and clusters until a on forehand chosen intra-cluster dissimilarity threshold is met. Parameter settings of the clustering method are fully adjustable, such as the relative weight of differences vs. similarities, as well as weight of the typologies clinical signs / affected animal categories / spatio-temporal proximity. The chosen classification method seems particularly suitable for the detection of clusters of atypical cases, as it is capable of finding clusters of cases without any prior information on the number of clusters to be obtained nor their features.

It is foreseen that the same classification principle will be used for the comparison of new records with reference records that describe typical cases of OIE-listed diseases, to avoid misreporting of possible notifiable diseases in the MoSS.

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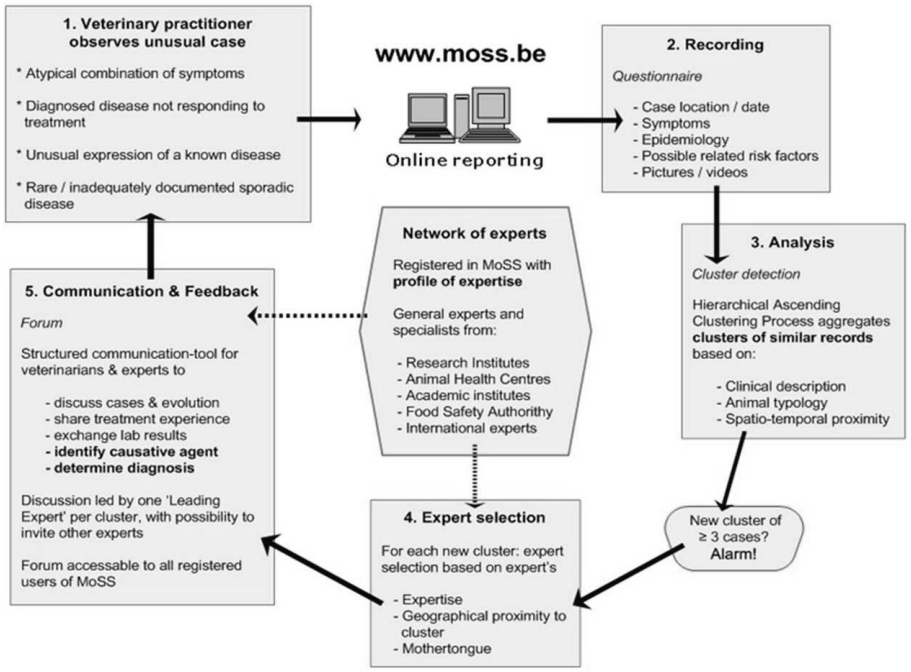
Figure 1: Schematic overview of online reporting of atypical cases and subsequent analysis and feedback in the epidemiological information system MoSS.



Aiming at fast disease identification and control, an alert signal provided by the onset of a new cluster will be followedby the selection and invitation of the 'best-fit' registered expert with regard to the content of the cluster in question. Subsequently, efficient communication between the veterinary field practitioners and expert – or several experts if needed - is organised on one dedicated forum page per identified cluster. The forum allows sharing structured information (lab analyses, evolution of symptoms) and will connect all levels of expertise to facilitate the diagnostic approach. The forum will be accessible to all registered users of the system and it is expected that the supply of information about similar cases will act as an incentive for both veterinary practitioners and experts to further use the MoSS.

Preliminary results

Although the MoSS-website is in the final stage of construction, records of atypical syndromes have been made yet by a number of veterinary experts who are acquainted with the system. The clustering and mapping processes were tested using real life data: records of Bovine Neonatal Pancytopoenia (BNP), a currently emerging syndrome in calves in Europe. Several records were registered in the system by different observers and subsequently compared with "noise" in the database. Figure 2 shows a classification result in MoSS at a certain time point. This cluster contains 78 BNP records made by 5 users, with a chosen intra-cluster dissimilarity threshold of 55%. It needs to be emphasized that a situation described here is not likely to occur in 'real life', as new clusters of 3 or more cases (and subsequent related cases) will be followed up and analysed by a (team of) expert(s) and therefore excluded from the ongoing clustering process.

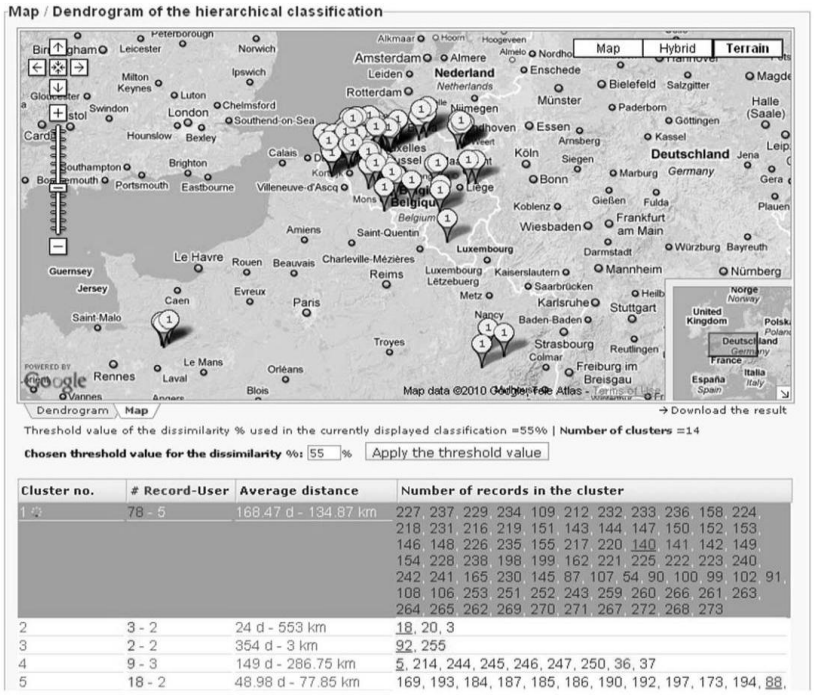
When the BNP cluster was created, none of the remaining records in the database concerned BNP, which shows the system's potential to discriminate the related BNP cases from the surrounding noise, irrespective of personal variation between the authors of BNP records.

Discussion

The preliminary results show an efficient identification of clusters by the hierarchical ascending clustering method implemented in the website. However, as MoSS' clustering method is fully adjustable, changes in parameter settings and intra-cluster dissimilarity threshold will lead to alternative classification results. Therefore, validation is needed to test and optimize MoSS' analytical characteristics. Internal validation and calibration should lead to the most optimum combination of parameter settings, to detect a potential emerging syndrome with maximum timeliness and sensitivity. External validation will be performed using historic data of model diseases, where identification of clusters of records grouped by model disease is expected.

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

Figure 2: Classification result of BNP records in the MoSS



Cluster no.	# Record-User	Average distance	Number of records in the cluster
1	78 - 5	168.47 d - 134.87 km	227, 237, 229, 234, 109, 212, 232, 233, 236, 158, 224, 218, 231, 216, 219, 151, 143, 144, 147, 150, 152, 153, 146, 148, 226, 235, 155, 217, 220, 140, 141, 142, 149, 154, 228, 238, 198, 199, 162, 221, 225, 222, 223, 240, 242, 241, 165, 230, 145, 87, 107, 54, 90, 100, 99, 102, 91, 108, 106, 253, 251, 252, 243, 259, 260, 266, 261, 263, 264, 265, 262, 269, 270, 271, 267, 272, 268, 273
2	3 - 2	24 d - 553 km	18, 20, 3
3	2 - 2	354 d - 3 km	92, 255
4	9 - 3	149 d - 286.75 km	5, 214, 244, 245, 246, 247, 250, 36, 37
5	18 - 2	48.98 d - 77.85 km	169, 193, 184, 187, 185, 186, 190, 192, 197, 173, 194, 88,

As for any system based on clinical observations, MoSS relies to a great extent on the experience, scientific knowledge and alertness of veterinary practitioners, as well as their goodwill to report unusual findings to contribute to animal/public health. Even when the success of the MoSS is proven by experience, it remains a challenge to keep veterinary practitioners motivated to continue submitting data after the newness of the surveillance system diminishes [3]. In addition, veterinary practitioners might initially be somewhat reluctant in reporting a case of a potential emerging disease, perhaps afraid for the consequences that it might have. These aspects may result in underreporting and thus an underestimation of the true incidence of clinical disease in the population, which is not uncommon in passive reporting strategies [4, 1]. Moreover, it creates a great importance of providing good training and education to both veterinary practitioners and experts on the MoSS project. Also, the various functions of the website, the added value for the veterinarian reporting a case in the MoSS and the foreseen communication and actions in the case of a potential emerging disease need to be constantly communicated in a comprehensive manner. In addition, providing fast feedback is not only an incentive to reporters; it is of great importance for both detection of a potentially emerging disease (identify possible causative agent as soon as possible and restrict negative consequences) as possible monitoring of endemic diseases (immediate information on disease incidences and history).

The MoSS focuses on production animals but is open to all species on request. The website allows for multilingual management and cross-border reporting and can be easily managed by non-informaticians. The system is based on the centralization and analysis of available information provided by veterinary field practitioners and will be a critical tool aiming at shortening the detection time of any health-related event of importance in domestic animals. It is a first significant step in the preparedness for detection of the 'unexpected', as well as monitoring of endemic and epidemic diseases.

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Temporal Outbreak Detection for Real-Time Animal Health Surveillance

L. Zhang[1]*, J. Berezowski[2], T. Herntier[2], I. Jamal[1], Y. Qu[1] and G. Zeng[1]

Abstract
A two stage processing method using Poisson regression and an Autoregressive Integrated Moving Average (ARIMA) model was developed to improve the performance of the temporal outbreak detection methods.

Keywords: event detection, real-time data analysis, control charts, outbreak detection, preprocessing, syndromic data.

Introduction
Control charts are widely used in outbreak detection [1]. The most common control charts are Shewhart, Exponentially Weighted Moving Average (EWMA) and Cumulative-Sum (CuSum) [1]. The essential assumptions of control charts are that the data should be normally distributed, and independent and identically distributed (i.i.d.) [1]. However, most real-time surveillance data have explainable patterns of seasonality, day of week (DOW), with significant autocorrelation and linear trends. The existence of these patterns in time series can reduce the effectiveness of control charts by causing either higher false positive rates or late detection [1]. Many methods have been proposed to remove or reduce these explainable patterns in order to improve the performance of control charts. Preprocessing methods such as regression, differencing and Holt-Winters exponential smoothing have been shown to improve the normality of the data and reduce the effect of explainable patterns [1].

One proposed approach for temporal event detection is to use various modeling techniques to model the time series data in a preprocessing step and then apply control charts to the residuals [1, 2]. Alternate methods include modifying the control chart method [3, 4] for example by modifying the moving window used to calculate the baseline mean and standard deviation.

We propose a 2-step preprocessing method followed by the application of control charts to the residuals. The first step fits a Poisson regression (PR) model [1] to the data, followed by an Autoregressive Integrated Moving Average (ARIMA) model.

Materials and methods
Outbreak detection methods: The test statistic for a Shewhart chart on day *t* is calculated as:

$$z(t) = (x(t) - \mu)/\sigma,$$

where x(t) is the daily count, μ is the baseline mean, and σ is the standard deviation. If $z(t) > N_z$, where N_z is the upper control limit, an alert is signaled.

The test statistics for an EWMA chart on day *t* is calculated as:

$$T(t) = \frac{y(t) - \mu}{[\sigma(\lambda/(2-\lambda))1/2]}$$

where $y(t)$ is the smoothed daily count defined as:

$$y(t) = \lambda x(t) + (1-\lambda)x(t-1)$$

with $x(0) = 0$. If $y(t) > N_y$, an alert is signaled.

The test statistics for CuSum chart on day *t* is defined as:

$$S(t) = \max(0, S(t-1) + \bar{x}(t) - k), S(0) = 0$$

where $\bar{x}(t) = (x(t) - \mu)/\sigma$, and k is the shift of the mean to be detected. If $S(t) > h$, an alert is signaled, where h is the control limit.

Preprocessing: The data used in this paper are cattle disease submissions to the Veterinary Practice Surveillance (VPS) system of the Alberta Veterinary Surveillance Network (AVSN). The data are daily counts of submissions by veterinarians in private practice for the period from January 1 2006 to April 9 2010. Particpating veterinarian's classified sick cattle into one of 13 syndromes at the time of examination. Since there were no major disease outbreaks observed in Alberta during this period we assumed there were no disease outbreaks in the data.

Figures 1 and 2 are the time plot, autocorrelation function (ACF) plot and quantile-quantile (QQ) plot for Respiratory syndrome submissions. This time series demonstrates patterns of seasonality, DOW, autocorrelation and violates the assumption of normality.

To improve the quality of this data and remove or reduce the negative patterns it contains, a two steps preprocessing procedure (algorithm) was developed. The 1st step uses a PR model borrowed from [1] followed by an ARIMA model applied to the residuals from the PR model.

The preprocessing algorithm can be described as following:

1. A PR model was fitted to the data to remove or reduce the seasonality and the DOW effect. We used 11 dummy variables for the 12 months of the year, and 6 dummy variables for the 7 days of the week. The PR model includes these 17 dummy variables and a linear trend variable, as follows:

$$\log(x(t)) = \sum_{i=1}^{17} a_i \mu_i(t) + Lt + \varepsilon(t),$$

where $\mu_i(t)$ is the dummy variable. Our purpose was to obtain the residuals $\varepsilon(t)$. Calculations were performed using R project [5].

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2. An ARIMA model was then used to remove or reduce the autocorrelations of $\varepsilon(t)$. This step was optional depending on whether the residuals $\varepsilon(t)$ were stationary or not. An ARIMA model was only used if the residuals were non-stationary.

Figure 1: Time and ACF plots of Respiratory syndrome submissions

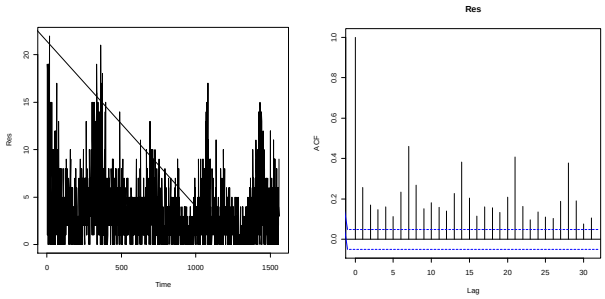
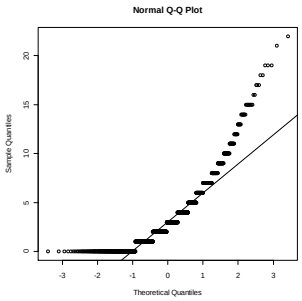


Figure 2: QQ plot of Respiratory syndrome submissions



The data was divided into training and testing data. Data from Jan 01 2006 to Dec 31 2008 were the training data and contained no disease outbreaks. Preprocessing models were derived fitting models to the training data for each syndrome. For Respiratory syndrome submission counts the preprocessing model was a PR model followed by an ARIMA (1,0,1). The time series, ACF and QQ (Figures 2, 4) for the Respiratory syndrome residuals suggest that these residuals were nearly normally distributed and i.i.d.

Residuals of daily counts: The outbreak detection method was applied to the daily count *x(t)* for each syndrome for each day of the testing period (Jan 1 2009 to Apr 9 2010). A residual *r(t)* between *x(t)* and the predicted value was estimated using a fitted pre-processing model and control charts were performed on the residual *r(t)*. The preprocessing model could be a PR model alone or a PR model followed by an ARIMA model. The algorithm for obtaining a residual *r(t)* for *x(t)* are as follows:

1. Predict the value $v_1(t)$ for the *t*th day using the PR model; compute the deviance residual between $x(t)$ and $v_1(t)$, denoted as $\varepsilon(t)$. If an ARIMA model is needed, go to 2); otherwise, $r(t) = \varepsilon(t)$.
2. Predict the value $v_2(t)$ for the *t*th day using the ARIMA model; compute the residual as $r(t) = \varepsilon(t) - v_2(t)$.

Figure 3: Time plot and ACF plot of Respiratory syndrome count residuals

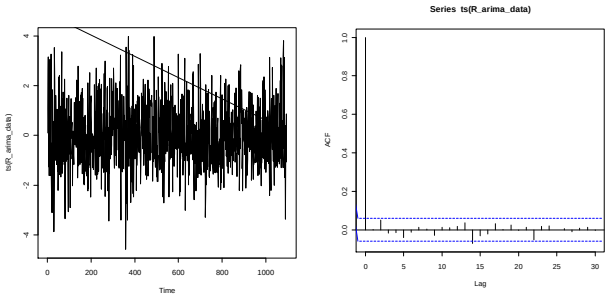
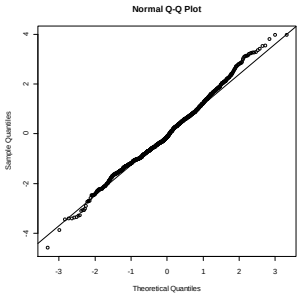


Figure 4: QQ plot of Respiratory syndrome count residuals



Outbreak simulation and insertion: Since there were no outbreaks in the training or testing data, outbreaks were simulated using the North American Animal Disease Spread Model [6] and inserted into the testing data for each syndrome. We used 9 different outbreaks, 3 slow, 3 medium and 3 fast outbreaks depending on the rate of disease spread. Outbreaks were inserted every second day for the entire 2009 calendar year resulting deleted in 1638 outbreak data sets for each syndrome.

Control chart methods were applied to the residuals during the testing period. With the false positive rate (FPR) fixed to one false positive in 1096 days, the sensitivity of each control carts was estimated, using the following two performance criteria:

1. Average Days to Detection (ADD): for one outbreak data set, the day to detection (DD) is defined as the number of days between the first day of the outbreak occurrence and the first day of alert since the first day of outbreak. ADD is the average of the DDs of the 1638 outbreak data sets.
2. Average Cumulative (ACum) number of herds: the number of herds infected during the DD is calculated for one outbreak dataset and the ACum is the average for all 1638 outbreak datasets. Generally, if a control chart has s shorter ADD, it usually has a smaller ACum. However, sometimes they are not consistent due to the speed at which the simulated outbreaks progress.

Performance was estimated for all dataset together and also for each of slow (ADDSlow, ACumSlow), medium (ADDMed, ACumMed) and fast (ADDFast, ACumFast) oubtrkeaks by averaging the results for each outbreak type.

Outbreak detection: For each outbreak data set residuals were calculated as in the section *Residuals of daily counts* above, for each day of the testing period, producing 1638 residual for each day. Control charts were applied to these residual sets to evaluate the performances in the following manner:

1. Parameter selection: With a fixed FPR, the parameter (N_z) of Shewhart chart was found by applying a Shewhart chart to the residuals of training data. μ and σ were computed as the mean and standard deviation of the residuals of training data.

   For EWMA and CuSum charts, there are many parameter pairs (λ, N_y) for EWMA, (h, k) for CUSUM corresponding to a fixed FPR. We chose to use the optimal parameter pairs such that ADD and/or ACum were the smallest with a fixed FPR.

2. Calculation of μ and σ: the training data was used to tune parameters for a fixed FPR. For outbreak detection, μ and σ were computed based on the baseline residuals from Jan 1 2006 to the previous day of detection. For example, if we perform detection for Jun 1 2009, then the baseline residual was from Jan 1 2006 to May 31 2009.

Results

The ADD and ACum for each control chart for the residuals of Respiratory syndrome submissions are presented in Table 1. For comparison, the ADD and ACum were calculated on the raw counts, without preprocessing (Table 2). For both, the FPR was fixed to 1/1096, which allowed one false positive alarm during the training period.

Discussion

Preprocessing improved the performance of all control charts significantly, for all three speeds of the simulated outbreak and for both measure of performance (ADD and ACum).

Table 1: Performance with preprocessing

Charts	Shewhart	EWMA	CuSum
ADD	39	32	32
ADDSlow	54	45	46
ADDMed	33	27	27
ADDFast	29	25	23
ACum	34	23	23
ACumSlow	31	21	22
ACumMed	34	22	23
ACumFast	36	25	23

Table 2: Performance without preprocessing

Charts	Shewhart	EWMA	CuSum
ADD	67	63	63
ADDSlow	89	85	85
ADDMed	57	52	52
ADDFast	55	52	52
ACum	196	145	145
ACumSlow	200	146	146
ACumMed	200	138	138
ACumFast	186	150	150

In the example given in this paper, data from Jan 1 2006 to Dec 31 2008 was used as training data and the data for the period after we used as testing data. Once the pre-processing and control charts are optimized, it will be possible to update the models every three to six months, using the most recent data.

Limitations to this work are that the PR model did not allow for the effects of holidays. The control charts were optimized using only one FPR. This assumes that there would be no difference in sensitivity of the control charts at differing FPRs. This may not be the case, and parameters making a control chart most sensitive at a FPR of 1/1096 may not make the control chart the most sensitive at a lower or higher FPR.

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Current and past management and staff of the Food Safety and Animal Health Division of ARD and participating veterinary practitioners in the Province of Alberta.

Optimization of Event Detection Methods for Disease Surveillance

L. Zhang[1], I. Jamal[1*], Y. Qu[1] and G. Zeng[1]

Abstract
EWMA and CuSum control charts are important methods for the surveillance of disease outbreaks. We propose a parameter optimization method for EWMA and CuSum to enhance the performance of the disease surveillance systems.

Keywords: monitoring systems, syndromic surveillance, pattern recognition, early warning systems, multiple sources of data data.

Introduction
Disease surveillance is the process of monitoring disease data in order to detect the outbreaks of an epidemic. The performance of the surveillance systems is very important to the decision makers. For example, one important criterion of the surveillance systems is the timeliness. If an outbreak can be detected earlier, then the decision makers can take some actions earlier to slow down the outbreaks (*e.g.*, isolation and immunization).

Control charts are widely used in the disease surveillance systems. The most common charts are Shewhart, Exponentially Weighted Moving Average (EWMA) and Cumulative-Sum (CuSum). In order to obtain an efficient surveillance system, there has been intensive work to compare the performances of different control charts and their modifications. However, there is no much effort in comparing the performances of one control chart with different parameters. These control charts have their own parameters which affect the performance of the surveillance systems dramatically. The purpose of this paper is to optimize the parameters of the control charts based on the evaluation of the performance of the surveillance systems with a fixed false positive rate (FPR), which is the ratio of false positive tests over all the tests performed.

This paper is organized as follows. In Materials and methods, the patterns of the disease data set are introduced, then the problem to be considered is given, followed by some preliminaries – outbreak insertion, preprocessing and residual deriving; in the Result part, the performance criteria are described, and the performance tables showing the performances caused by different parameters are given; the Discussion part includes some conclusions and possible future work.

Materials and methods
Data Introduction: A set of authentic disease data was used in this study. The data were the daily submissions of the infected herds from January 1 2006 to April 9 2010. There were no major disease outbreaks observed for this authentic data. The time plot and the autocorrelation function (ACF) plot are given in Figure 1, and the quantile-quantile (QQ) plot is shown in Figure 2. It can be observed that the data has the patterns of seasonality, the day of week (DOW), autocorrelation and the violation of normality.

Figure 1: Time and ACF plots of the data

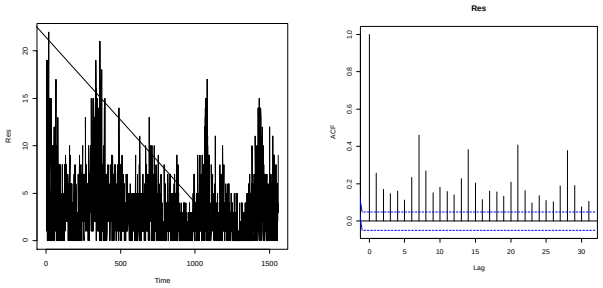
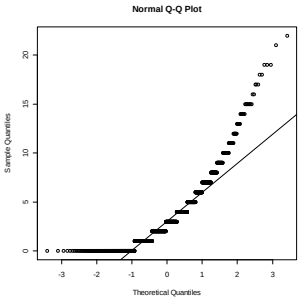


Figure 2: QQ plot of the plot



Problem description: The Shewhart, EWMA and CuSum control charts can be described as follows.

1. Shewhart chart: The test statistics on day *t* is calculated as
$$z(t) = (x(t) - \mu)/\sigma,$$
where x(t) is the daily count, μ is the baseline mean, and σ is the standard deviation. If $z(t) > N_z$, where N_z is the control limit, an alert is trimmed.
2. WMA chart: The smoothed daily count is defined as
$$y(t) = \lambda x(t) + (1-\lambda)x(t-1)$$
with $x(0) = 0$. Then the test statistics on day *t* is calculated as
$$T(t) = \frac{y(t) - \mu}{[\sigma(\lambda/(2-\lambda))1/2]}.$$
If $y(t) > N_y$, where N_y is the control limit, an alert is signaled.
3. CuSum chart: The test statistics on day *t* is defined as
$$S(t) = \max(0, S(t-1) + \bar{x}(t) - k), S(0) = 0$$
where $\bar{x}(t) = (x(t) - \mu)/\sigma$, and k is the shift of the mean to be detected. If $S(t) > h$, an alert is trimmed on day *t*, where h is the control limit.

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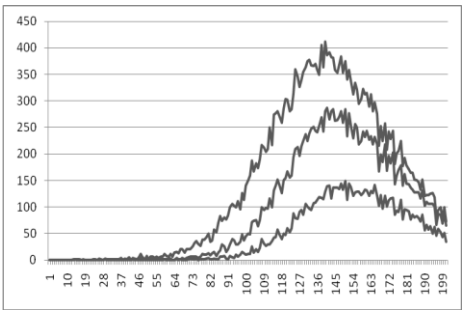
It can be seen that for Shewhart, since the baseline mean and standard deviation are available, the only parameter is

N_z. Given a FPR, N_z is then fixed, and no optimization is needed. However, for EWMA and CUSUM, there are two parameters, *e.g.*, λ and N_y for EWMA, h and k for CUSUM. In this case, parameter optimization is necessary in order to enhance the performance of the control charts. We use EWMA for illustration. Given a FPR, if λ is fixed as 0.1, we can find one corresponding N_y. Notice that there are many other λ (*e.g.*, 0.2, 0.3…) we can choose, which means that many parameter pairs of (λ, N_y) are available for one fixed FPR. Which pair is chosen so that the performances of the control charts are the best? This is the right goal of parameter optimization.

Outbreak simulation and insertion: Since there were no outbreaks in the authentic data, to evaluate the EWMA and CuSum charts, we simulated outbreak data and inserted them to the disease data set. We used a spatial simulation model, the North American Animal Disease Spread Model (NAADSM, 2), to get different outbreak data under the same scenario file. Because of the stochastic nature, different iterations may generate different outbreaks. Many iterations were run, and as a result, we could obtain many different outbreak data sets. According to the speed of the outbreaks, the outbreak data sets were classified as three categories: slow, medium and fast outbreaks. We chose 3 slow, 3 medium and 3 fast outbreaks for evaluation. Figure 3 demonstrates 3 outbreaks of one slow, one medium and one fast outbreak.

Figure 3: Time plots of different outbreaks



In order to avoid the effects of the patterns of seasonality DOW, and trends, these 9 outbreak data sets were inserted into the data set every other day from January 02, 2009 until the end of 2009 [1]. That means that the starting dates of outbreak insertions are on January 02 2009, January 04, 2009, and so on until the end of 2009. As a result, we inserted 182*9=1638 outbreak data sets into the authentic data set.

Preprocessing: EWMA and CUSUM were supposed to be applied to the 1638 data sets with outbreaks inserted. However, as stated earlier, the baseline disease data has some explainable patterns as seasonality, DOW, autocorrelation, linear trends and violation of normality. The efficiencies of the control charts depend on the assumptions of normality and independent and identically distributed (i.i.d) [3]. The existences of these patterns can reduce the quality of the control charts by causing a higher false positive rate or late detection [3]. In order to improve the quality, we needed to preprocess the baseline data by removing or reducing these negative patterns.

The baseline disease data was divided into training data from January 01 2006 to December 31 2008 and testing data from January 01 2009 to April 09 2010. It is noticed that in training data, there was no any counts caused by outbreaks. The preprocessing model was derived by fitting the training data. We proposed a two stage preprocessing method: fitting the authentic training data by a Poisson regression (PR) model to remove or reduce the seasonality, DOW, autocorrelation and improve the normality, and then followed by an Autoregressive Integrated Moving Average (ARIMA) model to further remove or reduce the autocorrelation. It was shown that the preprocessing improved the performance of control charts significantly. For the limitation of space, the details of preprocessing are omitted here.

Residuals of daily counts with outbreaks: Our purpose was to evaluate the event detection control charts by applying them to the 1638 outbreak data sets (data from January 01 2009 to April 09 2010 with outbreaks inserted). Thus we needed to obtain the residuals for all these 1638 outbreak data sets based on the preprocessing model derived from the training data. To this end, the following steps should be followed:

1. Predicted the values for time period from January 01 2009 to April 09 2010 using the preprocessing model;
2. Computed the residuals between the 1638 data sets with outbreaks inserted and the predicted values by Step 1.

By this way, we obtained 1638 residuals for the data sets with outbreaks inserted.

Result

Once the 1638 residuals of the data sets with outbreaks inserted have been obtained, it is ready to apply EWMA and CuSum control charts to these residuals. Before going on, some definitions are to be given as follows. With the FPR fixed, we can evaluate the control charts through their performances. We suggested use the following two performance criteria:

1. Average Days to Detection (ADD): for one residual data set, the day to detection (DD) is defined as the number of days between the first day of the outbreak occurrence and the first day of alert since the first day of outbreak. ADD is the average of these 1638 DDs.
2. Average Cumulative (ACum) number of herds: the cumulative number is the number of cumulative infected herds during DD. The average of the 1638 cumulative numbers is ACum. Generally, if a control chart causes a shorter ADD, it usually causes a smaller ACum. But sometimes, they are not consistent. If a control chart results in relatively

shorter DD on slow outbreaks, and relatively longer DD on fast outbreaks, its ACum could be larger since fast outbreaks can cumulate the number of infected herds much faster.

In order to see the performances of the control charts with respect to slow, medium and fast outbreaks, we also included ADDSlow and AcumSlow (ADD and ACum for slow outbreaks), ADDMed and ACumMed (ADD and ACum for medium outbreaks), ADDFast and ACumFast (ADD and ACum for fast outbreaks) in the evaluation tables. These performances were obtained based on the average of all slow, medium and fast outbreak data sets.

Now it is ready to introduce the evaluation process of the control charts. The control charts were applied to the 1638 residuals. The purpose of the evaluation is to find the best parameters among the possible parameters that can achieve the desired FPR so that the corresponding performance is the best. The evaluation process is illustrated as following:

1. Given a FPR, tuned the parameters based on the residual of training data: found the parameter pairs (λ, N_y) and (k,h) for EWMA and CuSum respectively. μ and σ were computed as the mean and standard deviation of residuals of training data.
2. Applied EWMA and CuSum with the parameter pairs tuned by 1) to the 1638 residuals and obtained the performances ADD, ACum, and so on. μ and σ were computed based on the baseline residuals from January 1 2006 to the previous day of detection. For example, if we perform detection for June 1 2009, then the baseline residual is from January 1 2006 to May 31 2009.
3. Evaluated the performance and found the best parameter pairs.

Table 1: ADD for CuSum of residuals

K	h	ADD	ADD Slow	ADD Med	ADD Fast
0.3	16.36	32	46	27	23
0.5	12.74	34	49	28	25
1.0	6.3	40	58	33	30
1.5	3.3	43	61	35	32
2.0	1.98	44	63	37	33
2.5	0.98	42	60	36	32

For the limitation of space, we only give the performance tables (Tables 1 and 2) of CuSum for the residuals of the 1638 data sets with outbreaks inserted under a fixed FPR=1/1096 (one alarm during training data period). The 1st column *k* was fixed first, and then *h* in 2nd column was tuned based on the residuals of training data to achieve the FPR.

Table 2: ACum for CuSum of residuals

k	h	ACum	ACum Slow	ACum Med	ACum Fast
0.3	16.36	23	22	23	23
0.5	12.74	27	26	27	27
1.0	6.3	33	32	31	35
1.5	3.3	37	36	35	40
2.0	1.98	42	40	41	44
2.5	0.98	39	36	39	42

Discussion

From the performance tables, it can be seen that the parameter pair (0.3, 16.36) results in the best ADD and Acum. It is optimal overall. On the contrary, (2.0,1.98) is the worst, and it causes 12 days later in ADD, and 18 more in Acum. By this way, the improvements in the performances by parameter optimization can be obviously seen. For slow, medium and fast outbreaks, (0.3, 16.36) is also optimal for this example. But it is not always so. It can be also observed that slow outbreaks are longer to be detected, but cause less ACums. Fast outbreaks give a reverse result.

It is noted that in our example, we have only inserted 9 outbreaks from different dates and obtained 1638 outbreak data sets. Since preprocessing has been proposed to remove the seasonality and DOW, in the future, we may increase the number of inserted outbreaks, but reduce the starting dates (now it is 182). By this way, the computation burden will not be increased, and the optimization will be more robust to different outbreaks.

So far we have only investigated the optimization of temporal surveillance methods. We are exploring the optimization methods for spatial and temporal/spatial data.

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The Ontario Farm-call Surveillance Project: Advantages of an active surveillance system

K. Zurbrigg[1*]

Abstract
Currently the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) relies on the surveillance of laboratory data to monitor livestock disease. Accurate and timely livestock disease surveillance requires additional data than what is currently available through the voluntary laboratory submission system. The Ontario Farm call Surveillance Project (OFSP) utilizes pre-diagnostic, farm-call data from private livestock veterinarians to identify outbreaks of disease in livestock. Compared to passive surveillance of laboratory data OFSP enhances the type and quantity of submissions to the laboratory, improves reporting on public health risks and strengthens farm veterinary practice.

Keywords: Veterinarian participation, value, cost-benefits.

Introduction

The OFSP was started in April 2009. The OFSP utilizes pre-diagnostic, health data to identify outbreaks of disease in livestock. The project is a pilot which has been funded for 4 years.

Currently the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) rely on the surveillance of laboratory data to monitor livestock disease. These data originate from voluntary submissions from livestock owners and are obtained easily and inexpensively. However livestock owners pay the full cost of diagnostic testing. Therefore the quality and quantity of data is dependent on the livestock owner's belief that testing is warranted. Additionally, a farm locator is often omitted on the sample submission form. Therefore spatial analysis of the data is not possible or is completed using the submitting veterinary clinic as a proxy for geographic region.

Accurate and timely livestock disease surveillance requires additional data then what is currently available through the voluntary laboratory submission system. The three objectives of the OFSP are to: 1) Determine if is it feasible to develop and maintain a surveillance system based on the farm calls of livestock veterinarians, 2) identify what advantages if any result from this type of surveillance system compared to the surveillance of laboratory data, and 3) determine if the operating cost of OFSP is warranted.

Materials and methods

Participating clinics-The veterinary clinics participating in the project are a convenience sample of food-production and equine clinics from across the province. Clinics were asked to participate based on their geographical location. Enrollment in the project is voluntary.

Data collection-Participating veterinarians record data including*:* the clinical signs and suspected diagnosis observed, the species and age of the animal, a farm locator, the date of the farm call, and the type of diagnostic samples that were submitted (if charged to the project's AHL account). Farm and owner name are confidential. A coding system set up by the clinic ensures confidentiality. Clinics submit data only on those farm calls where a health issue is observed. Data from routine farm calls (*e.g.* vaccinations or castrations) are not submitted, unless a sick/problem animal is examined.

Data Recording-The particular method of recording data is selected by each participating clinic. Recording of OFSP data is designed to enhance the existing system at the clinic and to make data recording as quick and easy as possible. Paper and electronic forms are used. Clinics are contacted if data is missing or illegible.

Data Transmission-Clinics using paper forms fax their forms to the project coordinator on a daily or weekly basis to a toll free number that sends the data as a PDF to the author's email address. Some clinics mail copies of the surveillance form once a week using prepaid express post envelopes. Other clinics submit data using the online form is downloaded weekly from a secure website. Iphone user farm reports are sent automatically via email as they are completed. Average length of time from completion of farm call until the OFSP receives data was calculated.

Analyses-Individual and groups of clinical signs (syndromes) are assessed by species and age group for temporal and spatial trends on a weekly basis using the Centre for Disease Control's (CDC) Early Aberration Reporting System (EARS) program. If the analysis generates a trend of interest, the veterinarians involved are contacted to determine if further investigation is warranted.

Compensation-Clinics participating in OFSP are compensated for their time through the ability to charge the cost of diagnostic testing at the AHL to the project's account. The rate at which a vet participating in the project elects to send in a diagnostic sample and charge it to the project's account was calculated (number of samples/total number of farm calls). The percent of livestock laboratory submissions that were from the OFSP as compared to total livestock submissions to the laboratory were calculated for April 2009–September 2010. The percent of livestock histology and necropsy submissions to the laboratory from the OFSP were also calculated as compared to the total livestock histology and necropsy submissions to

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the laboratory for the same time period. A list of all OFSP diagnostic submissions (from April 2009-Nov 2010) that resulted in a positive report for a zoonotic disease was generated. Veterinarians use the account at their own discretion. They are encouraged to use the account where the producer cannot or will not pay for testing and the veterinarian deems the case worthy of further diagnostic investigation. Participating veterinarians were contacted by the author and asked to describe cases where the ability to charge samples to the OFSP's account was uniquely beneficial to them. Any supplies or equipment that is needed to participate in the surveillance project are provided through the project's budget.

Cost Analysis-The project's operating costs to date were calculated by category and totaled.

Results

Twenty one large animal veterinary clinics (95 veterinarians) contribute data. Two are equine only, two are swine only and 17 clinics provide service for all livestock species. For all species, veterinarians participating in the project cover over 80% of moderate-high density livestock townships. The exception is small ruminants with only 30% of the moderate-high density livestock townships.

To date, data from 14,479 farms calls have been collected. The most common clinical signs for mature animals are: anorexia, depression, abnormal gut sounds, lameness and down. For preweaned animals the most common signs are: diarrhea, depression, anorexia, fever, down.

By species, the largest portion of farm call records is for dairy cattle (63.8%). By age, the largest portion of the records are for mature animals (84.5%). All farm call records can be mapped to a postal code region through the farm locator.

Fifteen clinics use a paper form to record surveillance project data. Four of the 21 clinics use an online electronic form and one uses an electronic form service paired with an iphone to record and submit data. One clinic created a report from their clinic software that contains the necessary surveillance data. The report is generated and emailed automatically. Forms with missing data are infrequent (less than 3%). However paper forms with illegible data are more common and associated with particular clinics.

The method of data transmission does not affect the timeliness of receiving the data but clinic does. Overall average number of days from completion of the farm call until the OFSP receives data is 14. Nine out of 21 clinics send data in 10 days or less from the completion of the farm call.

To date, no major outbreaks have been detected. Numerous clusters of data within a region or timeline have been discovered and followed up with participating veterinarians.

Clinics participating in OFSP submit a sample to the project's AHL account 8.5% of the time they complete a "disease-related" farm call. Participating veterinarians attest that samples submitted under the project's AHL account would not have otherwise been sent to the laboratory. OFSP submissions account for 3.5% (937/27021) of the total livestock submissions to the AHL from April 2009-September 2010. OFSP histology and necropsy submissions account for 6.3% (234/3712) of the total histology and necropsy livestock submissions from the same time period.

A number of zoonotic and federally notifiable diseases have been reported through submissions to the project's AHL account. These are found in Table 1.

Table 1: Zoonotic diseases reported through OFSP

Species	Disease
Equine	Eastern Equine Encephalitis
Bovine-Dairy	Cryptosporidiosis
	Botulism
Bovine-Beef	Salmonellosis (Salmonella typhimurium)
Caprine	Q Fever

Cases or situations where participating veterinarians found the ability to charge laboratory submissions to the OFSP account uniquely beneficial include:

1. While providing experience and training to veterinary students or foreign trained veterinarians waiting to write their board certification exams.
2. On two separate dairy farms, a practitioner sent in samples to determine the cause of cattle enteritis. In both cases the diagnoses were Bovine Viral Diarrhea (BVD). In both cases the veterinarian had set up vaccination protocols that included BVD. The veterinarian assumed that the herds were vaccinating for this disease but in both cases the producers had ceased this vaccination.
3. A participating veterinarian had several farms with wasting and/or unthrify sheep that had been in sick pens for a lengthy time. Diagnostic results determined that the animals had Johnes disease, which is considered incurable.

A minor compensation method for participating clinics includes the printing of invoicing/medical record forms that include surveillance data. Equipment examples include laptops, mobile internet devices, duplexing fax machines and iphones. Twice a year, gift baskets are purchased for each clinic to show appreciation for the time and efforts of veterinarians and staff.

The breakdown of expenses in Canadian dollars for year 1 and year 2 (to date) of the project are in Table 2.

Table 2: OFSP operating expenses to date

Category	Expense
Laboratory Diagnostics	$ 137,400
Personnel	$ 120,000
Equipment/software	$ 34,800
Supplies	$ 14,700
Gifts for clinics	$ 4,300
Total	**$ 311,200**

Discussion

Clinics participating in OFSP are located throughout Ontario, with focus on areas of greater livestock density. The low project coverage of townships with moderate-highly dense areas of small ruminants is challenging to address as few producers in this industry routinely use veterinarians.

Conversely, dairy cattle operations most frequently have routine veterinary visits and an established relationship with a veterinarian. This explains why the largest portion of farm call records is from this industry.

Farm locators such as postal code are not frequently listed on submissions to the laboratory. This means that a geographic analysis of laboratory data is based on the location of the submitting veterinary clinic. At best, this approximates the region for that submission. For clinics which cover a large territory, this approximation can be highly inaccurate. The OFSP data ensures that geographical analyses of farm calls are of greater accuracy than geographic analyses of passive laboratory submissions.

It is easy to list the benefits of electronic data. It reduces the frequency of illegible data and the amount of manipulation and time needed by project staff to enter data into a central database. However, having the clinic select a data collection and transmission format which veterinarians are comfortable with and that enhances the system already in place at clinics ensures that time involved in data recording is minimal and there is less frustration associated with new recording formats. Veterinarians that are not burdened by a project are more likely to continue their participation.

When livestock owners are responsible for the cost of diagnostic testing, the number of samples submitted to the laboratory is affected by the economic status of the industry and the owner's belief that the diagnostic testing is valuable. The OFSP removes the burden of cost of testing from livestock owners and allows participating veterinarians to perform diagnostic testing they feel is necessary. This increases submissions to the laboratory which enhances the laboratory data for surveillance and increases the potential for early detection and control of disease outbreaks.

Many emerging or re-emerging diseases in humans are zoonotic. [1] Inaccurate surveillance can increase human morbidity and mortality and greater cost to the healthcare system. The extensive distribution of the global animal population drives the need for effective local and regional surveillance systems for the early detection and prevention of zoonotic disease [2]. If zoonoses are reported by the laboratory, veterinarians can warn their clients of the potential human health risks as well as initiate or review biosecurity procedures to prevent the spread of disease within or between farms. Confirmations of immediately notifiable diseases can be shared with public health officials at the provincial and federal level.

Veterinarians participating in the OFSP have stated that the ability to offer diagnostic testing to a livestock producer free of charge has strengthened their veterinary practice through "value-added" service. Clinics that offer training to veterinary students can provide valuable practical experience in appropriate test selection, proper sampling techniques, interpretation of laboratory results, the translation of these results to the producer and decisions on treatment or resolution of the health issue. In Another example, laboratory confirmation of BVD in a herd that was assumed to be vaccinating for the disease emphasized the need to the veterinarian to routinely review preventative health protocols with clients and demonstrated to the producer the importance of following those protocols. Finally, it can be emotionally challenging to euthanize an animal [3]. This can result in these animals languishing in sick pens with no predetermined endpoint. Laboratory confirmation of disease may produce a new treatment regime or indicate that euthanasia is necessary. Either option promotes good animal welfare.

Laboratory testing as compensation for participating veterinarians is the largest cost centre of the OFSP however the return on this modest investment ($137,400 CAN) is quite high.

Accurate and timely livestock disease surveillance requires additional data then what is currently available through the voluntary laboratory submission system. An active surveillance system such as the OFSP is feasible. All the participating clinics find the method of compensation adequate for the time involved to record and submit the required data for the project. This combined with the low level of frustration in data recording and the belief that the OFSP strengthens their veterinary practice; contribute to the positive view of this project by veterinarians. This attitude increases the likelihood of long term sustainability of the project. Compared to passive surveillance of laboratory data OFSP enhances the type and quantity of submissions to the laboratory, improves reporting on public health risks and strengthens farm veterinary practice. These benefits more than justify the modest operating cost of the OFSP.

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