# ENDEMIC

# SAVSNET – The Small Animal Veterinary Surveillance Network: The '*Who What Where When* & *Why*' of the UK vet-visiting companion animal population

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## Abstract

The devolved nature of the small animal industry in the UK makes surveillance of diseases in this population extremely challenging. This information deficiency could hamper identification, treatment or containment of disease, especially if the aetiology is foreign, or a newly emerging pathogen. Here we describe the development of SAVSNET, the Small Animal Veterinary Surveillance Network, which seeks to use modern information technology to develop an extremely cost-efficient co-ordinated national surveillance strategy of companion animal disease in the UK.

**Keywords**: disease surveillance, diagnostic, prevalence, small animal, veterinary, risk factor

## Introduction

**What**: Disease surveillance can be described as the ongoing systematic collection and analysis of data, characterising the current health (disease) situation within a given population. The UK farmed livestock and bird populations are surveyed by VIDA (Veterinary Investigation and Disease Awareness), an initiative funded by government through collation of diagnostic results from the Veterinary Laboratories Agency and the Scottish Agricultural College [VLA, 2008]. For the equine population, the Animal Health Trust (AHT) captures diagnostic and post mortem results from commercial diagnostic laboratories and publishes them as Equine Quarterly Disease Surveillance (EQDS) reports [AHT, 2010].

In contrast, little is known about the level of disease in the UK small (companion) animal population, despite its size, estimated to be 10.5 million dogs and 10.3 million cats in 2007 [Westgarth *et al.*, 2007; Murray *et al.*, 2010]. This deficiency has been attributed to the relative lack of structured, coordinated disease surveillance targeting these populations [CAWC, 2008].

**Why**: This current shortage of structured surveillance represents a missed opportunity to better understand the current disease burden, both in terms of endemic disease, but also in terms of new diseases, such as the emergence of influenza H3N8 in dogs [Crawford *et al.*, 2005] and H1N1 in felids [ProMED, 2009].The lack of disease surveillance also has implications for human health, with reports indicating that approximately 75% of new and emerging diseases are zoonotic [Taylor *et al.*, 2001].

The need for surveillance in this sector has recently received publicity following the publication of the 'Independent enquiry into dog breeding', a report written by Prof. Sir Patrick Bateson in conjunction with the Kennel Club and the Dogs Trust. This paper called for 'the creation of a computer-based system for the collection of anonymised diagnosis from veterinary surgeries...' [Bateson, 2010].

**Who**: SAVSNET, the Small Animal Veterinary Surveillance Network, was established in September 2008 to address this gap in disease knowledge.

SAVSNET is a collaboration between University of Liverpool School of Veterinary Science and:

- Vet solutions, a leading practice management software provider, with 20% of the UK market share (>900 veterinary premises) nationwide.
- The Animal Health Trust.
- The National Institute of Biohealth Informatics (University of Manchester) who have developed the 'UK National Health Service e-lab' technology' which supports epidemiology and planning in human healthcare [Ainsworth & Buchan, 2009].

## Materials and methods

**How**: SAVSNET aims to use modern information technology to collect, collate and analyse disease data to develop a national coordinated programme of disease surveillance for the UK small animal population in two independent, yet overlapping projects.

Project 1: Diagnostic Laboratory Surveillance; in collaboration with the AHT, and collecting diagnostic results from commercial laboratories throughout the country.

Project 2: Real-time syndromic surveillance; in collaboration with Vet solutions, one of the leading practice management software providers in the UK, and collecting data from veterinary practitioners at the end of consented consultations.

**Ethical approval**: One of the big challenges in using clinical data, is obtaining ethical approval.

SAVSNET has received ethical approval from the University of Liverpool's Independent Ethics Committee to pilot both project 1 and 2. SAVSNET's project 2 pilot has also been approved by the Royal College of Veterinary Surgeons, the governing body for veterinarians in the UK.

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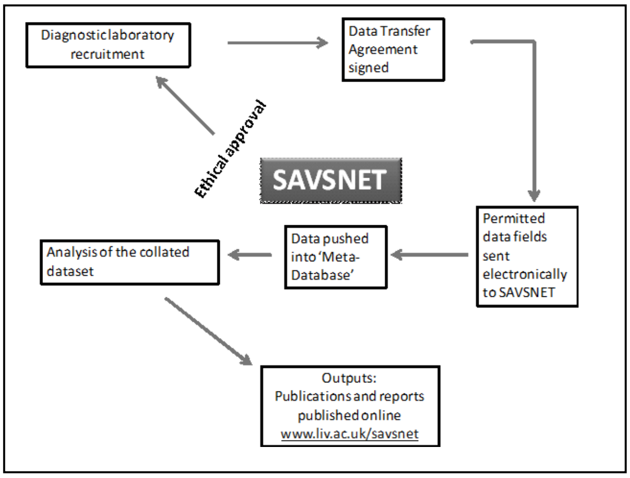
**Project** 1: Methodology

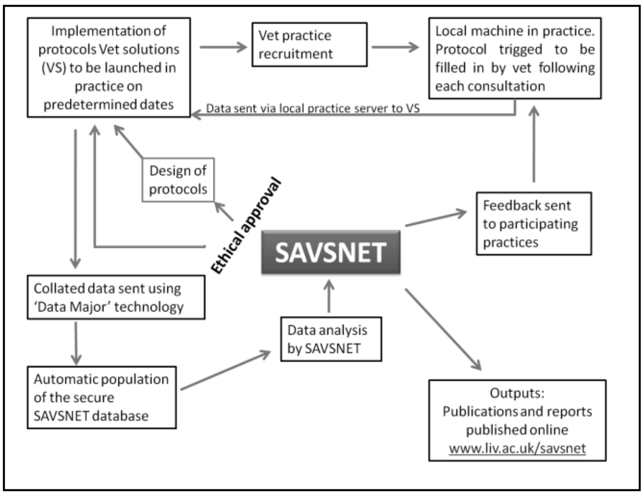


This ethical approval enables the collection of the following information types:

Project 1: Signalment, sample type, diagnostic methodology, test result, and the first two characters of the veterinary surgery submitting the sample for analysis.

Project 2: Signalment, reason for visit, treatment, answers to a syndromic questionnaire, and the full postcode of where the animal resides for spatial analysis (mapping will be carried out at the first half of postcode). Ethical approval for project 2 was granted providing all clients are informed about the project (waiting room posters, client FAQs, and SAVSNET contact details), and given an opportunity to opt-out should they wish.

**Project 2**: Methodology



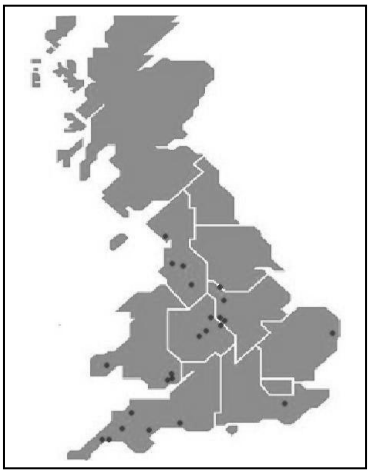
Agreements: Agreements were made with the laboratories and vet practices participating in SAVSNET to indemnify the data providers should any negative consequence result because of participation with SAVSNET. In addition for project 1, the agreement also allowed for the protection of any commercially sensitive information.

**When and where**: Project 1 was piloted between June-September 2010, with data from four UK national diagnostic laboratories. Project 2 was piloted in 32 veterinary premises in England and Wales from May-August 2010 (Figure 1).

Data collection in project 2 was followed by a survey to gather participating vets opinions on taking part in SAVSNET.

**Figure 1**:

Distribution of the 32 veterinary premises taking part in a three month pilot of data collection in small animal veterinary practice.



## Results

*Note: The results included here are examples of the type of data collected by SAVSNET and the types of analyses that SAVSNET will be able to perform. Since they are based on pilot projects with relatively small amounts of data, the actual results should be treated with caution.*

During piloting, data was collected on ~20,000 diagnostic tests carried from commercial laboratories over three months, and on ~30,000 consults in first-opinion practice (500 consults a day) from 32 veterinary premises.

**Figure 2**: Example of project 1 results: Breed as a risk factor for testing positive for Campylobacter spp.

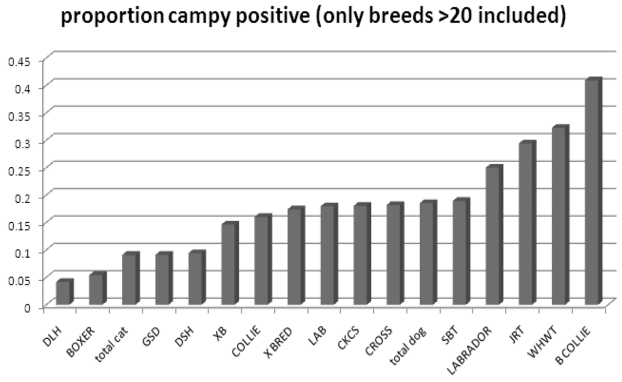
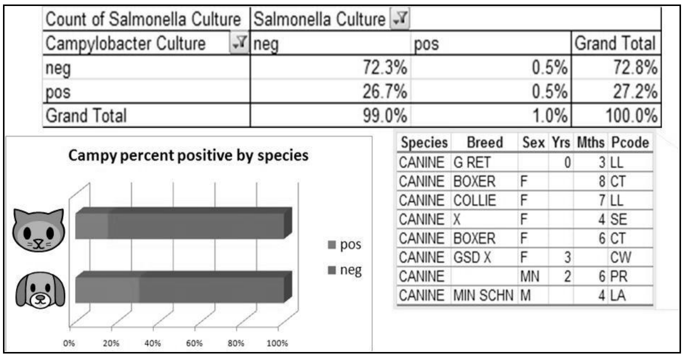


**Figure 3**: Example of project 1 results: illustrates co-infection with Salmonella and Camplylobactor spp. Further breakdown of the 0.5% co-infected animals are also explored.



| Count of Salmonella Culture | Salmonella Culture | | |
|---|---|---|---|
| Campylobacter Culture | neg | pos | Grand Total |
| neg | 72.3% | 0.5% | 72.8% |
| pos | 26.7% | 0.5% | 27.2% |
| Grand Total | 99.0% | 1.0% | 100.0% |

| Species | Breed | Sex | Yrs | Mths | Pcode |
|---|---|---|---|---|---|
| CANINE | G RET | | 0 | 3 | LL |
| CANINE | BOXER | F | | 8 | CT |
| CANINE | COLLIE | F | | 7 | LL |
| CANINE | X | F | | 4 | SE |
| CANINE | BOXER | F | | 6 | CT |
| CANINE | GSD X | F | 3 | | CW |
| CANINE | | MN | 2 | 6 | PR |
| CANINE | MIN SCHN | M | | 4 | LA |

Q. Is this the first time this animal has presented with pruritus? A. (i) First time (ii) presented previously (iii) unknown if this is the first presentation.

**Figure 4** (above): Example of project 2 results from a pruritus questionnaire, exploring breed as a risk for pruritus. The bar marked "TOTAL" acts as an average across the total population. Lurcher were seen least for pruritus. The Bichon Frise was seen most for pruritus.

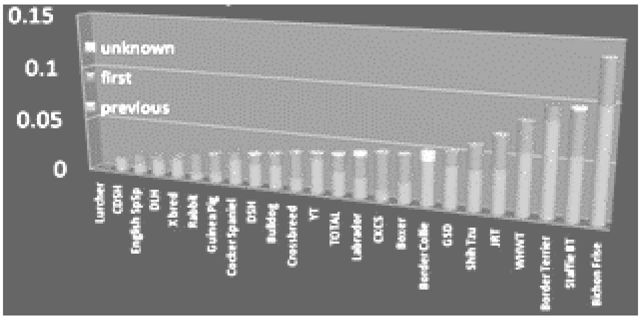
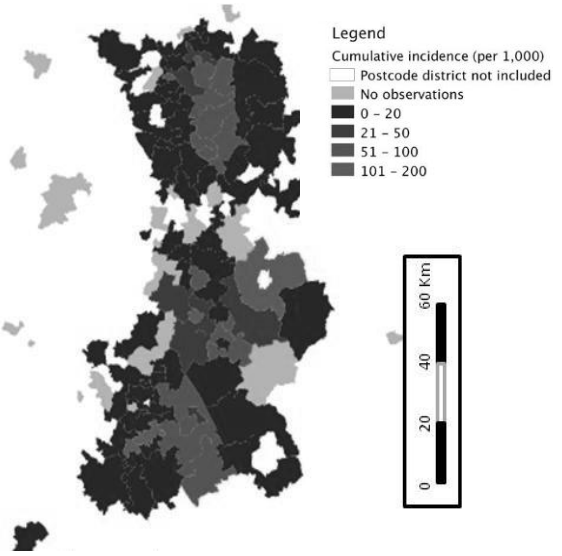


**Figure 5** (above): Example of project 2 results. Cumulative incidence of gastrointestinal disease in dogs in one region of the UK based on postcode districts during the first month (May-June 2010) of the pilot study. Analyses like these will be able to identify geographical hotspots of disease.



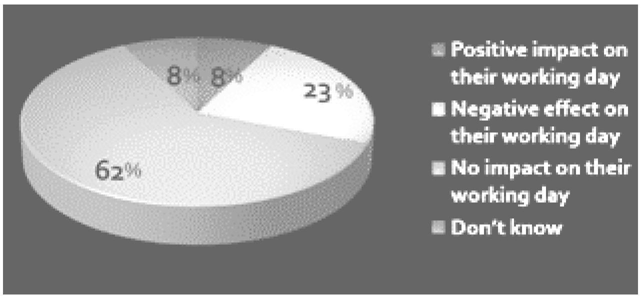
## Discussion

Collecting meaningful surveillance data from fragmented populations is extremely challenging, especially when responsibility for these populations is similarly fragmented and when funding is extremely limited.

SAVSNET has shown that using modern IT solutions to centrally collate ethically approved data is a cost efficient way to meet the demands of surveillance in such populations.

Plans are currently underway to further pilot the SAVSNET infrastructure in early 2011, increasing both the numbers of veterinary diagnostic laboratories and vets in practice submitting data. In addition, we are seeking additional funding to facilitate the scalability now needed to turn SAVSNET into a national resource.

**Figure 6** (below): Feedback from data-providers (vets) taking part in project 2. Vets found taking part in SAVSNET had



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### Acknowledgements

We are extremely grateful for the help, advice and patience shown by both the laboratories taking part in project 1 (Acarus, Biobest, Capitol, Nationwide, Test-a-pet) and the veterinary practices taking part in project 2, without whom, SAVSNET would not be possible. We would also like to thank the stakeholders (BSAVA, Defra, Dechra, IntervetSP, Merial, Novartis, Pfizer, Virbac and University of Liverpool) for their altruistic vision and faith in the project and funding the initial SAVSNET development.

# Validation of a cattle health monitor on routinely available data of specific dairy herds

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## Abstract

A monitoring system for the assessment of dairy cattle health based on routinely available data was developed in the Netherlands. This system served as an alternative for the compulsory quarterly farm visits. The validity of both systems relative to a herd check was assessed. This study showed that a monitoring system based on routinely available data served as a better diagnostic tool for detecting herds with insufficient cattle health than compulsory quarterly farm visits by the herds' veterinary practitioner.

**Keywords**: Validation, routine data, monitor, dairy cattle health.

## Introduction

Traditionally, cattle health is assessed during farm visits. However, farm visits are often time consuming, cattle health is assessed at only one point in time and inspection by many experts does not allow comparison within and between. These disadvantages of farm visits have caused a worldwide interest in monitoring cattle health and based on precollected data. A main advantage of precollected data is that they are uniformly gathered and registered throughout time. That makes comparison between dairy cattle herds possible and therefore these data could be used to develop reliable and effective tools for assessing cattle health and welfare.

A monitoring system for the assessment of dairy cattle health based on routinely available data, the Continuous Cattle Health Monitor (CCHM) was developed in the Netherlands. This system served as an alternative for the compulsory quarterly farm visits which were implemented in 2002 to meet the EU regulations RL64/432/EEG and RL97/12/EG, concerning the development of a compulsory network to control cattle health in dairy herds. However, before implementation of the alternative system, the validity of the CCHM and the compulsory quarterly visits relative to the real health status of the herd should be known. The aim of this study was to investigate the relative validity of a monitoring system based on routinely available data (CCHM) and the compulsory quarterly visits for detecting herds with prolonged cattle health problems.

## Materials and methods

### *CCHM Survey*

In 2007, a survey was conducted to validate the CCHM in practice. Dairy producers that were member of FrieslandCampina (about 9,500 members in 2007) could voluntarily participate in the CCHM. The condition to participate in the CCHM was that for each herd, all cattle health parameters within the CCHM had to be available from January 2006 to December 2007. Finally, 3,562 volunteers met this condition and participated in the study. Routinely available data of Dutch dairy herds were compiled from different organizations within the Dutch cattle industry. Data were obtained from the following sources:

- Identification & Registration organization (I&R), data concerning all on- and off-farm movements of cattle
- Milk control station (Qlip), data concerning bulk milk quality and number of lactating cows with cattle health disorders assessed during the compulsory quarterly visits
- Rendering plant, data concerning "fallen stock"
- Dutch cattle improvement organization (CRV Holding), data concerning milk production and udder health
- Animal Health Service, data concerning the certified status of the herd for diseases, namely bovine virus diarrhoea (BVD), infectious bovine rhinotracheitis (IBR), leptospirosis, paratuberculosis and salmonellosis.

The cattle health status of each dairy herd that participated in the survey was determined every quarter during a one-year period (2007). The percentage of herds with an insufficient cattle health status in the 4th quarter of 2007 was determined.

The parameters in the CCHM are presented in Table 1.

### *Herd Check and Compulsory Quarterly Visits*

In the first half of 2007, a herd check was assigned to 200 survey participants to assess the actual health status of these herds. A stratified random selection procedure was used to select sufficient herds with an insufficient cattle health status based on the CCHM. From the group with a CCHM's insufficient cattle health status, 50 herds were randomly selected and from the group with a CCHM's sufficient cattle health status 150 herds were selected.

The herd check consisted of the assessment of different cattle health aspects by two trained veterinarians.

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First, the general impression of the dairy cows (including condition, rumen fill, locomotion and cleanliness of skin and presence of skin injuries) was examined. Every aspect was scored on a scale from 1-10 points and an average score was determined for the general impression of the dairy cows. In addition, claw and leg disorders were scored on a scale from 1-10 points. Moreover, the percentage of cows with a body condition score <2 (i.e. very thin cows with no fat reserves), the percentage of lame cows and the percentage of loser cows were determined. Finally, the general impression of young stock (0-2 years old) was scored on a scale of 1-10 points. The average of all scored cattle health aspects (the overall herd check score, 1-10 points) was used to determine whether a herd had a sufficient or insufficient cattle health status.

**Table 1**: Overview of the parameters, thresholds and weighted scores within the Continuous Cattle Health Monitor (CCHM) to assess cattle health in dairy herds on a quarterly basis in the Netherlands.

| *Parameter* | *Threshold* | *Weighted score (in points)* |
|---|---|---|
| Cattle mortality (%/quarter) | ≤ 1.60 | 25 |
| Young stock mortality (%/quarter) | ≤ 8.5 | 10 |
| Incidence of subclinical mastitis (%/quarter) | ≤ 12.5 | 20 |
| Bulk milk somatic cel count (BMSCC in cells/ml per quarter) | ≤300,000 | 15 |
| Decrease in Standard Peak production (ΔSPP in %/quarter) | ≤ 10 | 15 |
| Closed farming system (no cattle moved on-farm in the previous 12 months) | | 10 |
| Certified free or unsuspected status for bovine virus diarrhoea (BVD), infectious bovine rhinotracheitis (IBR), leptospirosis, paratubercu-losis, salmonellosis (status/quarter) | | 5<br>(1 point per certificate) |
| **Total** | | **100** |

The threshold for an insufficient cattle health status was set at an overall herd check score <6 points. To obtain objective results, the two veterinarians that visited the 200 selected dairy herds did not know the cattle health situation or the CCHM's annual moving average of the dairy herds beforehand. In addition, the veterinarians visited the first ten dairy herds together to obtain agreement in their scoring. All participating herds were exempted from the compulsory quarterly visits. Nevertheless, in 92 herds from the 200 selected herds the quarterly visit was carried out. During these visits the numbers of lactating cows 1) that produce milk with abnormal organoleptic features, 2) with visible general health problems, 3) with abnormal uterine discharge, 4) with fever combined with diarrhoea, 5) with udder lesions, 6) with zoonotic diseases and 7) with notifiable diseases were registered. The total percentage of lactating cows with the above mentioned health disorders was determined for each herd.

*Data-analysis*

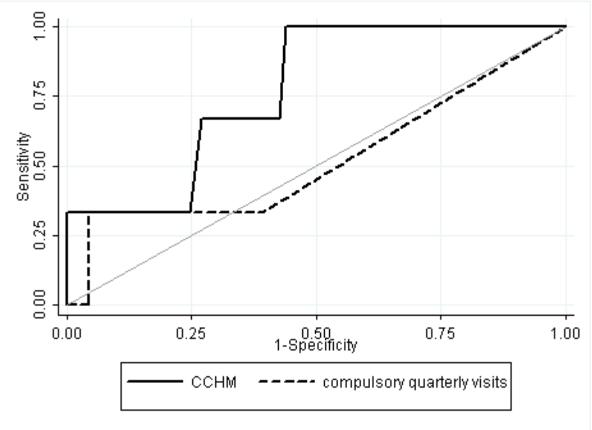
Non-parametric receiver operating characteristic (ROC) curves were used to examine the ability of the CCHM and the compulsory quarterly visits to discriminate between a sufficient or insufficient cattle health status, which was obtained with a standardized herd check.

The univariable associations between the CCHM's annual moving average and the cattle health aspects assessed during the herd check were screened using linear regression. Cattle health aspects with an association significant at $P \leq 0.25$ were included in a multivariable linear regression model as independent variables, the CCHM's annual moving average as the dependent variable. A backward selection procedure was used to select the cattle health aspects that were significantly associated with the CCHM's annual moving average ($P \leq 0.05$). Herd size (in classes: <25% percentile, 25%-75% percentile and >75% percentile of no. of cows >2 years) was forced in the model, because it was thought that herd size may be a confounder for the annual moving average.

## Results

The results showed that the area under the curve (AUC) for the CCHM was 0.81 (95% CI: 0.58-1.00) and was not significantly different from the AUC of the compulsory quarterly visits (0.52, 95% CI: 0.09-0.95). However, the AUC of the CCHM was significantly larger than 0.5, whereas the AUC of the compulsory quarterly visits was not significantly different from 0.5 (Figure 1).

**Figure 1**: Receiver operating characteristic (ROC) curves for the Continuous Cattle Health Monitor (CCHM) and the compulsory quarterly visits for the detection of herds with a (in)sufficient cattle health status. The grey solid line represents the 0.5 line, which is the limit for the ability of a test to discriminate between herds with a sufficient or insufficient cattle health status.



The results also showed that the routinely available data used in this study have a significant association with animal based measurements such as lameness, the presence of loser cows and the general impression of the health status of the dairy cows and young stock (Table 2).

**Table 2**: Significant associations between the CHHM's annual moving average/ insufficient CCHM's cattle health status and the cattle health aspects measured during the herd check.

| *Cattle health aspect* | *Class* | *CCHM's annual moving average* | |
|---|---|---|---|
| | | Estimate | 95% CI |
| Presence of lame cows | no | Ref. | |
| | yes | **-6.37*** | -11.19- -1.56 |
| Presence of loser cows | no | Ref. | |
| | yes | **-8.36** | -13.26- -3.45 |
| General impression of young stock<br>(per 1 point increase) | | **11.01** | 6.83-15.19 |
| Herd size<br>(no. lactating cows) | <47 | **-11.08** | -16.06- -6.10 |
| | 47-92 | Ref. | |
| | >92 | 0.67 | -4.28-5.62 |
| Intercept | | 0.09 | -32.42-32.60 |

*bold figures are significant ($P \leq 0.05$)

## Discussion

This study showed that a monitoring system based on routinely available data served as a better diagnostic tool for detecting herds with an insufficient cattle health status than compulsory quarterly farm visits by the herds' veterinary practitioner. All herds with an insufficient cattle health status were detected. Because specificity (51.0%) was relatively low, follow-up actions are needed for herds below the cut point of 69 points to confirm and, if necessary, to improve the cattle health status in these herds. Provided that the costs for not detecting a herd with an insufficient cattle health status can be ignored, the CCHM will be more cost-effective than the compulsory quarterly visits as only herds with a CCHM's insufficient cattle health status (<5% of the herds) have to be visited by a veterinarian instead of all dairy herds.

This study also shows that routinely available data have a relevant association with animal based measurements such as lameness, the presence of loser cows and the general impression of the dairy cows (including condition, rumen fill, locomotion and cleanliness of skin and presence of skin injuries) and young stock. Routinely available cattle health data could therefore be an efficient tool for preselecting herds with specified problems like lameness or poor condition.

Advisory services such as veterinarians and dairy co-operatives could use a tool similar to the CCHM to channel visits to those farms that are at risk for prolonged cattle health problems.

**Acknowledgements**

This study was financed and supported by the Dutch dairy co-operative FrieslandCampina. We would like to thank the Dutch organisations that provided data for this study. We also like to acknowledge the farmers that were involved in the survey.

# Active Surveillance and Control Programme for *Salmonella* Dublin in Cattle: Alternatives to Acceptance of Endemic Infection with Poor Control Options

**L.R. Nielsen**[1*] and E. Rattenborg[2]

## Abstract

This study illustrates how prevalence and incidence of *Salmonella* Dublin in cattle has been markedly reduced in dairy herds during active surveillance and a control programme targeting infected herds in Denmark from 2002 to 2010. The results suggest that this might by a good alternative to passive surveillance systems.

Register data might be useful for design of effective surveillance programmes for *Salmonella* Dublin in cattle in the future. Statistical analysis of register-based variables confirmed previously known risk factors for becoming test-positive (i.e. purchase of animals from test-positive herds, number of cattle in test-positive neighbouring herds, herd size and season), but also pointed out additional factors affecting the risk of dairy herds changing status from test-negative to test-positive (indicative of new or recurrent infection). Increasing geometric cell count measured through the mandatory milk quality assurance scheme was associated with increasing risk of becoming test-positive, while participation in a voluntary control programme for paratuberculosis, another bacterial infection with similar transmission patterns, was associated with decreased risk of becoming test-positive. This suggests that there might be synergistic benefits from running control programmes for both infections simultaneously. We did not find the risk of becoming test-positive significantly different between organic and conventional herds, nor between analysing laboratories.

Even when controlling for other risk factors, it was evident that the risk of changing to test-positive was significantly lower after the surveillance programme was supplemented by an intensified control period from October 2007 and onwards than during the surveillance period before 2007.

**Keywords**: *Salmonella*, cattle, surveillance, control, serology

## Introduction

The most common type of surveillance of *Salmonella* in cattle is passive surveillance in which samples are collected and submitted to laboratories upon suspicion of salmonellosis, sometimes supplemented by collection of extra samples and tracing efforts upon isolation of *Salmonella* in fresh meat surveillance and coincidental isolations of *Salmonella* serotypes.

Despite the fact that collection of samples at suspicion of salmonellosis is compulsory according to legislation in many countries, submission of samples is highly dependent on the compliance of farmers and local veterinarians and which consequences isolation of *Salmonella*-serotypes have for the farmer and his herd. Therefore, monitoring of prevalence or incidence of *Salmonella* in cattle is hampered by poor and varying sensitivity over time in passive surveillance systems.

A few passive surveillance systems imply rigid (and costly) control measures on test-positive herds [1]. However, most passive surveillance systems allow for little or no follow-up in the herd from which *Salmonella* is isolated, let alone effective preventive measures to stop transmission to other herds. Vaccination has been used and sometimes reduce clinical signs, but does not stop transmission of bacteria [2]. In general easy and effective control-measures are lacking. This leads to a pattern with endemic occurrence of *Salmonella* in the cattle population. Subclinical infection can be present for extended periods of time, and occasionally outbreaks of varying severity lead to production losses, and loss of animal health and welfare due to increased morbidity and mortality in the herd [3].

In Europe, the most commonly isolated serotypes in samples originating from cattle are *Salmonella* Dublin and *Salmonella* Typhimurium [1, 4]. Whereas *Salmonella* Typhimurium occurs in all animal species, *Salmonella* Dublin is host-adapted and mainly found in cattle and in cattle-premises, cattle abattoirs or where cattle manure/slurry has been spread. Furthermore, *Salmonella* Dublin appears to lead to fewer, but more invasive infections in humans making it difficult to treat and resulting in higher case mortality than other serotypes [5]. In the beginning of this century the situation was considered unacceptable by the Danish Cattle industry and the Veterinary and Food Administration, and a surveillance program targeting *Salmonella* Dublin was initiated in October 2002 to control and eventually eradicate this serotype through surveillance and effective control measures in the cattle population.

All dairy herds are classified into “level 1” or “level 2” based on the four most recent year-quarterly bulk-tank milk samples that are tested for antibodies directed against a *Salmonella* Dublin-antigen (serogroup-D-antigen). All non-dairy herds are classified according to the antibody results of the eight most recent blood samples tested with a similar antibody test. Finally, herds are classified as “level 3” if *Salmonella* Dublin is diagnosed from a herd with salmonellosis (i.e. clinical disease with symptoms caused by *Salmonella*).

The most effective control measure was assumed to be to reduced contact between infected and non-infected herds.

Therefore, the programme was constructed to include consequences (locked period in level 2) upon purchase of cattle from herds classified as level 2 or 3. From October 2007 an intensified control campaign was

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implemented in addition to the surveillance activities. It was mainly directed towards improved control of *Salmonella* through test-and management procedures in infected herds. The surveillance programme provides a unique source of data for analysing risk factors that might be used to improve the current system and support informed decisions on eventual surveillance and control of *Salmonella* in other countries and potentially other species.

Thus, the aim of this study was to illustrate incidence risk and prevalence over time before and after the surveillance programme was initiated. Furthermore, we aimed at analysing the effect of having an organic production system and being part of a voluntary paratuberculosis control programme on the risk of new *Salmonella* infections. Both of these factors might affect the biosecurity and management in the herd. Finally, we aimed at analysing if the risk of new infections were reduced after the reinforced control campaign was implemented in October 2007 compared to the period with surveillance from 2002 to 2007. The study is based on register data available from the Danish Cattle Database.

## Materials and methods

From the 1st of July 2001 to the 1st of July 2010, 7,528 Danish cattle herds were recorded as milk-producing for longer or shorter periods of time. Surveillance and register data were collected from all herds and were aggregated on year-quarterly periods, mainly because the *Salmonella*-status in the surveillance programme is based on bulk-tank milk samples collected automatically every 3 months. The national and regional year-quarterly incidence risk of changing test-status from negative to positive (indicative of new or recurrent infection) was calculated as the number of shifts divided by the number of year-quarters at risk.

Because of large structural changes in the cattle population the risk factor analysis was restricted to a dataset including surveillance and register data from 6,688 dairy herds with a herd size of at least 10 animals in each year-quarter and herds that had been milk-producing for at least one year during the study period. The study period was restricted to the 1st of January 2003 to 1st of April 2010 in order to be able to include purchase patterns and neighbour test-status in previous year-quarters as risk factors for the current year-quarter. All herds were assigned to one of eight regions in Denmark (according to their x- and y-coordinates). These regions were used for administrative purposes during the *Salmonella* control campaign, and were included in the analyses to account for differences in prevalence and level of centrally governed control efforts.

The following variables were studied for associations with shifts from test-negative in one year-quarter to test-positive in the following year-quarter:

**Continuous variables**:

*Log geo cell count:* the natural logarithm of the average weekly geometric cell count in bulk-tank milk in the current year-quarter.

*Neighbours*: Number of cattle in *Salmonella* test-positive herds within a 5 km radius in the previous year-quarter (divided by 1,000 for the analysis).

*Total herd size* measured as the average number of animals in the herd recorded over the three months of the current year-quarter (divided by 100 for the analysis).

*Time*: Number of days since the beginning of the surveillance programme (divided by 365 for the analysis).

**Categorical variables**:

*Region* (8 regions in Denmark)
*Period* (“Surveillance”: from Jan 2003 to Sept 2007 or “Control campaign”: from October 2007 to 2010)

*Production type:* All herds were categorised as either “Organic” or “Conventional” during the full study period, because it was not possible to obtain precise information about start and end dates for organic production.

*ParaTB-programme*: Part of the voluntary paratuberculosis programme in the current year-quarter or not.

*Purchase*: Number of animals purchased from *Salmonella* test-positive herds over the previous 6 months. Categorised into four levels: 0, 1-10, 11-50 and >50 animals purchased.

*Lab*: Indicates which one of two laboratories that analysed the bulk-tank milk samples for antibodies.

*Season*: Winter: December-January-February, Spring: March-April-May, Summer: June-July-August, Autumn: September-October-November.

After descriptive statistics and graphical exploration of all variables statistical analysis of risk factors for becoming test-positive was performed by logistic regression in SAS® v. 9.2 taking into account repeated measurements on herds using an autocorrelation structure between year-quarters. Back-wards stepwise elimination of variables not significant at a 1% level was performed. All possible two-way interactions between remaining variables and reintroduced excluded variables were evaluated.

## Results

Overall, there were 2,042 (1.6%) shifts from test-negative to test-positive in 1,515 herds out of all 131,462 test-negative year-quarters. In other words 5,173 herds (77.3%) of the herds never experienced a shift from test-negative to test-positive in the study period, while 22.7% of the herds had on average 1.3 shifts. Overall, the incidence risk (Figure 1) and the prevalence (Figure 2) of test-positive dairy herds reduced markedly in the years after the programme was initiated.

Table 1 shows the results of the final logistic regression model (parameter estimates $\beta$, $P$-values and odd ratios (OR) with 95% confidence limits for each level of the variables) including all significant main effects. Two factors were excluded from the final model due to too high $P$-values, “*Laboratory*” ($P$=0.6) and “*Production* *type*” (organic or conventional) (*P*=0.05). No interactions were found relevant.

**Figure 1**: Incidence risk for becoming test-positive per year-quarter from April 2002 to March 2010 in all Danish dairy herds.

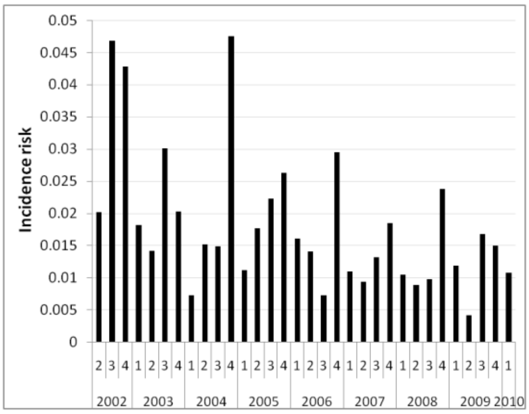
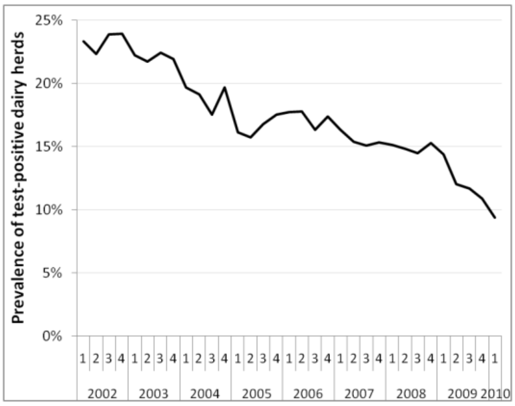


**Figure 2**: Change in national prevalence of *Salmonella* test-positive Danish dairy herds, July 2001 to June 2010.



## Discussion

*Salmonella*-incidence and prevalence data from beginning of 2002 were analysed. Large fluctuations in incidence risk between seasons were obvious. The incidence risk was reduced by two to four times and prevalence was reduced by more than 50% over the study period. There is no evidence in these analyses that the reductions were due to the surveillance and control programmes. However, it is likely that that they were the main reason for the success, because trading patterns and control activities against *Salmonella* Dublin improved markedly over these 8 years due to establishment of the programmes. With the structural changes in the cattle population towards fewer and larger herds, an increase in incidence and prevalence would otherwise have been expected as a consequence of movements of cattle, larger herd sizes and more complex contact patterns [1, 6].

Apart from previously known risk factors associated with herd size, purchase patterns and geography [6] it was interesting that geometric cell count was associated with increased risk of infection. This might be due to underlying factors such as hygiene and other diseases. Furthermore, it was interesting to see that herds that participated in the paratuberculosis-programme had reduced risk of *Salmonella*-infection. This is likely to be due to increased awareness of purchase risk and management directed towards transmission of bacteria via faecal matter.

**Table 1**: Model results from logistic analysis of risk factors for changing from test-negative to test-positive in the Danish cattle-*Salmonella*-programme

| *Risk factor and levels* | *β* | *P* | OR (95%CI) |
|---|---|---|---|
| *Intercept* | -10.3 | | |
| *Region* | | *<.0001* | |
| East Jutland | -1.12 | | 0.3 (0.2-0.4) |
| Himmerland | 0.09 | | 1.0 (0.9-1.3) |
| Islands | -1.17 | | 0.3 (0.2-0.4) |
| Mid Jutland | -0.15 | | 0.9 (0.7-1.1) |
| Midwest Jutland | -0.49 | | 0.6 (0.5-0.8) |
| North Jutland | -0.46 | | 0.6 (0.5-0.8) |
| South Jutland | -0.32 | | 0.7 (0.6-0.9) |
| Southwest Jutland | 0 | | 1 |
| *Period* | | *0.01* | |
| Control campaign | -0.23 | | 0.8 (0.7-0.9) |
| Surveillance | 0 | | 1 |
| *ParaTB-programme* | | *0.005* | |
| Yes | -0.27 | | 0.8 (0.6-0.9) |
| No | 0 | | 1 |
| *Purchase** | | *<.0001* | |
| >50 animals | 1.73 | | 5.6 (4.1-7.7) |
| 11-50 animals | 1.03 | | 2.8 (2.2-3.6) |
| 1-10 animals | 0.63 | | 1.9 (1.6-2.3) |
| No purchase | 0 | | 1 |
| *Season* | | *<.0001* | |
| Sept-Nov | 0.86 | | 2.4 (2.1-2.7) |
| Dec-Feb | -0.05 | | 1.0 (0.8-1.1) |
| Mar-May | 0.24 | | 1.3 (1.1-1.5) |
| Jun-Aug | 0 | | 1 |
| *Log geo cell count* | 0.48 | *<.0001* | 1.6 (1.4-1.9) |
| *Neighbours (per 1000)* | 0.18 | *<.0001* | 1.2 (1.1-1.2) |
| *Herd size (per 100)* | 0.15 | *<.0001* | 1.2 (1.1-1.2) |
| *Time (per year)* | -0.06 | *0.004* | 0.9 (0.9-1.0) |

*Purchase from test-positive herds in the previous 6 months

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# Surveillance of wildlife diseases in Belgium

**A. Linden**[1], M. Wirtgen, S. Volpe, A. Nahayo, J. Pirson, J. Paternostre and F. Grégoire

## Abstract

Eight years ago (2002), a wildlife health surveillance initiative was launched in Wallonia (Southern Belgium). The initial project dealt with paratuberculosis in wild cervids. Years after years, its scope and resources were progressively widened, leading to the current "WildScreen.be" surveillance network. This paper describes the organizational and managerial keys underlying the network and summarizes its first outputs.

**Keywords:** wildlife, Belgium, surveillance, methodology.

## Introduction

Wildlife diseases have gained considerable attention in recent years due to their impact on public health, economy and wildlife conservation. This was dramatically highlighted by the SARS, West Nile fever and H5N1 avian influenza outbreaks. A retrospective study of infectious diseases that emerged in humans between 1940 and 2004 revealed that 60.3% were zoonoses, 71.8% originated in wildlife and that the proportion of the latter increased significantly over time [1]. The role of wildlife as reservoirs of pathogens that are transmissible to livestock also becomes more and more problematic in countries where overabundance of wild species and extensive management of livestock increase the risk of interspecies transmission.

In these contexts, the need for surveillance and control programmes of wildlife diseases is becoming evident for a lot of countries. In Southern Belgium, such a surveillance initiative was launched in 2002. The initial project dealt with paratuberculosis in wild cervids. Years after years, its scope and resources were progressively widened, leading to the current "WildScreen.be" global surveillance network (Table 1).

**Table 1**: The WildScreen.be wildlife surveillance network, according to Dufour and La Vieille's criteria [2]

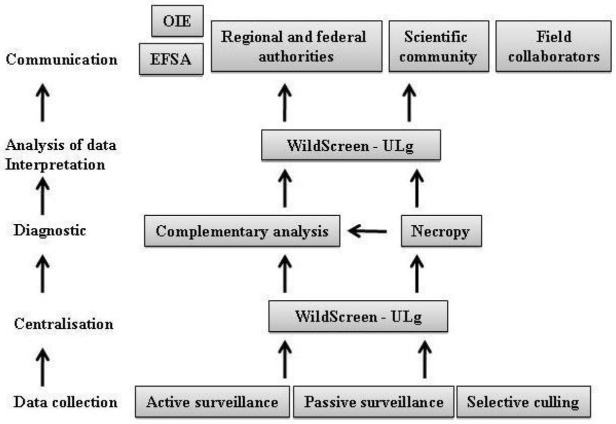
| | |
|---|---|
| Scope of surveillance | Regional: Wallonia, 16,844 km$^2$ |
| Type of surveillance | Global |
| Targeted diseases | Endemic and exotic diseases |
| Targeted populations | Wild ungulates, lagomorphs, carnivores<br>rodents, insectivores and birds to a lesser extent |
| Studied populations | Sample |
| Data collection | Passive and active |
| Mode of operation | Partly integrated and partly autonomous |
| Coordinator | University of Liege (ULg) |
| Collaborations | Reference laboratories for targeted pathogens |
| Funding sources | Regional government and ULg for maintenance of the Network<br>Federal government and European funds for targeted surveillances |

WildScreen.be relies on both epidemiovigilance and epidemiosurveillance and aims at (i) detecting new or exotic pathogens in wildlife to take adequate control measures as soon as possible, (ii) evaluating the true extent of infections due to specific pathogens* to inform and advise the Authorities in the decision making process to implement control measures, (iii) evaluating control plans and (iv) providing data for conducting research.

## Materials and methods

Samples and data are collected through both active and passive surveillance policies over the whole territory of Wallonia (southern Belgium) and are transmitted to the WildScreen.be's core facilities (storing fridges, autopsy room, diagnostic laboratory and offices) located in Liège (Figure 1).

**Figure 1**: Operating scheme of WildScreen.be



*Active surveillance* is performed on apparently healthy animals shot by hunters during each hunting season (October to December) throughout Wallonia, the current targeted species being *cervidae, suidae and anatidae.* In 2009, the populations of free-living red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*) were estimated to 11 000, 35 000 and 26 000 heads, respectively [3]. The Region is divided in 33 forest districts, each with specific environment and wild species population densities. Accordingly, whenever a random sampling is achieved, the number of contributing samples from each district is systematically proportional to its population density. The sample size is chosen according to statistical considerations, such as desired precision of estimated prevalence and expected prevalence of the disease targeted, but also according to other constraints related to the field conditions [4]. The study design includes a two –stage cluster sampling

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* Studies are carried out on prioritised pathogens chosen by the steering committee on the basis of impact on human health, rural economy and wildlife conservation. In Belgium, the decision process leading to prioritization and associated surveillance funding is shared by regional (livestock and wildlife health) and federal (public health) Authorities.

strategy. Some hunting areas are first randomly chosenin each forest district and some animals are randomly sampled in each chosen hunting area.

For each animal enrolled in the active surveillance scheme (*anatidae* excepted), postmortem examinations are performed in field conditions by veterinarians. A standard questionnaire (traceability code number, sex, age, body weight, body condition and macroscopic aspect of internal organs) is systematically completed. Afterwards, the blood and tissue samples that are the most suited for the detection of the pathogens targeted are collected, processed (whenever a special procedure is required for better conservation) and transported to the WildScreen.be's core facilities.

*Passive surveillance* is performed on found-dead animals collected all over the year in Wallonia. Targeted species are broader, including ungulates, lagomorphs, carnivores and, to a lesser extent, rodents, insectivores and birds. WildScreen.be has installed 20 freezers over Wallonia that are specifically dedicated to the storage of bodies from wild animals found dead by forest rangers, hunters and other field actors. On a regular basis, a shuttle is organized to empty the freezers and to bring the bodies to the WildScreen.be's core facilities in Liège where a standardized procedure for necropsic examination is implemented by the network's personnel, with the help of experienced veterinary pathologists whenever needed. A necropsy report is systematically written, gathering descriptions of macroscopic alterations, inventory of tissue samples sent to the laboratory and cause of death.

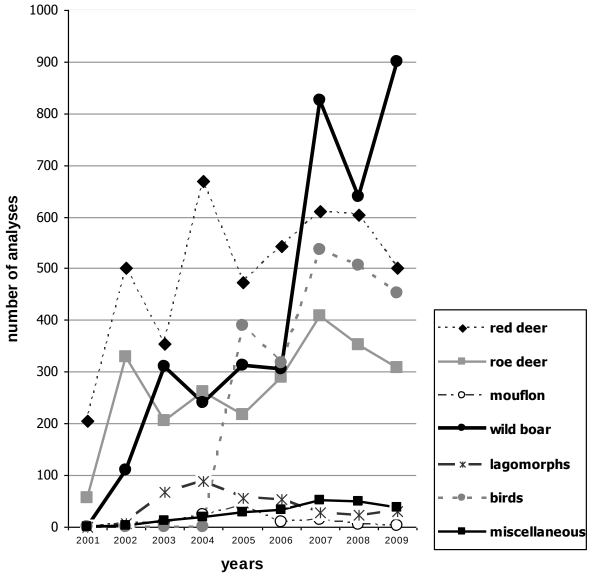
*Selective culling* also contribute to sample/data collection. This official control procedure aims at hasting elimination of diseased/ injured wild animals (see discussion section).

After centralisation in WildScreen.be's core diagnostic laboratory, samples are either processed on site or dispatched to specialized diagnostic facilities, depending of the pathogen targeted. To do this, collaborations were formally concluded with a series of institutions: University of Liege (Liege), Veterinary and Agrochemical Research Center (Brussels), Research Laboratory for Vector-Borne Diseases (Brussels), Institute for Tropical Medicine (Antwerp), ISP-Pasteur Institute (Brussels), ARSIA (Ciney), SPW-LFSC (Gembloux), AFFSA (Ploufragan, France), and Robert Koch Institute (Berlin, Germany). After the results are transmitted back and are duly interpreted in the context of necropsic and histopathologic results, a complete report is written by the WildScreen.be team and a tailored written feed-back is sent either to field collaborators, hunting associations, regional and federal Authorities or international bodies like OIE.

## Results

In active surveillance, more than 2 000 analyses are performed each year (Figure 2). The results from two specific surveys are summarized here.

**Figure 2**: Evolution of the number of analysed animals from 2001 to 2009



*Bluetongue in wild red deer* - Throuh the WildScreen.be's active surveillance program 1 620 hunter-killed red deer blood samples were gathered within 4 years. The sera were examined for anti-BTV antibodies. All 2005 sera were negative but seroconversion was detected from 2006 onward. The apparent seroprevalence was 1.5, 52.3 and 33.9% in 2006, 2007 and 2008, respectively. All spleen samples from red deer were vironegative in 2006 while 14% were positive in 2007.

*Classical swine fever (CSF) in wild boar* - Through the active surveillance program, 850 hunter-killed wild boars were sampled in 2009 (sampling fraction 3.3%). All sera were negative for anti-CSF antibodies and RT-qPCR did not detect viropositive cases.

In passive surveillance, almost 300 found-dead wild animals are necropsied each year. Two results are summarized here, (i) the distribution of the causes of mortality in roe deer (Figure 3) and (ii) among red deer (Figure 4). Passive surveillance led to the first detection of the EBHS calicivirus (European Brown Hare Syndrome) in hares in 2004 and the CDV morbillivirus (Canine Distemper Virus) in foxes in 2009.

Results of selective culling (Table 2) show that 203 out of 359 game species (56%) were eliminated for infectious or parasitic reasons.

**Figure 3**: Distribution of causes of death among roe deer (*Capreolus capreolus*) in Wallonia – necropsy of 213 found dead animals, between 2005 and 2009.

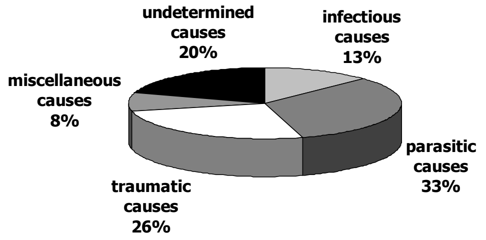


**Figure 4**: Distribution of causes of death among red deer (*Cervus elaphus*) in Wallonia - necropsy of 158 found dead animals, between 2005 and 2009.

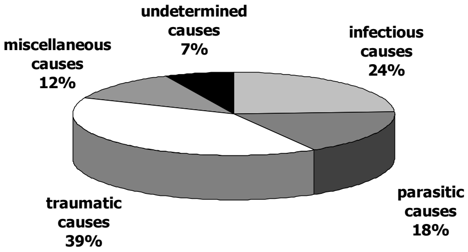


**Table 2**: Selective culling from October 2003 to December 2009: distribution of causes of morbidity among the analysed animals

| Species | Causes of morbidity | | | | |
|---|---|---|---|---|---|
| | Infectious | Parasitic | Traumatic | Miscellaneous | ND |
| **Red deer** | 75 | 11 | 72 | 11 | 5 |
| **Roe deer** | 15 | 47 | 16 | 7 | 27 |
| **Boar** | 8 | 42 | 11 | 3 | 2 |
| **Mouflon** | 2 | 3 | 0 | 0 | 2 |
| **TOTAL** | **100** | **103** | **99** | **21** | **36** |

ND, non determined. n=359.

## Discussion

For exotic diseases, the WildScreen.be network detected the first cases of bluetongue disease in wild cervids (2006), EBHS in hares (2004) and canine distemper in foxes (2009) in Southern Belgium [5 and 6]. Related to the surveillance in wild boars, the region is actually free of CFS. For bluetongue disease, the network has not detected massive mortality despite the dissemination of the virus over the entire region in 2007. The passive surveillance is strategic for early detection of exotic diseases. However, this surveillance is very difficult to organize on the field and interpretation of results is often difficult due to selection biases and poor quality of analysed tissues. To counterbalance this, a strong and sustained motivation of each field collaborator is crucial. To get this, it is very important to generate training sessions for them and to systematically and regularly provide them a scientific feed-back about the "fate" of each sample and about its pixel-sized but important contribution to the general picture. This is time-, money- and effort-demanding for a network, but it is the only way to maximize the probability that the network itself will be and stay in a good position to detect and manage emerging diseases.

Studies on targeted endemic diseases are continuously carried out to monitor changes. Results are transmitted to Authorities to help in the decision-making process. For instance, due to focal points of infectious problems in game species (namely paratuberculosis in cervids), regional Authorities enacted a law allowing immediate selective culling of obviously severely diseased animals (severe emaciation with/without diarrhea) to prevent pathogens from spreading. This official procedure can be implemented by hunters and forest rangers all over the year in the focal zones concerned. The legal procedure includes a mandatory necropsy by the team of the WildScreen.be network at the University of Liege. In wild boars, endemic situation for Aujeszky disease and brucellosis and the real risk of reintroduction of CSF from neighbouring countries associated with high density of wild boars in some forest districts must be taken in consideration in hunting management. Sensibilisation campaigns are also carried out by WildScreen.be to inform forest stakeholders about the infectious risks inherent to overabundance of some game species and advices are given to limit or suppress artificial feeding in the forest. Several years will be necessary to evaluate the impact of such control measures in the region.

In the future, WildScreen.be has to improve some points, including biases in passive surveillance and motivation of field actors. Surveillance has to be focused on zones where the interface between wild and domestic animals is the most likely and intimate. The array of partnerships should be extended and the network's communication to the society must be improved. Finally, additional financial support is still needed to go thoroughly into some priorities (bovine tuberculosis for example) and to anticipate new infectious zoonotic threats, especially in the context of climatic changes.

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# *Mycobacterium bovis* surveillance in European badgers (*Meles meles*) killed by vehicles in Northern Ireland: an epidemiological evaluation

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**D.A. Abernethy**[1], E. Walton[2], F. Menzies[1], E. Courcier[1] and P. Robinson[1]

**Abstract**
A survey of bovine tuberculosis (TB) in badgers killed by cars has been ongoing since 1999, with an annual mean of 78 badgers submitted for laboratory examination. The sample prevalence was 20.0% and temporal trends were similar to those of bovine herd prevalence. TB in badgers and surrounding cattle herds was spatially associated at distances up to five kilometres. The survey provides valuable epidemiological information and is a useful monitoring tool, but is constrained by several inherent weaknesses. Validation, through comparison with badgers captured at setts, is therefore required before the survey can be fully utilized.

**Keywords:** Bovine tuberculosis, badgers, surveillance, roadkills.

## Introduction

Bovine tuberculosis (TB) remains endemic in parts of the British Isles despite nationwide eradication programmes being in place for more than 50 years and significant progress in the early stages. Final eradication has proved elusive with lack of success attributed to a range of cattle-related factors and the presence of wildlife reservoirs, most notably the European badger (*Meles meles*).

Northern Ireland has an estimated population of 34 000 badgers with a mean sett density of 0.56 social groups per square kilometre [7]. Culling is not permitted and monitoring of TB has therefore been conducted through informal surveys of badgers killed by vehicles. In this paper, results of the current survey are presented and its value and limitations discussed.

## Materials and methods

Reports of badgers killed by vehicles and collection of carcases (“road traffic accident” badgers or “RTAs”) are managed by a Wildlife Officer within the Veterinary Epidemiology Unit of the Department of Agriculture and Rural Development (DARD). Reporting was initially limited to government employees to restrict reporting bias but later extended to farmers and members of the public. When collecting the RTA, the Officer confirms the map reference and identifies the two nearest bovine herds. Carcases are submitted to one of two laboratories of the AgriFood and Biosciences Institute (AFBI), a non-departmental government body, who undertake all laboratory procedures. Following a standardized *post mortem* examination, a range of tissue samples are routinely collected from lymph nodes (a “cranial” pool comprising parotid, submaxillary and retropharyngeal lymph nodes, prescapular glands pooled with popliteal, and the mesenteric lymph nodes), as well as faeces and urine. Samples from other lymph nodes or organs are taken when gross pathological changes are detected. Bacteriological culture is undertaken on all samples using standard methods and genotyping (spoligotyping, VNTR) performed where positive growth is observed, to confirm *M. bovis* to strain level.

Summary measures are reported for the period January 1999 to September 2010. Bovine TB test data were obtained from the central animal health database of DARD. Stepwise logistic regression was used to explore associations between TB-infected carcases and regional distribution. For this, locations were classified by county (one of six) and by veterinary administrative area (one of ten). Thereafter, spatial associations between TB in badgers and surrounding bovine herds was assessed at three levels. First, the risk of TB in the two herds nearest to infected badgers, for one year either side of the RTA collection date, was compared to those nearest TB-negative badgers. Second, the cumulative herd prevalence of TB in five concentric rings of one kilometre was compared in herds around infected badgers with those around TB-negative badgers. Third, the ratios of the nearest neighbour distance from a TB positive herd and a TB negative herd were calculated for each badger, based on Woodroffe *et al.* [6], and the ratios compared between infected and uninfected badgers using Wilcoxon rank sum tests. For the analyses, a positive bovine herd was defined as a herd having one or more skin test reactors or a culture-positive abattoir case. An infected badger was one confirmed as *M. bovis* by molecular means. Prior to 2006 however, confirmation was not possible for a minority (27%) of badgers, which were allocated a TB status based on the annual proportion of samples confirmed. These data were included in the cumulative and annual prevalence estimates but excluded from the spatial analyses. For the latter two spatial analyses, data were restricted to the period 2006 to 2009 to limit temporal variation. Mapping was undertaken in ArcMap version 9.3.1 (ESRI), logistic regression in SPSS 17.0 and nearest-neighbour distances in R version 2.12.0 using the spatstat package.

## Result

935 badgers were tested between 1999 and Sept 2010, an annual mean of 78 (range: 20 in 2001 to 134 in 1999). The sample prevalence was 0.20 with annual prevalence varying from 0.07 to 0.49 (2009 and 2002 respectively, Figure 1). The trend over the period was similar to that observed in the bovine population.

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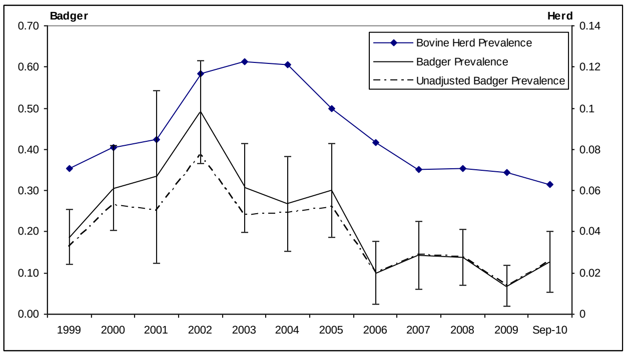
The prevalence by county varied from 0.10 (Co. Londonderry; 95% exact binomial confidence interval = 0.05, 0.15) to 0.29 (Co. Fermanagh; 0.16, 0.42) and, between 2006 and 2009, from 0.06 (Co. Londonderry and Co. Antrim; 0.01 to 0.16) to 0.25 (Co. Fermanagh; 0.03 to 0.65). No significant association (p>0.05) was detected between the infection status of badgers and location, at either county or administrative area level.

**Figure 1**: Annual herd prevalence and RTA survey prevalence (solid line = adjusted for samples not confirmed by genotyping, with 95% confidence intervals; broken line = samples confirmed by genotyping).



The risk of TB in the two herds closest to each RTA was significantly higher in those located near infected badgers than uninfected animals (Odds Ratio = 1.67; 95% confidence limits = 1.27 and 2.21; $\chi 2$ test, p<0.001). Herds located in each of five zones surrounding infected carcases had a significantly increased risk of TB between 2006 and 2009 compared to those around TB-negative badgers (Table 1).

The ratio of distances between herds (TB-positive, TB-negative) and RTAs (TB-positive, TB-negative) was significantly lower for infected than for uninfected badgers (p = 0.019), suggesting infected badgers were closer to infected TB herds than uninfected badgers.

**Figure 2**: Distribution of samples by county.

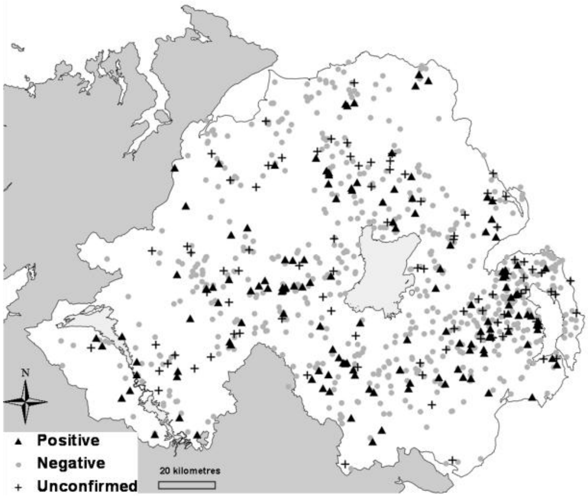
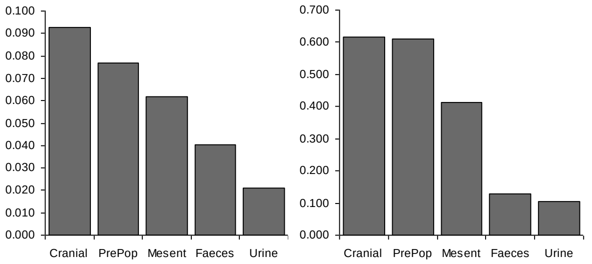


**Table 1**: Cumulative herd prevalence, 2006 to 2009, around infected and uninfected badgers and Z-test for difference in proportions.

| *Zone* | *Prevalence* | | *Z-test* | |
|---|---|---|---|---|
| | *TB-pos.* | *TB-neg.* | *Z-value* | *Confid.* |
| <1km | 0.382 | 0.303 | 2.374 | 98.2 |
| 1to2km | 0.331 | 0.264 | 3.326 | 99.9 |
| 2to3km | 0.310 | 0.273 | 2.275 | 97.7 |
| 3to4km | 0.278 | 0.235 | 2.999 | 99.7 |
| 4to5km | 0.274 | 0.224 | 3.473 | 99.9 |

The cranial pool of lymph nodes was the most commonly affected of the routinely sampled carcase sites (9% of sites, 60% of infected badgers, Figure 3). Of 141 badgers sampled at three or more carcase sites and confirmed as infected by spoligotyping, infection was disclosed at a single site in 77 (54.7%). Conversely, three or more sites were affected in 33 (23.4%).

**Figure 3**: Prevalence of infected sites in all badgers (left) and in infected badgers (right). Cranial = pool comprising parotid, submaxillary and retropharyngeal lymph nodes, Prepop = pool comprising prescapular and popliteal lymph nodes, Mesent = mesenteric lymph nodes.



## Discussion

Surveys of RTA badgers are a relatively inexpensive and ecologically friendly means of monitoring TB. They have been used previously in Great Britain, where prevalence estimates ranged from 0.12 to 0.21, with a mean of 0.15 [2], [3] and in Ireland where prevalences of between 0.10 and 0.14 were reported [5]. In Northern Ireland, a survey in the 1980s recorded a prevalence of 0.12 with TB-infected badgers more likely to be within 2km of an infected bovine herd than TB-negative badgers (n = 240; Odds Ratio = 5.52; 95% C.I. = 1.59 to 23.38; p <0.005; O. Denny, unpublished data). In an early analysis of data from the current survey (1999 to 2001; n = 254), the sample prevalence was 0.18 and no association was detected between TB in the badgers and that of the nearest two herds (Odds Ratio = 1.56; 95% C.I. = 0.76, 3.11; $\chi^2$; p = 0.193). However, the risk of TB in the previous four years in herds within three kilometres of a positive carcase was significantly higher than those around a TB-negative carcase (0.26 and 0.20 resp. $\chi 2$ test; p<0.001), [1].

The results of the survey are consistent with a close association between TB in badgers and cattle. Notwithstanding the small sample sizes in some years, the temporal trend in sample prevalence is similar to that of the bovine herd prevalence. Furthermore, a consistent, significant spatial association existed between infected badger carcases and TB in surrounding herds, from those closest to where badger carcases were collected, to the five kilometre limit of the analysis. The lack of association between RTA positivity and region may arise from a limited sample size and short-term variation but may also indicate that alteration of prevalence within one species may affect the other, but on a localized scale.

As these results do not indicate direction of transmission, they provide no information on the relative roles of badgers and cattle in the persistence of TB in Northern Ireland. Nevertheless, work elsewhere (for example, the Four Areas Trial in Ireland and the Randomised Badger Culling Trial in England) indicates a prominent role for the badger and risk mitigation measures have been implemented (*e.g.* badger culling and improving on-farm biosecurity) or are currently under trial (*e.g.* vaccination). RTA surveys prove a useful role in monitoring trends and providing epidemiological information. They may also assist in identifying potential areas for intervention or those with increasing infection to allow early remedial action or enhancement of measures already in place. However, surveys of this nature contain a number of inherent weaknesses. As a passive means of surveillance, they are highly dependent on the cooperation of the public. Even with quotas in the current survey, reporting varied greatly within the country and it is not clear if sufficient RTAs will be reported within a sufficiently short period to allow sufficient spatial precision. RTA badger surveys are also subject to bias; they are not a random sample of the population and may not therefore be typical of all badgers with respect to age, sex or severity of TB infection. Heavily infected badgers may be less likely to roam, resulting in an under-estimate of infection, which will also occur if younger badgers are more likely to range away from setts. The sample population is biased to areas surrounding roads and thus regions with a lower road density or with roads less used by cars will be under-represented. Conversely, high-speed roads provide few RTAs as carcases are often too damaged for retrieval.

A more serious bias results from the passive nature of the surveillance and consequent reporting bias whereby carcase sightings are more likely to be reported by farmers with infected herds than those from TB-free herds. This risk was initially addressed by limiting reports to government employees but was not maintained due to a need to increase the sample size and to farmers reporting RTAs through local government offices. In a field survey of setts in Northern Ireland, Menzies *et al.* [4] found that farmers with herds recently infected with TB were more likely to know the location of setts on their farm than those with no infection. If such farmers are more likely to report RTAs, the bias will lead to an over-estimate of prevalence and the spatial associations reported above. The decay in risk with distance from TB-infected carcases (Table 1) may point to causality but a similar trend with TB-negative badgers is more consistent with reporting bias.

These weaknesses have been long recognized [3] and may be partly addressed through strict controls on reporting, setting of regional quotas and proactive searching for badger carcases. However, where the purpose is to provide information on the geographic distribution of infection in badgers as a prelude to intervention (*e.g.* vaccination), the survey method needs to be first validated before the results can be applied [8]. This can be achieved through a series of comparisons with badgers captured at the set, to ensure the RTA badgers are representative of the population.

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# Description of causes of mortality in cattle over 2 years, foreseen interest for epidemiological surveillance

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## Abstract

We are currently implementing a cattle mortality monitoring system in France. Building such a system requires a good knowledge of the baseline mortality and its determinants. Thanks to a database gathering data about more than 40 000 deaths of adult cattle that occurred in France from August 2000 to March 2001, we described the causes of mortality according to temporal and individual factors. Besides its interest for animal production science, this descriptive work will help us to define the baseline against which anomalies could be detected in the future mortality monitoring system.

**Keywords**: Health indicator, syndromic surveillance, mortality, cattle, early warning system.

## Introduction

In most European countries, human health agencies have implemented mortality surveillance systems. These systems are designed to identify excess deaths by comparing the observed and expected mortality [1].

Such a monitoring system could be relevant for cattle surveillance, but the baseline mortality in this species is not well described yet. Indeed, despite important consequences for farmers (loss of production, loss of genetic, culling costs), mortality in adult cattle has been rarely studied.

Our study aimed at describing and quantifying the most usual causes of death in French cattle. Beyond its interest for production sciences, this work will help us to understand and model the baseline fluctuations of mortality, a necessary step towards the building of an anomaly tracking system.

## Materials and methods

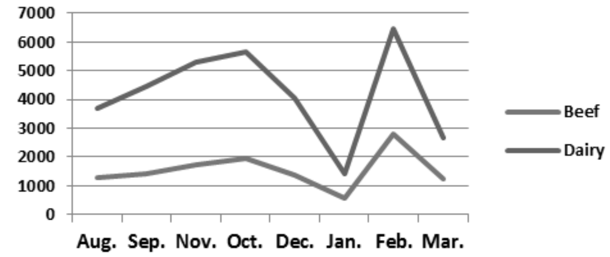
*Data collection:* Data were collected through a program initially designed for the Bovine spongiform encephalopathy (BSE) surveillance in 2000 [2]. At this time in France, every death of cattle over 2 years was investigated by a veterinarian, who had to fill up a form (“Fiche d’Information et de Suivi de la Mortalité”). In addition to data related to BSE testing, the forms included the following information: herd and animal identification, age at death, sex, production type, death type (euthanasia or natural death), associated syndromes (neurologic, genital, digestive…) and diagnosis. We analyzed the diagnoses reported by veterinarians for all the cattle deaths that occurred in Brittany, Lower Normandy and Pays-de-la-Loire (three French administrative regions in the western France) from 07/08/2000 to 16/03/2001 (with an interruption from 22/12/2000 to 29/01/01), since they were the only data that had been recorded in an electronic database. This part of France is an important region of production, especially for dairy cattle (mean dairy population in this region is 1,841,172 [3], i.e. about 40 percent of the French dairy cattle).

*Classification of death causes:* We defined an algorithm to classify the diagnoses according to keywords, syllables and words associations that were found in the character chains of the diagnosis field. For similar diagnoses, different expressions were taken into account (*e.g.* milk fever and parturient paresis), as well as familiar terms. When different causes were recorded, the algorithm selected a unique cause according to the priority order we previously defined. Only categories representing more than 2% of the total amount of deaths were kept for description. Others were grouped in the “Other causes” category. Comparisons between different groups (type of production, sex…) were done using Khi2 tests.

## Results

The program gathered data about 47,266 deaths of cattle, among which 12,779 beef cattle and 34,487 dairy cattle, mainly female (96%). Only 77 percent of the total had a diagnosis completed. Monthly number of death notifications by cause is represented on Figure 1. The very low number of deaths in January corresponded to the period of the interruption of the program.

**Figure 1**: Monthly cattle death notifications from August 2000 to March 2001 by production type



From the 36,640 recorded diagnoses, the algorithm could group 27,895 into 12 categories representing at least 2% of the deaths. The 8,585 diagnoses left, which had scarce occurrences, were grouped in the “Others causes” category.

Monthly proportion of each cause is shown on Figure 2 for beef and dairy cattle respectively. Relative proportions of death causes were not constant over the Study period. The proportion of unknown causes was

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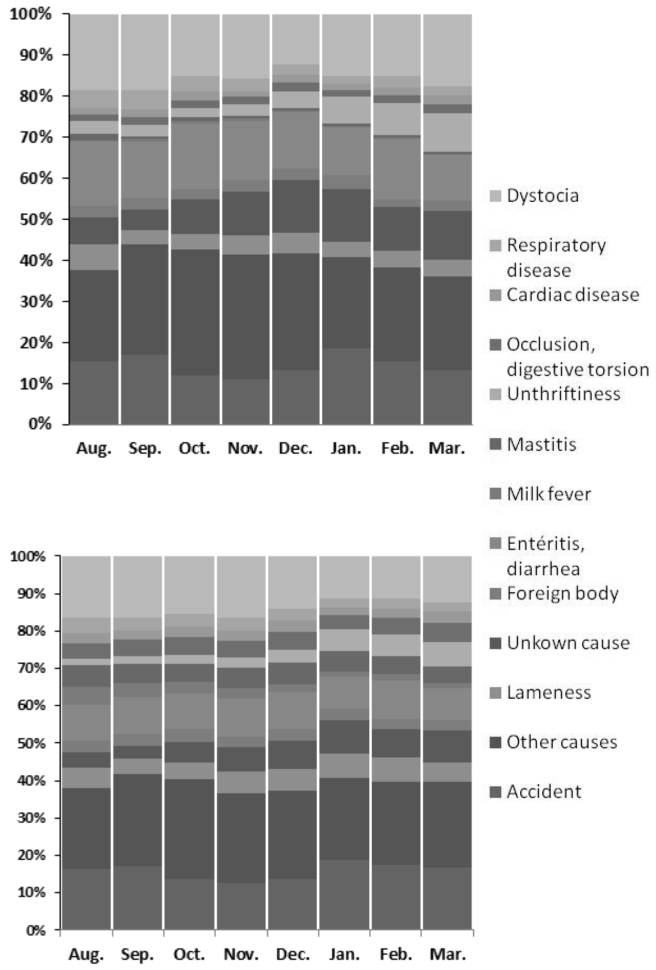
for example significantly different from December to March than from August to November.

**Figure 2**: Monthly proportion of each cause among notified deaths from August 2000 to March 2001 in beef (up) and dairy cattle (down)



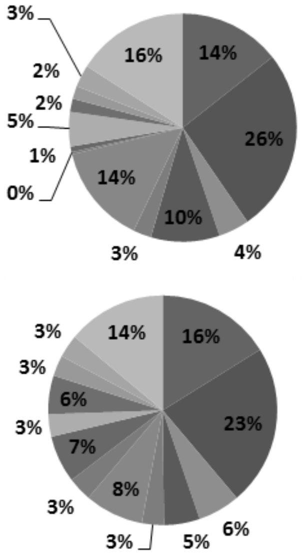
The main death causes (except "Others causes"), whatever the production type, are "Accidents" and "Dystocia" in similar proportions (between 14% and 16%), followed by "Enteritits and diarrhea" (Figure 3). The "Other causes" are rather numerous, representing between 26% (for beef) and 23% (for dairy) of the causes, highlighting the broad range of possible causes of deaths, and the difficulty to classify them.

Importance of causes varied according to the production type (Figure 3). The proportions observed in the two groups were significantly different.

As expected, "Milk fever", "Mastitis", "Lameness" and "Occlusion/ digestive torsions" were more frequent death causes in dairy than in beef cattle whereas "Unknown causes", "Enteritis/diarrhea", "Unthriftiness" and "Dystocia" were more frequent death causes in beef than in dairy cattle.

Death causes were significantly different in male and female ($p<0.0001$), even when discarding deaths related to reproduction ("Mastitis", "Dystocia" and "Milk fever"). Accidents were more often reported as cause of death in males (27%) than in females (20%).

**Figure 3**: Proportion of death causes among beef (left) and dairy (right) cattle



Besides the death for which the diagnosis was missing (i.e. 23% of the total deaths, that in some cases may have had an identified but not recorded cause), 4.8% of the recorded diagnosis indicated that a diagnosis could not have been reached. These deaths, from unknown causes, were most frequent in beef cattle than in dairy cattle (proportion was 1.7 times higher in beef than in dairy cattle), and in male than in female (proportion was 1.12 times higher in male than in female).

## Discussion

At the time of the survey, diagnosis was freely recorded by veterinarians. We managed to classify the diagnoses using an algorithm analyzing character chains. But besides languages matters, the algorithm had to prioritize determinants when the cause recorded was not unique, and choices had to be done, sometimes arbitrarily. Better classification could be have been reached if nomenclature as the Systematized Nomenclature of Human and Veterinary Medecine (SNOMED) or the Veterinary Nomenclature (VeNom) had been used to classify the death causes.

But in spite of its limits, the algorithm allowed us to classify and described the death causes that occurred in a cattle population of about 2.7 million individuals, monitored during an 8 months program. Importance of the different death causes varied according to the production type and sex of the cattle. As expected, causes of death in dairy cows were related to milk production and housing system, whereas beef cattle death are more often linked with poor health monitoring possibilities [4].

Moreover in each population group, the distribution of the causes was not stable in time but varied over the 8 months of the program.

Seasonality of crude cattle mortality rate has already been described [4, 5], but its determinants are not well known. In a previous work, we modeled the weekly evolution of crude mortality rate in cattle over 2 years and identified a strong seasonality. The periods with higher mortality rates matched the calving seasons but we had no data regarding causes of death. The present results confirm the important proportion of the deaths related to calving in cattle over 2 years: “Dystocia” and “Milk fever” represented in average 16% of the deaths in beef cattle and 19% in dairy cattle. However results also showed that other causes of death presented temporal variations which could not be explained by the calving rate. For example the proportions of “Unthriftiness” and “Unknown causes” increased from November to March in beef cattle, in parallel to the increase of the proportion of calving related causes of death.

Hence even if calving rate seem to represent a determinant factor for cattle death and could therefore be taken into account for modeling baseline mortality, the fluctuations we observed in the causes of death suggest the existence of other seasonal factors to be identified.

The system we plan to implement is based on a comparison between observed and excepted mortality rates, without information on causes. The results of this work will help us to understand and model the baseline fluctuations of the crude mortality rate in the different cattle population. However it could be interesting to add information on causes in the surveillance system. Indeed following the frequency of death causes, including “Unknown causes”, could allow detecting anomalies whenever the crude number of death remains in the ordinary limits.

The studied cattle population was not representative of the whole French cattle population and the program only lasted 8 months. To complete this description and better understand the determinant of the cattle mortality baseline, we are now carrying out a national postal survey, planned to last one whole year and collect weekly data about the cattle causes of death .

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# Risk factor analysis and possible control measures for Salmonella in Swiss breeding pigs within the framework of an imminent need for a national control programme

**S. Bruhn**[1*], J. Rushton[2] and M. Reist[3]

## Abstract

Nontyphoidal salmonellosis has a human health impact with between 10% - 20% of human salmonellosis cases in the EU thought to be attributable to pig meat. In order to reduce this societal impact a survey in Swiss breeding pig farms was conducted and risk factors and control measures at pre-harvest stage investigated within the framework of an imminent need for a national control programme.

**Keywords**: cross-sectional study, risk factors, Salmonella, national control programme.

## Introduction

*Salmonella* develops from a pre-harvest infectious agent and multiplies at harvest and post-harvest stages of the chain [1] causing human salmonellosis. The global human health impact is high with an estimated 93.8 million illnesses each year of which 80.3 million are thought to be foodborne [2]. In the EU there are 131,468 cases (2008) [3] and 10-20% of these are thought to be ascribable to pig meat [4, 5, 6]. Fatal outcomes have been reported [7, 8] and deaths are estimated to be 155,000 worldwide and several thousand in the EU [1, 2]. The above mentioned numbers are only the tip of an iceberg. Goldbach and Alban [9] assume that only 12,5% of all cases are reported taking a mid-value between 5% and 20%.

The importance of salmonellosis may rise with increasing multiple antibiotic resistance of pork-related serotypes[10, 11] and ageing populations as the young, old, pregnant, immuno-compromised (YOPI) population is more susceptible to disease [12].

In order to reduce human salmonellosis cases the EU Zoonoses Regulation (EC) No 2160/2003 asks each country to implement a National Control Programme (NCP) to achieve a reduction target for *Salmonella* infections in pigs set by the European Commission within 3 years, resting on results of the EU slaughter (2007) and breeding pigs (2008) surveys.

*Salmonella* control in breeders (particularly nucleus farms) was thought to have the biggest impact on the reduction of human salmonellosis cases [1]. Within the framework of an imminent need for a Swiss national control programme, risk factors for Salmonella infections in its target population were analysed based on the Swiss data of the European baseline survey in order to establish the most relevant control strategies.

## Materials and methods

In 2008, a cross-sectional study was conducted according to commission decision 2008/55/EC. Farms were divided into breeding (≥ 40% of gilts are sold for breeding purposes) and production farms (pigs mainly sold for fattening) and stratified by size as well as five regions according to the pig health service. Farms were selected proportionate to each stratum by simple random sampling. Sample size calculations were based on an annual expected prevalence of 50%, a 95% confidence interval, 7.5% accuracy and an applied finite population correction factor.

Per farm 10 faecal samples were collected - when possible from 10 different sample units with at least 10 animals. For detection and serotyping of *Salmonella* ISO 6579:2002 Annex D and the Kaufmann White scheme were used, respectively. A farm was *Salmonella* positive when at least one sample was positive. Information on 20 risk factors specified by commission decision 2008/55/EC for detection of *Salmonella* were collected using a farm-level questionnaire.

First, data consistency check and descriptive analysis were performed. For the statistical analysis Stata® v.11 was used. Data were accounted for their correlated nature by using the generalized estimating equations (GEE) method. Farm level variables were analysed using normal statistics and the Chi-squared test. To consider the number of positive sample units per farm Poisson regression was applied. Variables with a Salmonella infection association of a P-value $<0.3$ were selected and checked for major differences in percentages and for correlation among each other with a univariate logistic regression using GEE. The final model was obtained by manual backward calculation removing one by one the variable with the highest P-value confirmed by a Wald test. All variables with a P-value below roughly 0.05 were left in the model and checked for interactions.

In ArcMap v.9.2 density maps per square kilometer were created for positive and negative farms using a kernel smoothing tool (ESRI Inc., Redland, CA, USA) with identical bandwidth and an odds ratio extraction map was established. Spatial scan statistic SaTScan v.7.0.3 using circular scanning windows, the Bernoulli probability model and Monte-Carlo randomisation with 999 permutations was performed for high and low risk areas.

The *Salmonella* prevalence was estimated in an epidemiological decision tree spread sheet model built in Excel®. To obtain the overall prevalence, the fraction of each of the 40 branches (Figure 1) was determined based on the survey data, multiplied by the relative risk for each branch and adding up the values of all branches.

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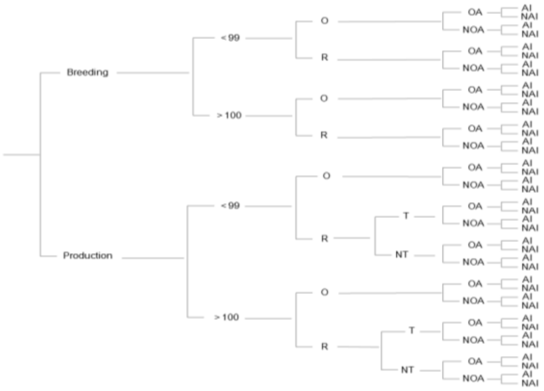
The relative risks of boar management were obtained from own survey data. For feeding organic acid and all-in/all-out management published odds ratios were recalculated into risk ratios [13]. The according risks in the exposed and unexposed farms related to the survey's overall prevalence estimate were then obtained by using the percentage of farms feeding organic acid and applying all-in/all-out management from the survey data. To estimate the reduction in prevalence the fractions of the branches without a particular intervention were set to zero by adding them to the fractions with this particular intervention. The input parameters were modelled stochastically using distributions generated by @Risk 5.0 for Excel® and running 1000 iterations with the Monte Carlo simulation. For validation purposes it was checked if the model output without applying distributions matched the overall prevalence. Sensitivity analysis was performed for each scenario to find the input parameters with the biggest impact on prevalence reduction.

To assess the impact of tested negative boars it was assumed that between 1 and 5 boars (with a mode of 2) were bought per year having also a 13% Salmonella infection prevalence. The test systems' specificity and sensitivity was supposed to be 100% and 60%-90%, respectively, accounting for test and sampling (intermittent shedders) uncertainty. The prevalence reduction in farms buying negative-tested boars was calculated separately for the two farm sizes by taking the estimate from farms with own boars as baseline and adding the difference in prevalence between farms with own and bought boars times the risk of not detecting *Salmonella* despite testing.

**Figure 1**: decision tree underlying the Excel® spread sheet model to estimate the effect of the tested interventions on prevalence reduction



Legend: <99 = farms with < 99 pigs; >100 = farms with >100 pigs; R = farms buying boars; O = farms keeping own boars; OA = farms feeding organic acid; NOA = farms not feeding organic acid; AI = farms with all-in/all-out management; NAI = farms without all-in/all-out management; T = boars pretested before purchase; NT = boars not pretested before purchase.

## Results

The ideal sample size was 231 (158 production and 73 breeding) farms. 223 of the 231 farms gave valid results. 29 farms (13%, 95%CI 8.9%-18.1%) and 89 sample units (4.0%, 95%CI 3.2%-4.9%) were *Salmonella* positive. The prevalence was slightly higher in breeding than in production farms, with overlapping confidence intervals [15.5% (8.0%-26%) versus 11.8% (7.2%-18%)]. In total, 14 different serovars were found, with *S.* Thyphimurium (n=6) and *S.* Derby (n=4) being the most frequent.

The fact that single pens (meant to be pens containing only 1 pig which thus has no direct contact to other pigs) incorporated up to 60 animals showed that this variable inconsistently interpreted. Seven of 20 variables (number of pigs in a sample unit, production stage, age, gender, feed origin, feed supplement) contained subcategories with <10% of the data. In the cross-tabulations it was striking that bigger farms were more likely to be *Salmonella* positive than smaller ones (22.1% versus 8.2%).

Nine of 11 possible risk factors with p<0.3 were included into the multivariable model as two of them were excluded: production subtype due to its correlation with production type and single pen because of inconsistency and its strong association with all-in/all-out and production stage (85,4% of all pens with all-in/all-out management and 93.8% of the maternity production stage pens were kept in single pens). The final GEE model included farm size with p<0.001, replacement boars with p~0.05 and production type. After adjusting for production type and boar replacement, there was strong evidence (p<0.001) that larger farms (100-999) had an almost 6 (95% CI 2.43 to 14.0) fold odds of *Salmonella* infection compared with smaller farms and some evidence (p=0.06) that farms purchasing boars >90% had a 2.4 (95% CI 0.97 to 5.95) fold odds compared with those having only own boars or lower purchase quota. As the estimate for farm size never changed >4% when removing variables from the model as well as when checking all remaining variables in the final model there was no indication for possible confounding. The subsequent Wald tests were all in favour of the simpler final model. As the percentages of positives in production type stratified by replacement boars was among producers 12.9% purchasing versus 3.6% having own boars and among breeders 11.1% versus 20.5%, respectively, interaction was tested as an alternative within the final GEE model revealing a small P-value (p=0.04) but huge confidence intervals of the estimate (1.07-235). The Wald-test comparing this interaction model with the final model was indifferent (p=0.08). Validation with the Poisson regression resulted in an even smaller P-value (p=0.014) for this interaction, but still, although narrower, a huge confidence interval (1.74-123). In the likelihood ratio test (LRT) the interaction model appeared to be the better one (p=0.001). However, using normal logistic regression considering a farm only to be either positive or negative, this interaction was no longer apparent.

In the spatial analysis no effects could be seen in the extraction maps. Also in SaTScan no clusters were identified (all p>0.58). All interventions lead to a 2.3% to 8.5% reduction in the mean prevalence, assuming causality, with an increasing effect in the order feeding organic acid, all-in/all-out and their combination. The sensitivity analysis showed that the prevalence of S*almonella* was the most critical input parameter with correlation coefficients between - 0.33 and - 0.8.

Adopting the model for the upper (18%) and lower (9%) confidence interval prevalence to further examine the prevalence impact revealed that the higher the prevalence the higher its absolute reduction would be.

## Discussion

The sample size was large enough to estimate the prevalence of Salmonella infection with an accuracy of 5% as the detected prevalence was below 50%. The apparent overall prevalence estimate is accurate as the study was representative for the pig breeding population and the 95% confidence interval width was moderate. The sample unit prevalence of 4% needs to be taken with caution as multi-sampling of same groups was sometimes necessary in smaller farms, which was not assessed.

The risk factor analysis yielded only limited information for control strategies as farm size was the only evident risk factor which was already well described in literature [14, 15, 16]. The latter is no option for interventions, however the information might be used in a risk-based control programme. Why no other risk factors were detected was not clear. These findings may be true, due to bias (non-differential misclassifications may dilute effects), insufficient power for risk factor detection or chance (Type I-error). Bias was possible as data inconsistency in the single pen variable existed. Gathering artificially 10 animals per sample unit in smaller farms increased indifferent data in mixed subcategories and sample size might have been too small to detect smaller effects, especially in variables with several subcategories and in those lacking sufficient positive results. Furthermore, many variables contained almost the same data for the 10 sample units per farm creating difficulties in the calculations in the correlated dataset next to the often skewed data within the variables. In addition, other risk factors for which no data were collected in this survey may exist. Whether the interaction replacement of boars among production farms can be interpreted to be a risk factor is in question. The evidence was weak (Wald test p=0.08 and huge confidence intervals). The artificial multiplication of this variable in the overall correlated dataset might have overestimated its effects. However, this possible interaction was statistically validated with a Poisson regression model - where no artificial multiplication on sample unit level took place, assuming causality. As in surveys only associations are assessed, further studies with higher causal relations need to be conducted to elucidate this possible interaction.

In Switzerland no spatial pattern of *Salmonella* infection in pigs was found, in contrast to results based on serological data obtained for UK and Denmark [17, 18]. As the 30 isolated strains belonged to 14 different serotypes, no geographical relationship of the single serovars was investigated, although they might occur [19, 20].

Typically, SIR transmission models are used to estimate changes in prevalence. However, with choosing the decision tree approach to model the effect of control strategies on Salmonella prevalence, the own survey data reflecting the Swiss breeding pig structure best could be incorporated. Disadvantages were restrictions to risk factors looked at in this survey as well as unwieldiness and imprecision if too many interventions were included. The fact that the reduction in prevalence was greater when interventions were combined indicated that the model worked as also shown in the validation step. The greater effect of the all-in/all-out interventions can be explained by its bigger risk ratio. As sensitivity analysis revealed that the Salmonella prevalence were crucial for the model output and adopting the starting prevalence confirmed its impact on the amount of reduction determining the true prevalence could be a possible improvement. In addition, there was uncertainty around the used published risk factor prevalence which were each based only on one or two studies, some with lacking or vague definition. To obtain more information, new risk estimates based on data analysis of the same survey on EU level should be published by EFSA soon.

A cost benefit analysis is currently being carried out by the European Commission as part for the target setting process for Salmonella control in pigs. For a successful implementation of a control programme, cooperation with the pig industry, agreement on who bears which costs and mechanisms to transfer benefits across the food chain will be essential, as benefits of Salmonella control occur mainly in human health.

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# Surveillance for Post-Weaning Sheep Mortality in Extensively Managed Australian Sheep Flocks: Challenges and Practicalities

**A.J.D. Campbell**[1*], C. Hill[1] and C. Bell[2]

## Abstract

Weaner illthrift is a syndrome of poor growth and production and excessive mortality that occurs in sheep in the first 12 months after weaning in many production systems and environments. It is of major economic and welfare significance but is often poorly detected by farmers. An intensive survey of two farms in Victoria, Australia was conducted over 6 months to investigate the causes of weaner illthrift and mortality. The major causes of mortality were cutaneous myiasis and ruminal acidosis, although these are often considered to be secondary causes of the syndrome. Accurate and timely detection of deaths was difficult to perform under the extensive grazing system used on the farms and that is characteristic of most Australian sheep farms, and the challenge such obstacles present to effective endemic disease surveillance is discussed.

**Keywords**: sheep, weaner, mortality, surveillance, illthrift, blowfly strike.

## Introduction

In sheep production, the term ‘weaner’ usually refers to a sheep between weaning, which may occur from 2–3 months old, and about 12 months of age. In Australia, many farmers have difficulty successfully managing weaners, especially Merinos [1, 2]. In extensively managed sheep production systems, such as occur in Australia, accurate stock tallies are not always kept and weaners often die unnoticed [3]. This may reduce the effectiveness of monitoring of endemic disease and the detection of exotic or emerging diseases of sheep.

‘Weaner illthrift’ has long been recognised as a syndrome characterised by poor growth and production, and excessive mortality [4]. Multiple aetiologies for weaner illthrift have been described, with poor nutrition and gastrointestinal parasitism frequently implicated as important, but not exclusive, causes in many cases. However, these descriptions have tended to precede the adoption of strategies that have significantly improved Australian farm productivity in the last few decades, such as the use of improved perennial pasture species, changed times for lambing and weaning, and preventive sheep health programs [5].

A maximum mortality of 4% in the year after weaning has been suggested as a target for weaners [6, 7]. However, in a national survey of 1,410 Australian sheep producers in 2008, 44% of farmers reported having annual average weaner mortality greater than the target maximum of 4% [8]. Fourteen percent reported mortality was 10% or greater. Similar figures have also been estimated from incidentally or indirectly reported data from field experiments and suggest that death rates have not decreased in recent times. Similar post-weaning mortality has also been reported in other parts of the world in widely varying production systems [7].

Recent studies have quantified important associations between decreased mortality, and increased bodyweight and average growth rate of the weaner flock [3, 9], which could be used to develop predictive mortality models and decision support tools to help farmers alter management and nutrition of the weaner flock to improve post-weaning survival. However, these analyses have relied on bodyweight monitoring at relatively infrequent intervals and have been unable to measure weight change approaching time of death of weaners that actually die.

Weaner mortality was recently estimated to cost approximately AUD$89 million annually and was ranked the fourth most costly endemic health problem in the Australian sheep industry [10]. Furthermore, the problem of weaner illthrift and mortality extends beyond pure economics because the current scenario of represents a significant animal welfare issue. The economic and welfare aspects of weaner mortality mean its effective monitoring is an important issue for the wider sheep industry and is currently a major area of policy development and research [8, 11]. Weaner illthrift also presents challenges for conducting effective surveillance-the subjects frequently die unnoticed over potentially large areas on private farms. Limiting animal health resources in the private and government sectors, as well as farm labour, exacerbate the difficulty of detection.

Thus, the aim of this pilot study was twofold. Firstly, it sought to document causes of death of weaners in two farms in western Victoria, Australia, managed according to routine district practice. Secondly, it sought to gather more detailed bodyweight data in these flocks to improve survival models used in farmer decision support tools. This paper reports the mortality results and comments on the logistics of undertaking the surveillance.

## Materials and methods

Two farms grazing Merino sheep and producing fine wool near Shelford in the Western District of Victoria, Australia (38.01º S, 143.96º E) took part in this study. Both farms were clients of the Mackinnon Project, a whole-farm advisory service based at the Faculty of Veterinary Science, University of Melbourne. The farms were chosen because of willingness by the farmers to participate in detailed monitoring of their weaner sheep flock and relative proximity (about 75 minutes by car) to veterinary facilities at the University of Melbourne.

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The farms were about 15 km apart and in a similar environment, with a roughly uniform annual rainfall pattern that averaged about 430 mm per annum (2000–2010). Farm A is about 5,000 ha in area and produces fine wool and lambs for slaughter from Merino and Merino-cross sheep, and cereal grains from broadacre cropping. Farm B is about 400 ha in area and produces fine wool from purebred Merinos. On both farms, Merinos lambed over a 5-week period commencing in September. About 2,000 and 850 lambs from farms A and B, respectively, were weaned at the start of December. Weaners then grazed paddocks 10–20 ha in size containing a brassica fodder crop interspersed with pasture (Farm A) or a pure brassica sward (Farm B). Supplementary barley grain was provided to weaners on Farm A in self-feeding troughs at an average rate of 210 g/sheep.day.

About 750 weaners were identified with individually numbered ear tags in late December 2009 on both farms, comprised of male weaners only on Farm A and both males and females on Farm B according to the farms' management policies. The farmers undertook to carefully inspect the weaner flock on a daily basis. Ear tag numbers of animals that were unwell or had died were noted, and the latter to be submitted for necropsy by a veterinary pathologist. All weaners were weighed monthly by the researcher. Pasture availability and quality in the grazed paddock were assessed visually each month. If visits had not been necessary for collection of animals for necropsy, the researcher visited the farm weekly to further inspect the flock. Observations ceased in May 2010, after the seasonal break when mortality risk in weaner flocks tends to decline sharply [3].

## Results

The causes of death that could be established on the two farms are listed in Table 1. On Farm A, nine dead weaners were found in the first two months of monitoring, prior to the onset of a blowfly strike outbreak (see below). A diagnosis could be established at necropsy in 4 of these animals, however rapid autolysis in the hot summer environmental conditions precluded a diagnosis being made in the remainder. Ruminal acidosis was the most prevalent cause of death. Soon after the first weighing, the weaner flock on Farm A was re-sorted by the farmer and other animals escaped through a broken fence into another group of sheep, so that an accurate tally of animals could no longer be made.

On Farm B, 9 weaners out of 808 (1.1%) died over the 4 months of monitoring from late December 2009 to late April 2010. All missing animals were accounted for. The most prevalent cause of death was blowfly strike, associated with the outbreak described below.

The incidence of blowfly strike increased on both farms in association with warm, moist environmental conditions in late March. At this time, the farmer on Farm A ceased monitoring the weaner flock closely and, although he reported a number of sheep dying of blowfly strike, he did not record tallies of affected animals. However, thirty-eight out of 576 animals went missing between the last two weighings with no animals being removed to other paddocks by the farmer. Thus, up to 6.6% of weaners may have died during the blowfly strike outbreak.

**Table 1**: Causes of death in weaner flocks from farms A and B.

| *Cause of death* | *Number of animals* | |
|---|---|---|
| | *Farm A* | *Farm B* |
| Blowfly strike (cutaneous myiasis) | 7 | 38† |
| Bacterial enteritis | 1 | |
| Aspiration pneumonia | 1 | |
| Ruminal acidosis | | 4 |
| Inanition | | 1 |
| No diagnosis | | 5 |

†estimate only

## Discussion

Although conducted in a convenience sample of farms over one season, this study did not identify poor nutrition or gastrointestinal parasitism as major causes of death in the monitored flocks. However, the diseases we observed have been reported as components of weaner illthrift in other surveys [12]. On both farms in our study, seasonal conditions favoured outbreaks of blowfly strike that were the major cause of death. These conditions probably helped to maintain pasture quantity and quality, ameliorating potential malnutrition. Nonetheless, mortality exceeded the industry target on one farm. This farm's manager reported being aware that the incidence of flystrike was increasing but does not appear to have reacted with sufficient speed to mitigate its effects on the flock. It is interesting to speculate that a more timely intervention may have been made if the rate of flystrike development had been better quantified.

The second most important cause of death was probably ruminal acidosis, which occurred only on Farm A. Examination of feeding records revealed that no introductory period of limited access to the barley grain was used when supplementation started, and the acidosis probably occurred in dominant weaners that consumed excessive amounts of grain soon after it was introduced.

The ruminal acidosis cases highlight important issues that are relevant to surveillance of endemic disease. Most of the sheep dying of acidosis were detected by the researcher, rather than the farmer. About half the carcases found around this time were severely decomposed and difficult to find without careful searching of the paddock. Thus, under normal management, it is possible that an important management-related cause of death may have gone undetected, and undiagnosed, until more animals had died. This suggests that, in extensively managed sheep production systems where labour is limiting, effective surveillance for endemic disease is difficult for conditions associated with management risk factors, and not only pathogens.

Detailed surveillance was performed more effectively on a smaller farm (Farm B), compared to a large one, even though the availabilities of labour per dry sheep equivalent were similar on both properties. On Farm B, the size of the farm meant the farmer could more actively monitor the weaner flock and check through the paddock more carefully for moribund or dead animals. Even with a researcher performing additional checks on Farm A, a number of dead weaners went undetected until it was too late to obtain an accurate diagnosis of the cause of death. Thus, on both farms, availability of labour appears to have been important in determining the success of the surveillance activity. However, the trend on Australian sheep farms is that labour units are allocated over increasingly larger numbers of animals.

The challenges encountered in undertaking this pilot study illustrate the difficulties of conducting effective surveillance in extensively managed livestock systems, and particularly in groups of young animals. These animals may often not be closely examined by the farmer for many months and will not enter an abattoir or be processed in some other way that would facilitate the accurate observation of disease or collection of reliable data. Yet, such groups of animals are often vulnerable to disease, because of factors such as specific nutritional requirements or naïve immune status, and disease or mortality problems that go largely unobserved contribute to health, productivity and welfare issues of major industry importance. New surveillance systems must take account of these aspects of disease that are unique to particular production systems or groups of animals. Furthermore, the importance of disease control that relies on strategic disease and nutritional management, such as bodyweight and growth rate manipulation to reduce mortality risk, rather than tactical responses to monitoring data, should not be overlooked.

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# Bee mortality and bee surveillance in Europe

**M.-P. Chauzat**[1], M. Debin[2] and P. Hendrikx[3]

## Abstract

The bee surveillance project sought information on both the prevalence of honey bee colony losses, and the surveillance systems operating in 27 European countries. Through a standardized questionnaire, information was obtained about the operation of the surveillance systems from 24 countries, relating to 25 systems. Existing data about colony losses were also collected from these countries. In addition, a thorough literature search of the existing databases, as well as relevant grey literature about causes of colony losses was completed, and the literature evaluated.

The main conclusions from project activities can be summarized as follows:

- General weakness of most of the surveillance systems in the 24 countries investigated;
- Lack of representative data at country level and comparable data at EU level for colony losses;
- General lack of standardisation and harmonisation at EU level (systems, case definitions and data collected);
- Common consensus of the scientific community about the multifactorial origin of colony losses in Europe and in the United States and insufficient knowledge of causative and risk factors for colony losses.

The project made 20 recommendations in the following areas:

- Implementation of a sustainable European network for coordination and follow-up of surveillance on colony losses to underpin monitoring programmes;
- Strengthen standardization at European level by harmonization of surveillance systems, data collected and by developing common performance indicators;
- Build on the examples of best practice found in existing surveillance systems for communicable and notifiable diseases already present in some countries;
- Undertake specific studies that build on the existing work in progress to improve the knowledge and understanding of factors that affect bee health (for example stress caused by pathogens, pesticides, environmental and technological factors and their interactions) using appropriate epidemiological studies (case control and longitudinal studies).
- The set up of a coordination team at European level.

This coordination team should be organized in such a way so as to ensure its sustainability and to enable effective surveillance program activities at the European level.

**Keywords**: Honey bee mortality, colony losses, overwintering mortality, surveillance system.

## Introduction

The French agency for food, environmental and occupational health safety (Anses, ex AFSSA) set up a consortium of seven European bee disease research institutes in order to answer the European Food Safety Authority (EFSA) call to assess existing surveillance systems, and to collate and analyse existing data and publications related to honey bee colony mortality across Europe [1, 2]. This consortium gathered partners representing the following countries: France, Germany, Italy, Slovenia, Sweden, Switzerland and the United Kingdom. The project was divided into three work packages: (i) a description and critical analysis of surveillance programs that measured colony loss, (ii) the collection and analysis of the epidemiological datasets on colony losses, (iii) a critical review and selection of relevant literature on the possible causes and risk factors of colony losses [3].

## Materials and methods

### *Description and critical analysis of surveillance programs*

A standardized surveillance network assessment tool (SNAT) was developed to analyse the European colony losses surveillance programs. Twenty-seven countries were selected to be part of the study. Twenty-five SNATs from 24 countries were completed, received and processed. Some Member States completed several SNATs, two countries had no surveillance system in place (Ireland and Portugal) and one country provided no answer (Romania). The SNAT analysis allowed the Countries to be classified into four categories: those with (i) a very good level of compliance with the standards of a good operating system (1 system), (ii) an upper intermediate level of compliance (4 systems), (iii) a lower intermediate level of compliance (12 systems) or (iv) a low level of compliance (8 systems).

### *Collection and analysis of the epidemiological datasets on colony losses*

Data from surveillance networks were collected and standardised in order to allow analysis at the European level. The only indicator that appeared to be commonly used was the “global colony loss rate” during the over wintering period. Therefore, all aspects of colony losses (such as summer losses) could not be addressed through this study. Temporal and geographical analyses showed an important variability in colony losses. However, such trends are difficult to interpret considering the wide variation in the quality of the systems that produce these data.

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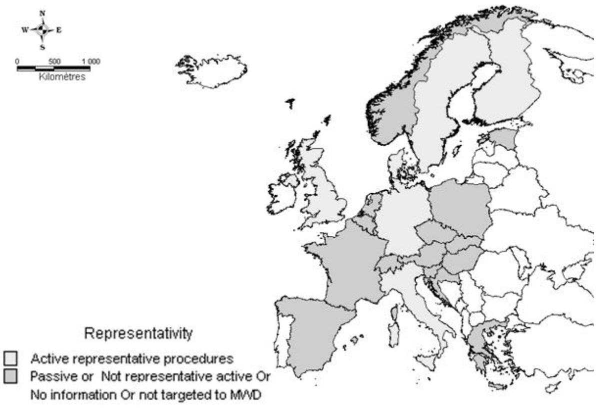
*Critical review and selection of relevant literature*
The literature review work package provided the opportunity to develop a specific methodology for literature search and analysis. The "priority 1" references selected and reviewed validated the objectivity of the literature search which is expressed through the variability and the balanced topics included (none of the topics appeared to be over-represented).

## Results

*Description and critical analysis of surveillance programs*
Eighty percent of the surveillance systems were found to comply with less than 50% of the 40 items covered by the questionnaire. This generally low level of compliance reflects a broad margin for improvement in most of the European surveillance systems considered within the project. Concerning surveillance procedures and protocols, of the 18 systems stating that they have in place active surveillance procedures, only 6 can be considered as valid active systems able to produce representative figures of the true colony loss situation r the considered countries (Figure 1).

**Figure 1**: Representativeness of the colony losses surveillance in Europe

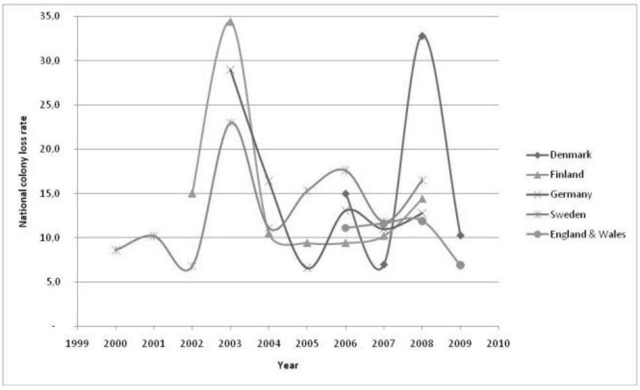


It was found that colony losses surveillance systems in Europe are characterised by a variety of the approaches and operational methodologies. Nevertheless, the majority do share common aspects, in particular the weakness of the systems implemented, and the lack of representative data produced.

*Collection and analysis of the epidemiological datasets on colony losses*
The project noted (i) a baseline colony loss rate around 10% each year at the European level and (ii) a higher level of colony loss in some countries during the years 2003 and 2008.

This analysis clearly highlights an absence of shared epidemiological indicators, collected following common surveillance procedures and based on comparable populations. Trend analysis and mapping suggests some periods of higher colony loss rates, but these findings should not be over interpreted (Figure 2). They serve to illustrate the fact that existing data collection systems are not robust enough for between country comparisons across Europe, or the analysis of trends at the European level.

**Figure 2**: National percentages of colonies lost during winter from 2000 to 2009 in Denmark, Finland, Germany, Sweden, England and Wales



*Critical review and selection of relevant literature*
The results of this work regarding risk and causative factors involved in colony losses have to be taken as a "snap shot" of the scientific community's opinion as it was at the moment of the work (beginning of 2010); these are "time sensitive", and evolving due to the amount of ongoing research which is likely to lead to new findings and a better understanding of the factors involved in the coming months or years.

There is a common consensus amongst the scientific community that the causes of colony losses in Europe and in the United States are likely to be multifactorial (in the two aspects of this term: combination of factors at one place and different factors involved according to place and period considered- [4]). Factors implicated include beekeeping and husbandry practices (feeding, migratory beekeeping, treatments and so forth- [5]), environmental factors (climate, biodiversity- [6]), chemical factors (pesticides) or biological agents (Varroa, Nosema, *etc.*) which together create stress, weaken bees' defense systems allowing pests and pathogens to kill the colony (*e.g.* one or several parasites, viruses- [7]).

Questions remain about the sequence of events that lead to colony mortality, and future studies should be designed and conducted to address this.

There are many inconsistencies in the ways in which "colony losses" are defined. Up to 17 different definitions for CCD exist in the literature. This means that reports may not always be referring to the same phenomenon, and this creates confusion when trying to explain the origin of what has been identified in the field. The described pathology is varied, with authors using the same descriptions for different sets of circumstances. A specific study should be undertaken to clearly categorise and quantify the various expressions of colony losses in the field. This study should be closely linked to the strengthening of surveillance systems.

High concentrations of pesticides have rarely been identified in relation to colony losses (CCD in USA and winter colony losses in Europe- [8]) although acute events of pesticide toxicity are well described during the production season (and clearly differentiated from CCD and winter colony losses). However, the questions of possible synergistic effects of various pesticides and the effect of chronic exposure to sublethal doses of pesticides remains [9], and requires further investigation. Biological agents such as parasites, viruses or bacteria, alone or in combination, have clearly been identified as important factors in colony losses [10]. Nevertheless, there is still a lack of knowledge about the exact mechanisms and/or interactions involved, this must also be addressed. Even though the multifactorial origin of colony losses is well acknowledged, the respective role of each factor as a risk or causative agent is unknown, and no hierarchy of relative threat posed by each one has been established. These matters require further investigation using appropriate epidemiological studies (case control and longitudinal studies).

## Discussion

The project advocates the improvement of the surveillance systems and has produced a set of 20 recommendations, designed to enhance honey bee surveillance systems at the European level. To enable these recommendations to be realised, the consortium proposed the setting up of a multidisciplinary scientific and evidence analysis (monitoring) group designated with developing common guidelines for the organisation, implementation and follow-up of national surveillance systems.

Harmonisation of surveillance procedures at a European level should lead to the establishment of a consistent and robust set of epidemiological indicators, calculated following the same rules and protocols in all countries, and produced by comparable active surveillance procedures applied across comparable populations. This recommendation is essential, as not only this will allow accurate comparisons to be drawn between the status of different European countries, and thus facilitate the objective assessment of fluctuating colony losses within Europe. An appropriate tool to monitor colony losses at a European level is important since it will provide National and European decision makers, and also the beekeeping industry, with accurate figures about colony mortality which, in turn could focus control and research activities.

The scientific monitoring group should be implemented at the European level to harmonise surveillance systems, and should also be responsible for the implementation and follow-up of the European data collection, management and interpretation activities.

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# Geographical atlas and temporal trends of all causes mortality in adult dairy cattle in Italy

**M.I. Crescio**[1]*****, R. Desiato, F. Ingravalle and G. Ru

## Abstract

Livestock mortality data are useful to syndromic surveillance provided that we understand the local specific patterns of mortality. Here we describe the epidemiology of mortality in adult (≥24 months) dairy cattle in Italy during the period 1/01/2002-31/12/2008, by means of spatial and temporal trends analysis.

**Keywords**: mortality, dairy cattle, temporal trend, spatial analysis, syndromic surveillance.

## Introduction

Conventional surveillance systems have been the primary tools of public health in detecting and responding to infectious disease outbreaks. However, traditional surveillance often operates with considerable delay, thus complementary surveillance systems are required to decrease the lead time [6].

A relatively new application of surveillance methods for animal health, food safety and public health is syndromic surveillance. .

Syndromic surveillance systems use electronic health data from a variety of sources to look for unexpected changes, with the assumption that such information may provide early indications of a disease outbreak. The name syndromic comes from the fact that the earlier version of these systems organized the data by grouping records with similar symptoms in the so-called syndromes [4].

Recently syndromic surveillance has been applied to animal health using data from farms, sales-yards, veterinary practitioners and abattoir condemnation data [2, 10, 11, 12].

Mortality data in human medicine as in veterinary science represents a useful indicator of the health of a population: in Italy the National Bovine Registry makes cattle mortality data available with a minimum delay after death (maximum 7 days).

Previously conducted studies, applied to human health data (*e.g.* influenza and hot weather), showed that mortality data were useful to syndromic surveillance systems [7, 9].

The use of mortality data for syndromic surveillance, however, can be limited as mortality can be influenced by a variety of factors and understanding changes in patterns of mortality in herds or flocks requires prior understanding of locale-specific mortality [3].

Consequently the objectives of this study were to describe the epidemiology of all causes mortality of adult (≥24 month) dairy cattle mortality in Italy, during the period 1/1/2002-31/12/2008, by means of both spatial and temporal trends analysis.

## Materials and methods

The National Bovine Registry provided the following individual information:

- Numerators: ear tag number, breed, sex, birth and death date, holding identification code of all adult cattle (over 24 months) that died, were culled or slaughtered on farm during the study period.
- Denominators: number of cows in each holding, split into 12 yearly age-class (24-35, 36-47, 48-59, 60-71, 72-83, 84-95, 96-107, 108-119, 120-131, 132-143, 144-155, 156 and over months) on 01/01 and 01/07 of each year of the study period. The holding identification code provided also the geographical location of the farm. The herd size was estimated from the average number of cattle on farm and classified in 4 classes: little (1-19 cows), medium (20-99 cows), big (100-499 cows) and huge (500 cows and more).

Firstly we described both geographically and by year some features as age distribution, herd size distribution and type of production (milk or mixed production).

We then calculated crude and specific mortality rates for macro geographical areas (North, Centre, South and Islands) and year of study.

A geographical analysis was then carried out at a district level on directly standardized rates (by age-class and herd size) and at a municipality level on standardized mortality ratios (SMR).

To take into account the uncertainty of the local measurement and the spatial dependence between neighbouring measurements, empirical bayesian smoothing was applied, adapting a Generalized Linear Latent And Mixed Model (GLLAMM).

Clustering of mortality was then evaluated using global (Moran's I, Getis and Ord G) and local (Moran's $I_i$ and Getis and Ord $G_i$ and G*) autocorrelation indexes.

Time trends were analyzed on macro geographical areas: directly standardized rates (by age-class and herd size) were obtained and trends and seasonality was evaluated by smoothing with moving averages (annual and quarterly, respectively), moreover seasonal indexes (specific and typical) were obtained to evaluate the seasonal changes during a specific year and over a number of years respectively.

All analyses were conducted using the software Stata 10 (StataCorp,College Station, TX)

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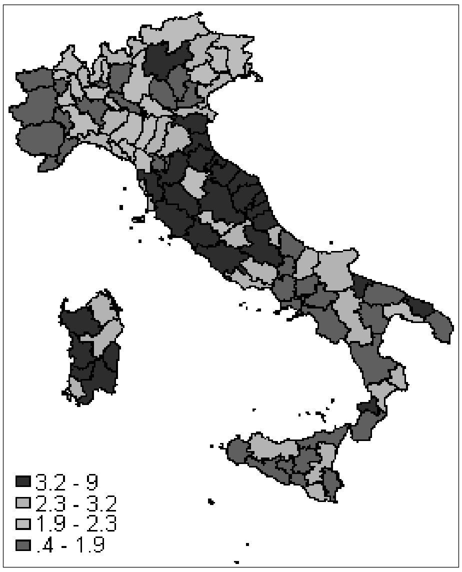
## Result

Dairy cattle population in Italy shows differences in age, herd size and type of production distribution if examined geographically, no differences were seen when those characteristics were examined by year.

Crude mortality rate for the whole study period in Italy was 2 death cattle/ 1000 cow-years. Differences ($\chi^2$ test for homogeneity $p<0.001$) were seen in specific mortality rates by year of study, macro geographical areas and herd size.

**Figure 1**: Mortality rate (N° death/1000 cow-years) in 2002-2008 in Italian districts, after direct standardization for age-class and herd size. Choroplet representation quartile



As shown in Figure1, the differences seen in the district specific mortality rates, after direct standardization for age category and herd size, when represented as quantile choroplet map are grouped at a geographical level higher than the district level (regional, macro area level) with higher values in the Centre and in the Sardinia region. At the municipality level adjacent SMRs and Bayesian smoothed SMR were quite dissimilar.

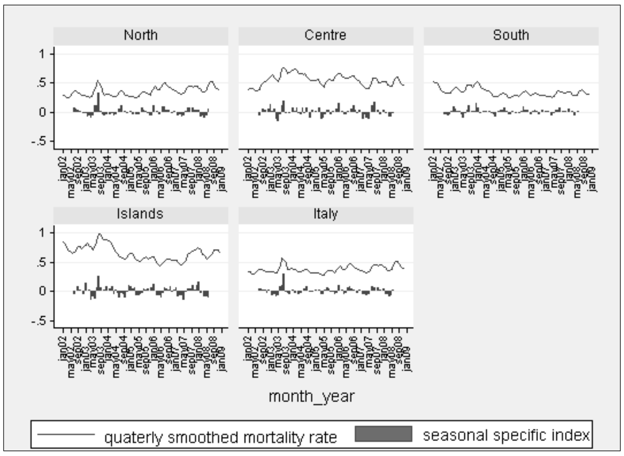
The absence of clusters of all cause mortality at the municipality level was confirmed by global and local autocorrelation indexes.

Long-term trend analysis with annual smoothing showed 2 increases in mortality during the study period, the first one in 2003 and the second one in 2006. This situation, though with some differences, was seen even when macro geographical areas were examined separately.

When evaluating the seasonal trend, applying a quarterly smoothing, and the seasonal specific index (Figure 2) we see that in all macro geographical areas in 2003 the increase in mortality was related to the summer period (May-September for the north, June-September for the southern areas). Conversely, the 2006 increase in mortality involved only the North and the Centre, while was delayed to 2007 in South and Islands, and the specific seasonal index didn't show specific changes in mortality in a certain month or season.

Finally the typical seasonal index shows differences in the seasonality of mortality over the macro geographical areas: in northern Italy seasonal mortality is related to summer months, while in the other areas it is related to winter also.

**Figure 2**: Mortality rate (N° death/1000 cow-years) in 2002-2008 in Italy and in italian macro geographical areas, after direct standardization for age-class and herd size. Trends after quarterly smoothing (line) and seasonal specific index (bars).



## Discussion

Mortality is not evenly distributed in Italy and geographical differences seems to be related to differences at regional or macro areas level more than at lower level (district or municipality level).

Mortality shows a strong seasonality too.

The seasonal mortality pattern varies depending on the geographical areas where cattle were breed.

Italian geographical areas are characterized by different climate as well as different kind of farming: the northern area has a continental climate and most of intensive farming, while the other areas have a Mediterranean climate and a more extensive farming. Direct standardization for herd size (considered as a proxy of the kind of farming) and age-class allowed us to remove at least part of confounding coming from age and kind of farming. However high values of temperature and humidity have been proven to raise the risk of mortality [5]. Therefore the mortality peak of 2003 is attributable to the heat-wave that affected Europe during the summer months.

The mortality rise in 2006, however, seems not to be related to a particular month or period. In France a similar increase in mortality was attributed to the Blue Tongue Virus serovar 8 epidemics [8], but in Italy we detected only a few cases [1].

An alternative hypothesis could be a progressive turning to insurance programs covering the costs of disposal of death on farm animals, that started to rise in this period, at least in the northern regions an then spread towards the southern ones.

In conclusion the use of mortality data enabled us to identify retrospectively abrupt variations in mortality, identifying the effects of the 2003 heat wave, confirming their potential usefulness in syndromic surveillance. On the other hands mortality data are influenced by a number of not biological factors (*e.g.* changes in laws, economics) that have to be taken into account when syndromic surveillance is applied.

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# Monitoring goat's diseases in Poitou-Charentes, France: Interest of using mandatory health assessment reports

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## Abstract

As part of a dairy goat's disease surveillance network developing in Poitou-Charentes, a group of veterinarians, farms' representatives and scientists has designed a form to harmonize epidemiological data collected during the annual health assessment visits done in every farm. A new decree regulating the use of drugs by farmers actually imposes the record of syndromic data. The forms collect by veterinarians and their centralization and analysis by the network central unit is being tested in order to improve and validate this endemic diseases monitoring system.

**Keywords**: surveillance, syndromic, endemic, goats; veterinarians.

## Introduction

Dairy goat herds are concentrated in Poitou-Charentes region with 43% of France milk production. Besides endemic diseases impact on milk production and quality, stakeholders are particularly aware of the threat caused by emerging diseases since the Netherland Q fever epidemic.

Considering the lack of reliable information on goats' health situation to guide decision-makers on diseases surveillance, control and research priorities, a regional health committee representing goat milk industry, health managers (departmental veterinary services and animal health groups), veterinarians and laboratories has decided to support the creation of a regional surveillance system that would allow sharing and analyzing existing health data. Coordination of the project and data analysis was assigned to a "central unit" based in Niort Anses laboratory, because of its recognized expertise in goats' diseases. The central unit is composed of one epidemiologist, three researchers and one IT professional.

The first step of the project was to implement a complete inventory of sanitary data collected on a regular basis by goat farms technicians and veterinarians, and produced by laboratories.

The main protagonists of disease surveillance in Poitou-Charentes were interviewed in order to determine precisely their activity and the motivations and limits of their participation to this surveillance system. The health data effectively or potentially collected were identified and qualified in terms of population under surveillance, reliability, representativity, accuracy, and adequacy with surveillance objectives. The data availability was determined in terms of cost, time and developments required for the communication of these data to a central database managed by Anses.

Veterinarians were interviewed and their data qualified according to the latter criteria. Different types of data recorded were identified, such as farm visits purposes, laboratory results, drugs delivered and farm health assessment reports. The latter appeared to be the most relevant information to collect in order monitor the main syndromes observed in farms.

Since 2007, farmers have to implement every year one or two health assessments with their veterinarians in order to be allowed to treat their animals on their own against specific diseases, respecting a precise protocol. The corresponding report must include specific data described in the ministerial decree n° 2007-596 [1]. However, the requirements don't allow enough standardization of data collected and thus, reports written are not appropriate for disease surveillance.

This study presents the development of a form that would allow harmonizing epidemiological data collected by veterinarians during health assessment visits in goat farms and make it possible to assess prevalence and trends of goat endemic diseases.

A feasibility study is being implemented with five veterinarians in order to assess the functionality of such a system for diseases monitoring. Supported by the analysis of the first data and the improvement of the form, the second step will be to ask a significant number of veterinarians to participate to this surveillance system.

## Materials and methods

### *Form conception*

The form was designed with the objective of providing data for surveillance of endemic diseases. Detailed objectives were determined within the regional health committee, considering the limits in the precision and sufficiency of data available in health assessment reports.

Available data for the implementation of the health assessment are:

- Farmer health notebook: details of all health events (date / animals identification / age / diagnostic or suspicion) and treatments (product / dosage / withdrawal time / operator) recorded by the farmer and the veterinarian;
- Complementary exams results: laboratory and necropsies;

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- Milking performance assessment: milk production; monthly cell enumeration; number of death and renewal;
- Any other information related to health;
- Based on the information required by the decree n° 2007-596 [1] and some examples of forms filled by veterinarians, it was decided that the surveillance would not assess specific diseases but syndromes occurrence. Information about the etiology can be added when confirmed by a laboratory tests, or strongly suspected by clinical and epidemiological information. However, it won't be possible to quantify specific diseases.

A bibliographic investigation of goats' endemic diseases and pathogens in the region allowed to list the most common diseases corresponding to the syndromes recorded by farmers [2, 3].

For each syndrome and each etiology, a single terminology and case definitions were suggested. The form finalization was done within the "veterinarians working group" composed of practitioners and scientists.

*Form content*
Farm characteristics:

- Type of production: milk / cheese / kid / breeding goat;
- Breed / Presence of other species;
- Herd size: does / bucks / renewal herd / kids;
- Number of sold / bought / dead animals;
- Feeding (kids and adults) and water supply;
- Facilities;
- Performance monitoring: milk production and quality.

Qualification of the general precision of results:

- Health documents management;
- Farmer ability to detect and diagnose health events.

Health events observed in three age groups between birth, weaning, first parturition and culling:

For each syndrome (digestive/respiratory/nervous/skin /musculoskeletal/reproductive/mammary/general)

- number of sick animals,
- number of dead animals,
- main diseases and pathogens suspected,
- complementary tests performed.

Principal causes of culling and number of culled animals

Control measures applied against priority diseases:

- Screening / culling or isolation
- Medical prophylaxis: vaccination, endectocides…
- Basic treatments: antibiotics, endectocides…
- Supplementary treatments: vitamins, minerals, trace elements, kaolin, homeopathy; herbal medicine…
- Sanitary measures: disinfection, equipment check-up, litter removal, ventilation, order of milking, isolation of affected animals, colostrum thermisation…

*Data centralization*
First forms are sent to the central unit by e-mail or fax and are then recorded in a database.

If the system proves its functionality, a distant recording system will be developed to facilitate data standardization, recording and transmission.

*Data analysis*
Descriptive data analysis will allow calculating the syndromes prevalence for each age category in Poitou-Charentes dairy herds. It will make it possible to compare results according to the production types, the feeding system, the breed, the herd size...

Presentation by quartiles of within-herd syndromes prevalence and death rates will provide farmers and veterinarians with average and reference values necessary for the interpretation of their own farms performance.

The long-term monitoring should allow identifying trends in herd and within-herd syndromes prevalence, as well as death rates.

Specific diseases monitoring will not be precisely quantified within herds, but the system will allow identifying the main diseases diagnosed in relation with syndromes observed. This will provide a good guidance for diagnostic on the field and identification of priority diseases by health managers.

Measure of associations between farm characteristics and diseases occurrence could provide information on potential risk factors of diseases. Complementary studies could be implemented to confirm these hypotheses.

## Results

First forms collect is being implemented as part of the test period conducted with five veterinarians willing to support the project. A few forms have been transmitted to the central unit and did not yet allow the interpretation of results.

The objective of the test period is to assess the feasibility of such surveillance system and to improve its functionality. Veterinarians were asked to report the farmers' observations, as well as their personal difficulties encountered during the collect and recording of information (time spent, results validity and precision …). This information is being analyzed by the working group and already allowed some modifications of the form. The following discussion includes observations done by field veterinarians.

## Discussion

*Data validity and precision*
The quality of data collected depends on the farmers' ability to describe health events in his farm since the last health assessment, one year ago.

The precision of farmers' description is assessed by specific indicators of the quality and regularity of data recording by farmers. The use by farmers of specific health notebook and the preparation of the assessment by the calculation of specific indicators will facilitate the assessment and improve its precision. The implementation of an intermediate health assessment will also be a strong indicator of precision.

In order to estimate the validity of the syndromes identification, veterinarians have to assess if the farmers in charge of the health monitoring are able to detect affected animals and make a syndromic diagnostic. Actually, even syndromes identification requires experience and knowledge, especially for diseases causing several clinical signs, or facilitating other infections.

At the veterinarian level, the validity of the diseases identification can vary. The implementation of complementary tests must be mentioned to qualify the validity of the results.

Most general practitioners have a pretty limited knowledge of goats' diseases from veterinary schools. Besides, goat farmers often manage their animal's diseases on their own, which limits the opportunity for the veterinarians to develop skills in goat medicine. In a first period, only most experienced and motivated practitioners will be selected for the monitoring. For other veterinarians willing to participate, training in necropsy and goats' diseases is required. Diseases case definitions also give guidance for collecting valid data.

For most practitioners, the assessment main objective is to identify the main diseases and make recommendations about these diseases. Diseases of a lesser importance will be underestimated or left out. Therefore, the interpretation of results will be more reliable for the main diseases.

*Representativity*

The farm assessment covers a one year period and thus, it allows the representation of affections for every age, physiological status and season.

Farms sample providing valid information for the surveillance system won't be representative of the whole population. Actually, making a random selection of farms is not currently possible, because of the number of farmers who doesn't properly complete their health assessment.

If this surveillance system becomes operational in Poitou-Charentes, an extension to national scale could be initiated. This will allow comparison between regions of intensive production and more traditional production areas.

*Availability and sustainability*

Encoding of farmers' identity makes it possible to use potentially any farm health assessment report.

Most veterinarians interviewed gave a provisional agreement to share the data they collected during farm health assessment. However, questionnaires currently used by veterinarians for their assessment don't always allow filling every items of the form designed for the surveillance system. Some veterinarians only target the main diseases which is not compatible with participating to the surveillance system. An option could be to simplify the questionnaire and investigate only main diseases, so that more veterinarians could participate.

On the farmers' side, some are still reluctant to spend time for a continuous collect of all health events, which limits the quality of the assessment.

The central unit will have to accompany the evolution of practices regarding health assessment visits and clearly demonstrate the benefit of participating to the surveillance system: comparison of individual results with average values, access to training and assistance for diagnostic, guidance for implementation of health assessment visit …

The animation of the surveillance system should be permanent in order to maintain forms transfers. It should provide feedback on syndromic surveillance as well as complementary information from other networks (diagnostic laboratory network, sentinel farms network, antibiotic resistance surveillance network…) and results of specific investigations done by the central unit.

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# Are surveys about the last two antibiotic treatments helpful tools to monitor antimicrobial use in ruminant livestock?

**E. Gay**[1*], G. Cazeau[1], N. Jarrige[1], M. Chazel[1], C. Sala[1] and D. Calavas[1]

## Abstract

We analysed in this paper the relevance of an approach consisting in several retrospective cross-sectional surveys gathering data about the last two antibiotic treatments to monitor antimicrobial use in ruminants. This type of surveys allows to qualitatively study real antibiotics use in field conditions in each animal sectors. It provides information about antimicrobial compounds used given the disease and type of production of the animal, and quantifies extra-label use. Hence such an approach is useful, but should be used as a complementary tool of quantitative analyses measuring the amount of antimicrobial drugs use per animal sector. A legislative framework would be needed to better collect data about antimicrobial use in France.

**Keywords**: antimicrobial use, monitoring system, population structure, antimicrobial resistance.

## Introduction

In the current context of great concern about antimicrobial resistance, antibiotic use is considered as a major driver of the phenomenon [1]. Animals are often pointed out as important contributors to the antimicrobial resistance burden. Hence antibiotic consumption by animals, and especially food-producing animals, is considered as an important risk factor [2]. Policy-makers already implemented several risk management measures such as the ban of use of antibiotics as growth promoters since 2006 in the European Union. But there is a need to go further, and recommendations and prudent use guidelines are more and more numerous. To better target these recommendations, to assess their implementation and efficiency and provide data to guide policy decisions on antimicrobial use and availability, it is necessary to monitor antimicrobial use.

In France sales of veterinary medicinal products containing antimicrobials are monitored annually since 1999 by the National agency for veterinary medicinal products (ANMV), part of the French agency for food, environmental and occupational health safety (Anses) [3]. Monitoring of sales is very useful quantitative tool, but it lacks some qualitative information at the animal sector level. Few other data are available, as in France there is no record-keeping system at the national level to gather information about antibiotic prescription and use in farms. Hence several teams set up new approaches to collect this information [4-6].

In this paper we analyse one approach, and consider the utility of a design consisting in several surveys gathering data about the last two antibiotic treatments carried out in ruminant livestock.

## Materials and methods

The objective of these surveys is to qualitatively describe antimicrobial use, identifying the main antibiotic drugs employed and their condition of use. The study design consists in several retrospective cross-sectional surveys in the different ruminants sectors in France. Both veterinarians and farmers are targeted. All the population of interest is meant to be investigated if possible, but the surveys can be conducted using representative samples. Each survey aims at being carried out during one whole year to include all diseases, some of them been influenced by season. Hence one twelfth of the population or sample is randomly selected each month. Participation of farmers and veterinarians is on a voluntary basis.

Information is collected using anonymous questionnaires, mailed or given by surveyors. These questionnaires include three different parts:

- Information about the farm or the veterinarian: geographic localisation, type of activity, size of the farm / veterinary practice,
- Last two antibiotic prescription/use: type and number of animal(s) treated, disease, products' names and for veterinarians dose, frequency and duration of the treatment(s) prescribed,
- Context of the prescription: request for laboratory analysis for veterinarians, contact with the veterinarian and availability of a prescription for farmers.

The statistical unit is the antibiotic treatment.

## Results and discussion

Three of these surveys were carried out by the Anses Lyon so far, and two others are ongoing or about to start:

- Antibiotic use in cattle via prescriptions by veterinarians between 2006 and 2007: 25% response rate, 1,200 veterinarians enrolled, 2,345 prescriptions described,
- Antibiotic use by farmers in cattle between 2007 and 2008: 15% response rate, 1,487 farms enrolled, 2,341 treatments described,
- Antibiotic use by ovine farmers in 2007 in the frame of a case/control study on scrapie: 87% response rate, 279 flocks enrolled, 388 treatments described,
- Antibiotic use by goat breeders started in 2010 and ongoing in 2011,
- Antibiotic use in goats via prescriptions by veterinarians planned between 2010 and 2011.

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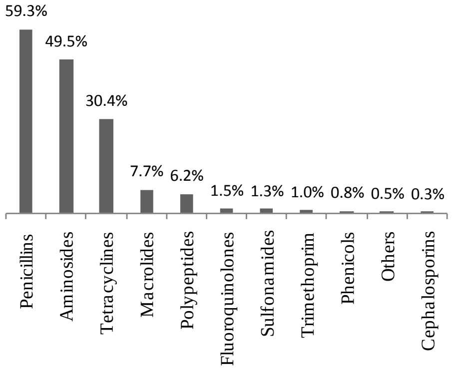
*What type of information such surveys can provide?* Unlike analysis of antimicrobial sales volumes, this type of surveys allows to study real antibiotics use in field conditions in each animal sectors specifically.

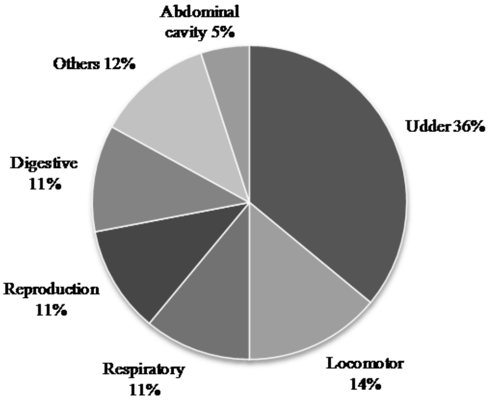
The main information collected is the distribution of the antibiotic molecules used, usually grouped by class (Figure1). It is measured by the proportion of total number of treatments including a compound of each antimicrobial class. This indicator allows comparisons between classes as it gets rid of differences of dose and duration of the treatment, which are very different between molecules and can depend on the weight of the animal treated [7].

**Figure 1**: Proportion of treatments described by ovine farmers including compounds of each antibiotic class



These surveys also provide information about the disease context (Figure 2): which disease lead to antimicrobial use? Description of the antibiotic classes used can hence be specified by disease (Table 1), and/or by production type (Figure 3). These features are important to better target for instance the conditions of use of the most critically important antimicrobial agents.

**Figure 2**: Disease context of antibiotic use by farmers in cattle in 2007



Addressing both famers and veterinarians enables to study the complementary aspects of prescriptions and use. For a same disease group, they may not use the same classes (Table 1), as the veterinarian usually deals with more severe cases or recurrent diseases, and/or preserve some antibiotic classes for its own practice.

Another important figure of antimicrobial use is the estimation of extra-label use. Misuse is an at risk practice towards selection of resistant bacteria. Three aspects of extra-label use are measured through these surveys: authorisation of the commercial product for the species treated (Table 2), agreement of the declared disease to the indications, and for veterinarians' surveys agreement of the therapeutic scheme with the indications (Table 3).

**Figure 3**: Proportion of treatments described by cattle farmers including compounds of each antibiotic class, by production type

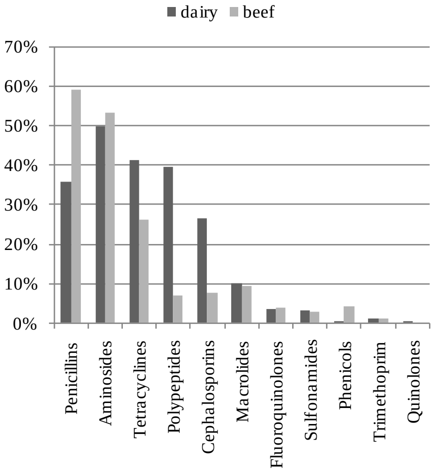


**Table 1**: Main antibiotic classes used (in % of total number of antibiotic treatments) by disease classes by farmers and veterinarians in cattle

| | Farmers | | Veterinarians | |
|---|---|---|---|---|
| Udder | aminosides | 54 | fluoroquinolones | 52 |
| | polypeptides | 53 | penicillins | 43 |
| | tetracyclines | 49 | cephalosporins | 31 |
| Locomotor | aminosides | 38 | aminosides | 38 |
| | penicillins | 37 | penicillins | 36 |
| | tetracyclines | 33 | tetracyclines | 31 |
| Respiratory | macrolides | 29 | fluoroquinolones | 39 |
| | phenicols | 27 | phenicols | 18 |
| | tetracyclines | 20 | macrolides | 17 |
| Reproduction | penicillins | 84 | penicillins | 78 |
| | aminosides | 72 | aminosides | 70 |
| | tetracyclines | 20 | cephalosporins | 15 |
| Digestive | penicillins | 39 | fluoroquinolones | 37 |
| | sulfonamides | 36 | penicillins | 33 |
| | polypeptides | 33 | polypeptides | 28 |

**Table 2**: Extra-label use: agreement for species and diseases treated

| | Ovine farmers | Cattle farmers | Veterinarians for cattle |
|---|---|---|---|
| No authorisation for the species | 16% | 0% | 0% |
| No agreement with disease indications | 8% | 7% | 13% |

In France a prescription is required to use antimicrobial drugs, even if the veterinarian does not examine the animal, which means he has at least to be contacted about the treatment. Such surveys among farmers give an estimate of the veterinary intervention and prescription rates (Table 4). This figure is important to evaluate the change level represented by veterinarians.

**Table 3**: Extra-label use: agreement with the indicated therapeutic scheme of the treatment prescribed by the veterinarian in cattle

| | Under | Correct | Over |
|---|---|---|---|
| Dose | 5% | 79% | 16% |
| Administration rythm | 9% | 86% | 5% |
| Duration of the treatment | 7% | 72% | 21% |

**Table 4**: Veterinary intervention and prescription rates for antimicrobial treatments conducted by farmers

| | Cattle farmers | Ovine farmers |
|---|---|---|
| Vet contacted | 52% | 27% |
| Vet examination | 33% | NA |
| Presence of a prescription | 88% | 50% |

*What type of information such surveys cannot provide?* These surveys are designed to qualitatively describe antimicrobial use. Hence they cannot estimate the amount of antibiotic drugs used at the farm or animal level, a key feature. That is why such qualitative surveys should be used as a complementary tool of quantitative analyses measuring the amount of antimicrobial drugs use per animal sector.

The link between antibiotic consumption and antimicrobial resistance cannot be estimated either by such a design. It provides descriptive patterns but very few analytic perspectives can be derived. A possible one is the study of the risk factors for extra-label use by veterinarians, considering the disease, the number of animals treated simultaneously, the use of laboratory analyses, the curative or preventive purpose of the treatment and the type of production.

Even if veterinarians' prescriptions and farmers' use of antibiotics are both addressed, the study design does not allow to estimate the compliance (prescription and use are not recorded at the same time on the same animals).

*What are the advantages and drawbacks of such surveys?* Such a study design involves a severe drawback: as it relies on a voluntary basis and collects declarative data, many selection and measure biases are to be expected. They both tend to under-estimate "bad" practices such as extra-label use or treatment without prescription because: i) respondents may represent a non-random sample of users, usually mores concerned by the subject and being good and/or prudent users of antibiotics, and ii) respondents may indicate what must be done rather that what they really do. These biases also imply non-representative results for the distribution of the antimicrobial classes. The analysis of the data collected has to include biases examination, especially selection biases linked with the voluntary basis, the declarative mode, and/or the use of a sample designed for another study.

Another difficulty is to identify the population of interest. It can be difficult to draw a list of the famers or veterinarians involved in each production sector, and to have reliable contact information (address, phone number).

On the other hand, such surveys are easy to implement, not too much time consuming for both organisers and participants, cost-effective, and have a good acceptance. The evolution to electronic surveys is going to even more emphasise such points.

## Conclusion

Cross-sectional surveys about the last two antibiotic treatments carried out by farmers and veterinarians are a helpful tool to contribute to monitor antimicrobial use, bringing in qualitative aspects in complement to quantitative estimate of antimicrobial sales at the farm or animal sector level. But if such surveys pretend to play a role in the monitoring of antimicrobial use, they have to be repeated regularly. Such a process should be carried out by scientists and policy-makers, a legislative framework being needed to better collect the data.

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# Surveillance of resistance to beta-lactams in *Escherichia coli*: results from the Resapath surveillance network in France

**E. Gay**[1*], M. Chazel[1], E. Jouy[2], M. Haenni[1], D. Calavas[1] and J.Y. Madec[1]

## Abstract

The Resapath is the French surveillance network for antimicrobial resistance in pathogenic bacteria of animal origin created by Anses in 2000. We analysed the Resapath findings about the resistance of *Escherichia coli* – the most common bacteria isolated from diseased food-producing animals – to beta-lactams – an antimicrobial class of major importance for both human and animal health – for the period 2006 to 2009. Resistance to amoxicillin is high in food-producing animals (45 to 77%), even if the effect is partially restored by clavulanic acid, but the phenomenon is stable for the past years. Resistance to last generation compounds of cephalosporin class is much lower (1 to 12%), but of major concern as it is increasing rapidly over the last few years in all animal sectors, mainly due to the presence of extended spectrum beta-lactamases. Resapath is an essential tool to provide the scientific community and authorities with reliable information on resistance trends by bacterial species and animal sectors in France, in order to implement recommendations and control measures.

**Keywords**: antimicrobial resistance, laboratory network, multiple diseases and species surveillance, *Escherichia coli*, beta-lactam.

## Introduction

Antimicrobial resistance is an issue of concern for both scientists and policy-makers of human and animal health, since emergence and spread of resistant micro-organisms can lead to treatment failures (already described in human health). In the past, science was able to stay ahead of this phenomenon through the discovery of new classes of antimicrobials, but the process slowed to a virtual standstill since the 80s. Therefore, effort should be made to slow the emergence and reduce the spread of resistant bacteria.

Food-producing animals are often pointed out as important contributors to the antimicrobial resistance burden [1]. Policy-makers already implemented several risk management measures such as the ban of use of antibiotics as growth promoters since 2006 in the European Union. In addition to that, there is a strong need for antimicrobial resistance monitoring, to measure the phenomenon and assess the effects of control measures [2].

The French agency for food, environmental and occupational health safety (Anses) created formally the Resapath in 2000, with the objective to monitor antimicrobial resistance in pathogenic bacteria of animal origin in France. This surveillance network formerly addressed cattle, pigs and poultry sectors, and had recently been extended to all farm and companion animals. All bacteria are targeted, but *Escherichia coli* (*E. coli*) is the most common bacteria isolated from diseased animals in cattle, sheep, pigs, poultry and rabbits populations. Moreover, this bacterium presents numerous antimicrobial resistance phenotypes, among which resistance to several beta-lactam antibiotics.

Resistance to third and fourth generation compounds from the cephalosporin class (3GC, 4GC) is of major concern, these antibiotics are critically important for human health. The resistance is increasing drastically in human medicine, and the situation in veterinary medicine seems to be moving in the same direction, especially in *E. coli*. In France the first identification of *E.coli* producing extended spectrum beta-lactamases (ESBLs) in food-producing diseased animals was achieved in the frame of the Resapath network in 2003 [3].

This study presents the analysis of resistance to beta-lactams in *E. coli* through the data collected by the Resapath network.

## Materials and methods

*Resapath surveillance system*: The main objective of the Resapath network is to monitor antimicrobial resistance in pathogenic bacteria of animal origin. Resapath is managed by two teams form the Anses: Lyon and Ploufragan-Plouzané laboratories. A steering committee including representatives from all partners (public and private diagnostic laboratories, policy makers, veterinarians) gathers annually. French public or private veterinary diagnostic laboratories participate to the Resapath on a voluntary basis. They send the results of the antibiograms they performed for veterinary practitioners to the surveillance network via electronic or paper forms. The antibiogram technique used is the disc diffusion method, and ring trials are organised annually to enhance method standardisation. The pathogen is classified as susceptible (S), intermediate (I) or resistant (R) regarding its inhibition zone diameter, according to the approved French breakpoints recommended by the veterinary Antibiogram committee of the French society of microbiology (CA-SFM) [4].

*Resistance measure*: From an epidemiological point of view, a strain is considered resistant as soon as there is a shift in the wild-type susceptible population, even if the clinical resistance is not fully acquired yet. Hence, the epidemiological resistance gathers all the non-susceptible strains, i.e. the combination of the R and I groups.

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Amoxicillin and the association of amoxicillin and clavulanic acid are used to estimate resistance of *E. coli* to the group of aminopenicillins. Ceftiofur is used as the indicator of resistance to last generations of cephalosporins. The enzyme involved in the resistance are not further explored with other laboratory analyses in the frame of the network, but the diameter for cefoxitin disk of the strains non susceptible to ceftiofur enables to turn toward an assumption about the mechanism: strains susceptible to cefoxitin tend to have an ESBL, and strains resistant to cefoxitin tend to overproduce a cephalosporinase.

Resistance to the several antibiotics were analysed from 2006 to 2009; Mantel-Haenszel Chi-Square were used to test for linear trends during the time period.

## Results

In 2009, 57 diagnostic laboratories submitted to the Resapath a total of 23,810 antibiograms addressing the different animal sectors: cattle, pigs, poultry, sheep, goats, rabbits, fish, horses, dogs and cats. *E. coli* stood for 46% (n=10,991) of all these antibiograms, and were the most frequent bacteria for the cattle, pigs and poultry, representing respectively 46% (n=3,443), 57% (n=1,387) and 74% (n=4,393) of the antibiograms collected in each sector.

Resistance to amoxicillin is high in food-producing animals, reaching more than 70% for cattle (Table 1). Variations among this species are rather high as the resistance among strains isolated from young cattle with digestive pathology was 87%, but was only 26% for strains isolated from mastitis in adult cattle. The activity of the amoxicillin is partially restored by the clavulanic acid, a beta-lactamase inhibitor, decreasing the resistance to one in two *E. coli* isolated form cattle, but this resistance level remains quantitatively important (Table 1). These patterns of resistance are stable over the last four years.

**Table 1**: Resistance (%R+I) of *E. coli* strains to amoxicillin and amoxicillin + clavulanic acid in the different food-producing animals sectors, 2006-2009

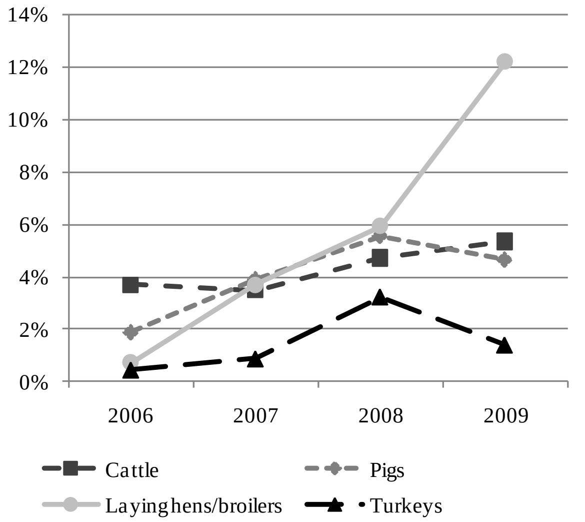
| | 2006 | | 2007 | | 2008 | | 2009 | |
|---|---|---|---|---|---|---|---|---|
| | AMX | AMC | AMX | AMC | AMX | AMC | AMX | AMC |
| Cattle | 77 | 59 | 73 | 52 | 72 | 50 | 75 | 51 |
| Pigs | 57 | 30 | 62 | 23 | 60 | 21 | 63 | 23 |
| Hens/ broilers | 45 | 20 | 48 | 21 | 51 | 26 | 56 | 25 |
| Turkeys | 61 | 33 | 60 | 39 | 59 | 37 | 61 | 30 |

AMX: amoxicillin AMC: amoxicillin + clavulanic acid

Resistance to 3GC is much lower, but of major concern as it is increasing rapidly over the last few years in all animal sectors ($p<0.05$ for cattle, pigs and hens and broilers) except turkeys (Figure 1).

The resistance to 3GC varies a lot within the various animal sectors. Indeed, in 2009, while the proportion of *E. coli* isolates resistant to ceftiofur was only 1.4% for turkeys, and less than 6% for pigs and cattle, it reached 12% for layer hens and chicken, twice the 2008 value. Furthermore, variations can be huge within the same animal sector too. For cattle, calves contribute much more to the resistance to ceftiofur than adult cows, with 7% of resistant isolates in calves (mainly with digestive pathology) and only 1% in adult cows (mainly with mastitis) in 2009.

**Figure 1**: Resistance (%R+I) of *E. coli* strains to ceftiofur in the different food-producing animals sectors, 2006-2009



As cefoxitin is not always tested by antibiograms, only data for cattle are numerous enough to give information about resistance mechanisms (Table 2). Among strains resistant to ceftiofur, half of them were resistant to cefoxitin in 2006, but the susceptible ones increased significantly ($p=0.007$) over the past years. It can be assumed that ESBLs are now the more frequent resistance mechanism encountered in 3GC resistant strains.

**Table 2**: Resistance of *E. coli* strains to cefoxitin among strains resistant to ceftiofur in cattle, 2006-2009

| | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|
| Cefoxitin S (%) | 48 | 58 | 58 | 73 |
| Cefoxitin R+I (%) | 52 | 42 | 42 | 27 |

## Discussion

Resapath is a passive or 'event-based' surveillance network; its laboratories participate on a voluntary basis, and its analyses examine only samples sent on the decision of veterinary practitioners. And yet bacterial isolation and antibiograms are not analyses that are routinely mandated, they are generally used for the most severe cases and/or after treatment failure. The data collected by the network therefore tend to over-estimate the antimicrobial resistance of pathogenic bacteria. Nevertheless, the significance of monitoring antimicrobial resistance lies in its ability to detect the most resistant bacteria and estimate trends. In that sense, the information provided by Resapath is relevant and makes it possible to highlight overriding trends related to antimicrobial resistance of pathogenic bacteria in France.

Level of resistance of *E. coli* to amoxicillin - an "old" and first-line antibiotic widely used in food-producing animals - is rather high. Moreover, resistance to 3GC is emerging and spreading, especially in chicken production. The same phenomenon had been described in the Netherlands [5]. In France, the constant raise of 3GC resistance over the last years is mainly due to the increased spread of ESBLs and confirms the persistence of an animal reservoir of the enzyme.

Resapath is an essential tool to provide the scientific community and authorities with reliable information on resistance trends by bacterial species and animal sectors in France. As resistance of *E. coli* to beta-lactams do not tend to decrease, other control measures should be implemented. One of the change levers is to better control the use of antimicrobial agents. Yet, resistance to antimicrobials is a natural biological phenomenon, but it had been amplified by extensive antimicrobial use [6]. Increasing use of 3GC and 4GC in animals is not the only risk factor for *E. coli* resistance to cephalosporin compounds, as use of other antibiotic classes (such as phenicols) can produce a co-selection of resistance to 3GC [7, 8], but the overall antibiotic consumption is involved. To avoid interdiction of veterinary use of drugs considered as vital for human medicine, it seems necessary to better control and monitor antimicrobial use in food-producing animals, and to monitor the effect of the measures implemented through surveillance networks such as Resapath.

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# Structured Passive Surveillance from Laboratory Routine: An Example for PRRS-Screening in German Pigs

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## Abstract

Non-structured passive surveillance is an important tool for disease detection, especially if serious events occur and non-representative data is not of any importance. For ubiquitary events like porcine reproductive and respiratory syndrome (PRRS) in pig herds this concept does not hold true. If data from laboratories is used to report prevalences or similar measures of interest, these results may be affected by serious selection bias. But, PRRS is one of the most important diseases in pig herds. This work therefore analyses the results of ELISA and PCR tests from a laboratory within a structured reporting system by introducing additional epidemiological information. The analysis of the differences in the outcome of PRRSV depending on vaccination and reason for examination or other factors like farm types are essential to add further information about virus' epidemiology within an usual farming population.

**Keywords**: laboratory-prevalence, PRRSV, PCR, ELISA, vaccination.

## Introduction

For many pathogens there are as yet no sufficiently exact studies available with reliable information about the most important risk factors. But, veterinary epidemiological studies are often time-consuming and expensive, particularly if sampling is to be representative, recent, and sufficiently large.

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in pig herds. The PRRS virus (PRRSV) causes severe economic losses due to reproductive failures in sows and gilts and respiratory distress in piglets and fattening pigs [1]. Therefore it is of basic importance to know about the associated risk factors for PRRS even on the population level of usual farming practice.

Setting up primary epidemiological studies is expensive. Therefore the secondary epidemiological use of data already existing from laboratory routine is an issue of a surveillance system.

This work analyses the results of ELISA and PCR tests from routine diagnostics depending on vaccination status and reasons for examination.

## Materials and methods

A total of 27,077 serum samples from pigs in Germany were analyzed from October 1, 2007, to September 30, 2009. Antibodies against PRRSV were detected by IDEXX Herd Chek*PRRS 2XR Antibody ELISA as described by the manufacturer. This test was interpreted as a measure of general contact to PRRSV. Therefore this outcome may be used for general risk factor description on the farm level.

Three to five individual serum samples from a single farm were pooled, and these pooled samples (6,246) were also analyzed by PCR based on the ORF7 gene [2]. In order to compare the diagnostic methods, the individual ELISA results from each pool were averaged to give a pooled ELISA result.

The samples from laboratory routine were identified either as part of a monitoring program with no actual health problems in the herd or reproductive problems, respiratory symptoms or other health problems were noted in the herd. Therefore within the study period the sample history was reported mandatory by describing shortly items on

- reason for examination
- animals' vaccination status
- farm production type

These data are available for 4,162 samples, i.e. 15% of the entire study population. With this information post-stratification and a post-hoc screening for risk factors was possible. This was used to set up frequency tables for single risk-factor analyses as well as for multi-factor analyses via stratified and conditional logistic regression with reason for examination as stratification variable as well. All statistical evaluations were conducted with SAS® Version 9.1 (Cary, USA) in a Windows environment.

## Results

Overall data from 27,077 serum samples are available. This data are related to an approximated number of 2103 farms with an average number of animals of 541 overall, of 274 for sow farms, of 714 for piglet farms, and of 1,498 for fattening farms, respectively.

Looking for the optical density as a measure of the ELISA-test it yields in a log-normal-like distribution of the entire study population with a substantial amount of non-positive samples (Figure 1).

From 4,162 samples a short sample history was collected by means of a mandatory form, which was introduced during the study period to enhance the epidemiological information of the data and to strengthen the epidemiological outcome of the data. 2,526 samples (60.7%) were sent to the laboratory due to farm-internal monitoring of the health status, 541 (13.0%) due to reproductive problems, and 1,095 (26.3%) due to respiratory symptoms respectively.

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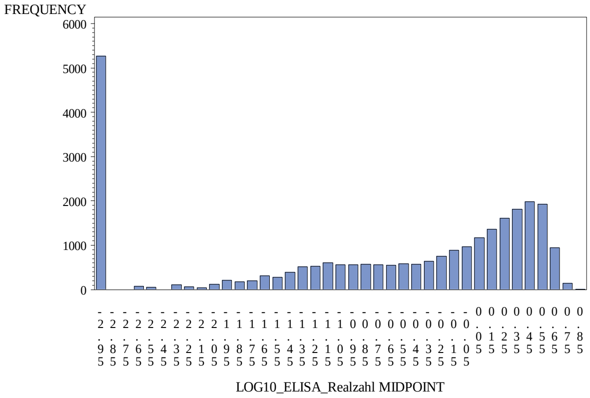
**Figure 1**: (relative) optical density (log scale) of PRRSV-ELISA from 27,077 samples from pigs in Germany



Taken these reasons for examination under account there is a considerable difference of positive samples between these groups. Within these differences, there is a substantial amount of positives even within the "baseline group" of samples, which were introduced to the laboratory for monitoring purposes, with 49.1% PRRS-positives in ELISA-testing and 34.4% PRRS-positives in PCR-testing (Table 1).

**Table 1**: PRRSV-ELISA- and -PCR-positive samples by initial reasons for examination

| Examination’s reason | ELISA | | PCR | |
|---|---|---|---|---|
| | % + | n | % + | n |
| Monitoring | 49.1 | 2,526 | 34.4 | 529 |
| Reproduction problems | 64.3 | 541 | 12.9 | 140 |
| Respiratory symptoms | 65.6 | 1,095 | 44.6 | 258 |

This difference causes the necessity to stratify all analyses by means of the examinations’s reason. Two major issues were studied following this stratification concept. First is information on vaccination, i.e. the information if the vaccination status of the farm is known, and if this is known, if a vaccination for PRRSV was performed. Therefore the statistical analysis was done as a two-step process.

Table 2 shows amounts of PRRS-positives by vaccination status stratified by the reason for examination. Within the ELISA-tests for monitoring samples the amount of positives differ between samples with known vs. unknown status of vaccination. This is not true for samples from farms with health problems. These patterns are not observed for PCR-diagnostics.

Taken into account the knowledge, if a vaccination occurs, roughly three quarters of the samples show a positive ELISA-result. This effect is independent from the reason for examination. In samples with no documented vaccination there are less ELISA-positives from samples due to monitoring than from samples due to health problems. This clear pattern is generally true for the PCR-test-procedures omitting the results from samples with no vaccination in the group with reproduction problems (Table 3).

**Table 2**: PRRSV-ELISA- and -PCR-positive samples by initial reasons for examination and vaccination status

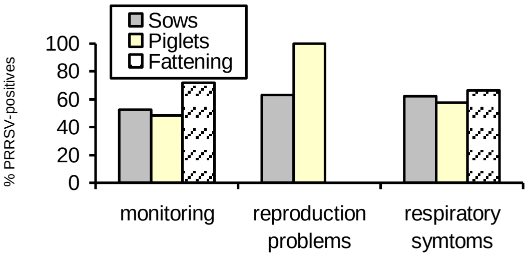
| Examination’s reason | vaccination status | | | |
|---|---|---|---|---|
| | known | | unknown | |
| | % + | N | % + | n |
| ELISA | | | | |
| Monitoring | 54.8 | 1,774 | 35.5 | 752 |
| Reproduction problems | 65.8 | 380 | 60.9 | 161 |
| Respiratory symptoms | 65.0 | 762 | 67.0 | 333 |
| PCR | | | | |
| Monitoring | 36.7 | 368 | 29.2 | 161 |
| Reproduction problems | 14.3 | 98 | 9.5 | 42 |
| Respiratory symptoms | 46.9 | 175 | 39.8 | 83 |

**Table 3**: PRRSV-ELISA- and -PCR-positive samples by initial reasons for examination and vaccination (for known vaccination status only)

| Examination’s reason | vaccination | | | |
|---|---|---|---|---|
| | yes | | No | |
| | % + | n | % + | N |
| ELISA | | | | |
| Monitoring | 74.9 | 833 | 37.0 | 941 |
| Reproduction problems | 73.9 | 257 | 48.8 | 123 |
| Respiratory symptoms | 73.9 | 260 | 60.4 | 502 |
| PCR | | | | |
| Monitoring | 49.1 | 169 | 26.1 | 199 |
| Reproduction problems | 13.4 | 67 | 16.1 | 31 |
| Respiratory symptoms | 41.4 | 58 | 49.6 | 117 |

As PRRS may generally be denoted as a multi-factor associated disease, the production type (sows, piglets, fattening) is of general interest for the different outcomes under study. Figure 2a show a clear difference between the samples from different production types for monitoring as well as for reproduction samples.

**Figure 2a**: % PRRSV-ELISA-positive samples by initial reasons for examination and production type



This effect does not appear for PCR-testing, where generally PRRSV is more often detected in samples with respiratory symptoms as the reason for examination (Figure 2b).

All this general patterns are not free from interaction within a framework of multiple association of the PRRSV-status. Therefore homogeneity was tested between the strata of examination's reason as well as for samples with known vaccination status (data not presented here). Due to a strong heterogeneity between these strata logistic regression analyses were performed separately within the combined classes (Table 4).

**Figure 2b**: % PRRSV-PCR-positive samples by initial reasons for examination and production type

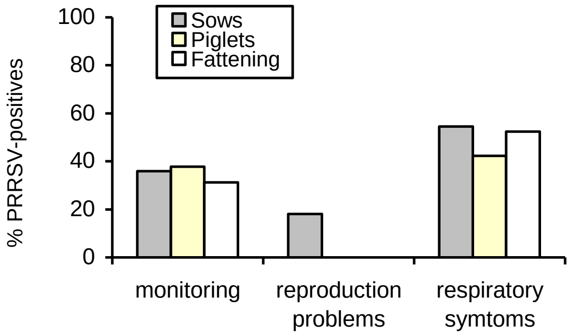


**Table 4**: Odds Ratios for PRRSV-ELISA-positives comparing production type to the reference group sows within the combined strata initial reason for examination and vaccination

| Examination's reason | vaccination | | | |
|---|---|---|---|---|
| | yes | | No | |
| | OR | p | OR | p |
| comparing piglets vs. sows | | | | |
| Monitoring | 1.089 | 0.752 | 1.012 | 0.953 |
| Any health problems | 1.206 | 0.488 | 0.420 | 0.018 |
| comparing fattening vs. sows | | | | |
| Monitoring | 2.326 | 0.181 | 3.845 | <0.001 |
| Any health problems | 4.449 | 0.002 | 1.252 | 0.294 |

Table 4 shows odds ratios for comparing production type piglets or fattening with the reference group within the combined strata of examination's reason and vaccination. There is a clear effect of the production system. The odds for a positive PRRSV-ELISA strictly increase from sow, to piglet and fattening production type. But, this effect strongly depends on the reason for sample's examination as well as the vaccination.

## Discussion

For many pathogens there are as yet no sufficiently exact studies available with reliable information about the most important risk factors. Therefore the secondary use of data already available from laboratory routine is a major issue of setting up surveillance systems to save money and avoiding primary or active epidemiological surveillance systems. But, usual laboratory data is due to severe selection, and additional efforts are necessary to make use of these data.

Comparative analyses of the subpopulations for the features "reason for examination", "pig farm type" and "animals' vaccination status" show connections and distinctions that contribute to our understanding of the epidemiology of infection pathogens.

We therefore add some basic epidemiological information to our data by adding a short questionnaire on the sample's acknowledgement form. Although this is not usual in (German) laboratory practice, these informations were collected without any substantial non-response.

As expected, we found that vaccination had a very strong effect on the number of positive ELISA tests in pigs. PCR was also affected by the vaccination status of animals, but to a lesser degree. Nevertheless, 57.3% of the unvaccinated pigs tested positive by ELISA and/or PCR, and thus were shown to be infected with PRRSV.

These analyses show, that data from routine diagnostics is applicable to a surveillance system if additional epidemiological data is collected, e.g. by means of short sample history. Here the reason of examination and basic knowledge of the vaccination status is essential to use data for a surveillance system. It is therefore recommended, that basic epidemiological information is added on sample's acknowledgement form to enhance the possibility of data use.

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# Estimated prevalence of *Mycobacterium avium subsp paratuberculosis* (Map) infection in French dairy goat herds

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## Abstract

A prevalence study on Map infection in dairy goats was carried out in France by using a serological ELISA. One hundred and five herds were randomly selected in different regions and all the goats of at least 6 months were sampled (n= 11,847). As the accuracy of the ELISA test was previously estimated, true prevalences were estimated by conversion from apparent prevalences via the Rogan-Gladen estimator. As herd level sensitivity and specificity are a function of sample size, and as the herd size varied considerably, the herd true prevalence was estimated using a weighted average according to herd size strata. At herd level apparent prevalence and estimated true prevalence were 55.2% and 62.9%, respectively. In positive herds, the average within-herd apparent prevalence was 5.9% and estimate of the average within-herd true prevalence was 11.1%. Results provided information about prevalence of Map infection in French goat herds, with respect to designing a Map infection control program in French goat herds.

**Keywords**: *Mycobacterium avium* subspecies *paratuberculosis*, paratuberculosis, goats, prevalence, ELISA test.

## Introduction

*Mycobacterium avium subsp paratuberculosis* (Map) causes a subclinical infection in various mammal species, including goats [1]. Some infected animals develop the clinical form (paratuberculosis) after several years. Control programs for paratuberculosis are based on hygienic measures to break the adult-juvenile transmission and on whole-herd testing of adult animals with culling of individual reactors to reduce contamination [2]. The humoral immune response appears lately and is considered as an indicator of a persistent infection.

The prevalence of an infection is often a key issue when decision or policy makers determine whether the infection should be considered important or not, and which measures to apply. Prevalence estimates are also often required as input parameters in epidemiological models used for simulation of spread of the infection or in risk assessments. In a review [3] it was showed that no estimate of animal level true prevalence was conclusive in Europe for goats although Map was isolated from animals in most of the studies. At herd level, the estimates of true prevalence were also deemed to be non-interpretable for most of the studies, except for estimates in Switzerland and Spain (above 20%). The prevalence of Map infection in dairy goat herds in France is unknown, although paratuberculosis is considered as a major [4].

The present study was carried out to estimate the prevalence of Map infection in dairy goat herds in France by mean of a serological survey. Despite its relatively low sensitivity, a serological technique was chosen because of the convenience of sample collection, rapid laboratory turnaround time and relatively low cost.

## Materials and methods

*Study design:* The target condition under study was infection with Map. The definition of “infection with Map” is any condition where entrance and persistence of Map have lasted long enough to give an immune response at any time [5]. This status can therefore be estimated by detection of antibodies by ELISA test [6].

The target population consisted of all French goat herds having goats of at least 6 months. The goat herds population was divided into six regions (Poitou-Charentes, Rhône-Alpes, Pays-de-Loire, Centre, Midi-Pyrénées, others) which contained 14%, 20%, 9%, 8%, 8% and 41% of the herds respectively.

Based on an estimated prevalence of 50%, at least 97 herds were needed to obtain a relative precision of 20% with 95% confidence [7]. The study population included 105 herds randomly selected out of the six regions. Blood samples from all goats of at least 6 months (n= 11,847) were collected in each herd by practitioners in the framework of the national notifiable disease control scheme. All sera were sent for testing from each official veterinary laboratory to the Niort Laboratory of Anses.

*Enzyme-linked immunosorbent assay (ELISA) procedure:* The serum samples were analysed using a commercial ELISA according to the instructions provided by the manufacturer (ELISA Paratuberculose anticorps bicupule®, Institut Pourquier, Montpellier, France). This ELISA measures serum antibodies to Map, using an absorption step with sonicates from an environmental mycobacterium, *M. phlei*, to remove non-specific antibodies as recommended [8]. A positive sample was defined by a Sample to Positive ratio (S/P) greater than or equal to 0.6. With this cut-off, specificity (Sp) of 100% (95% CI = [99-100]) and sensitivity (Se) of 53% (95% CI = [38-70]) were estimated for detection of infected goats, based on Bayesian methods [9, 10].

*Data analysis:* As all goats were tested in each herd, it was possible to calculate the within-herd prevalence, in addition to the prevalence at herd level. On herd level, the cut-off definition of a positive herd was 1 serologically-positive goat. The apparent prevalence at herd level (HAP) was calculated as the number of serologically-positive herds among the total number of herds tested.

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At animal level, the true prevalence (TP) can be estimated from the apparent prevalence (AP) via the Rogan-Gladen estimator [11]:

$$AP = Se \times TP + (1 - Sp) \times (1 - TP) \leftrightarrow TP = \frac{AP + Sp - 1}{Sp + Se - 1}$$

The herd level counterparts of Se, Sp and TP can be termed HSe, HSp and HTP, respectively. The conversion between HAP and HTP requires that HSe and HSp is known. As an imperfect animal level test has been used as an aggregate to classify a herd, estimators for HSe and HSp were proposed [12]. The HSe can be calculated as: $HSe = 1 - (1\text{-}P)^n$, where P is the apparent prevalence within a positive herd, and *n* is the number of animals tested in the herd. The HSp can be calculated as: $HSp = 1 - (1\text{-} Sp)^n$.

Both sensitivity and specificity at herd level are a function of sample size (*n*). In this study, the sample size per herd varied within a wide range (range: 14 - 840, first quartile: 50; median: 89; third quartile: 145). Therefore, the true prevalence at herd level was estimated using a weighted average of stratum specific estimates [13]. Strata were defined based on herd size, and the weight per stratum was the number of herds in that stratum divided by the total number of herds (spread sheet on request).

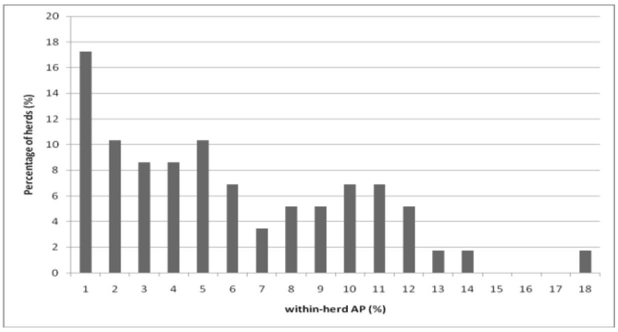
The within-herd apparent prevalence was calculated only in serologically-positive herds (n=58), as the number of serologically-positive goats among the total number of goats tested. The Rogan-Gladen estimator was used to derive within-herd true prevalence from within-herd apparent prevalence.

## Results

*Herd level prevalence:* Fifty eight herds were serologically positive herds. The apparent prevalence (HAP) was 55.2% (95% CI [45.3-64.7]), and the estimate of true prevalence (HTP) was 62.9% (95% CI [41.4-84.4]).

*Within-herd prevalence:* In serologically-positive herds, the average within-herd apparent prevalence was 5.9% (range: 0.7% to 17.6%, first quartile: 2.2; median: 4.8; third quartile: 9.3) (Figure 1). The average estimate of within-herd true prevalence was calculated to 11.1% (range: 1.1% to 33.1%, first quartile: 4.1; median: 9.0; third quartile: 17.6).

**Figure 1**: Distribution of within-herd apparent prevalence in Map positive French dairy goat herds (n=58)



## Discussion

Results of this study confirmed that Map infection is widespread in France in goat herds (HTP estimated at 62.9%). As the herd apparent prevalence (HAP) was calculated at 55.2% on a sample of 105 herds, the calculated relative precision ($=2\sigma/HAP$) was 18%, better than the desired value. In the current study, the number of herds in each region provided a study population representative of the target population but was too low to make comparison between regions. This study was the first study on prevalence of Map infection in French goat herds and it was carried out according to Nielsen and Toft recommendations [3].

To estimate true prevalence, we used the Rogan-Gladen method as recommended by Nielsen and Toft [3] to estimate the true prevalence when the test accuracy is known.

Despite low sensitivity, an absorbed ELISA was chosen for this study because of the convenience of sample collection, rapid laboratory turnaround time and relatively low cost. Moreover, this test was suitable to detect Map infected goat and its accuracy (Sp of 100% and Se of 53%) was assessed by Bayesian methods in a previous study [9, 10] and was consistent with literature data [5]. At herd level, according to Martin *et al.* [12], the estimate of the true prevalence is only dependent on the specificity of the test. A specificity of 100% could be questioned, while the data of Nielsen and Toft [5] gave a range of value between 93% and 100%. In the present figure, the lowest value of 93% would give an estimate of the true prevalence of 44.8% instead of 62.9%. However this value (44.8%) is included in the 95% confidence interval of the true prevalence estimated at herd level in the present study.

The average estimate of within-herd true prevalence was calculated to 11.1%. However, the estimated within-herd true prevalence varied within a wide range (1.1% to 33.1%) and 25% of herds had a within-herd true prevalence greater than 18%. The within-herd prevalence of Map infection varied greatly between herds.

The within-herd prevalence was calculated in serologically positive herds. As the test sensitivity is low, some serologically negative herds may be false-negative. However, in these false-negative herds, the within-herd prevalences were probably low, and had no major influence on the average estimate of within-herd true prevalence.

The results obtained in this study are difficult to compare with those of other studies because of the differences in tests used, the difference in sample size and the differences in study population.

The present study has been scheduled on the purpose of documenting the prevalence of Map infection in French goat herds, with respect to choosing, designing and budgeting Map infection control programs in French goat herds. As suggested by Nielsen and Toft [3], prevalence at herd and goat levels will influence the control program of Map infection. In France, Map infection is widespread in goat herds and many measures are needed to reduce the percentage of infected herds and goats. Control of clinical signs, with vaccination and management practices, should be considered in herds with high within-herd prevalence, management practices should be considered in herds with middle within-herd prevalence and surveillance should be considered in case of the likely absence of Map infection.

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# Increase of methicillin-resistant *Staphylococcus aureus* in Swiss slaughter pigs from 2009 to 2010

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## Abstract

A representative monitoring survey on prevalence of methicillin-resistant *S. aureus* (MRSA) in slaughter pigs in Switzerland was started in 2009 using methods recommended by EU guidelines. From January to December 2009, nasal cavities of 406 randomly selected pigs at slaughterhouse were screened for the presence of MRSA. Nine MRSA were found, which represents a prevalence of 2.2% (95%CI 1.0-4.2). The following year, 19 MRSA were isolated in 301 pigs tested between January and September 2010, which represents a prevalence of 6.3% (95%CI 3.8-9.7). Such an increase of MRSA carriage in 2010 was not expected considering the situation in 2009 and demonstrated the necessity of ongoing monitoring to get reliable data for surveillance approaches and modeling. Molecular typing should help to elucidate the reasons for this unforeseen development.

**Keywords**: MRSA, monitoring, resistance.

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged in animals in the last years worldwide [1, 2, 3, 4, 5]. In Europe, MRSA is mainly associated with non-symptomatic carriage in pigs [6, 7]. MRSA recovered from dust samples in pig production holdings in Europe show a prevalence of 26.9% varying between 0% like in Switzerland and 51.2% among the EU member states [8]. Recently, animal-associated MRSA has also been causing infections in pigs and horses, mastitis in cattle and infections in humans [9, 10, 11, 12, 13].

The analysis of dust samples from pig husbandry in Switzerland revealed no MRSA in 2008 [8]. Similarly, no MRSA has been detected in previous studies conducted before 2009 in swiss slaughter pigs [14] as well as in swiss pig carcasses [15]. In 2009, a study on 800 nasal swabs from slaughter pigs from two different slaughterhouses in Switzerland revealed 10 MRSA positive samples (1.3%)[16]. The same year, an official monitoring program on MRSA was started in Switzerland. Representative samples from pigs were taken at slaughterhouse and analysed for the presence of MRSA according to the guidelines recommended by EFSA [17].

Studies on prevalence of MRSA are conducted frequently worldwide whereas publications with aspects of chronological sequences are rare. This study aimed to determine the prevalence of MRSA overall the slaughtered pig population in Switzerland and to know the evolution of incidence of this zoonotic pathogen over the next decade.

## Materials and methods

Representative samples were taken in the framework of a yearly national monitoring program on antimicrobial resistance in food animals [18]. In 2008, 2'643'714 pigs were slaughtered in Switzerland. Over 85% (2'250'859) were slaughtered at nine abattoirs. Based on these data, a random sampling plan for 2009 and 2010 was conducted. Nasal swabs (Oxoid Ltd, Basingstoke, England) were taken from 406 fattening pigs in 2009 and from 301 pigs in January to September 2010 at the nine biggest slaughter houses in Switzerland. Each slaughter establishment in the sampling frame collected a number of samples proportional to the number of pigs slaughtered at each establishment per year.. With one exception in 2009, where a holding was probed twice on different dates, only one sample was taken per holding.

Samples were taken with transport swabs from the nares of the pigs just after stunning by officials of the Swiss abattoir authorities and were sent immediately after sampling to the laboratory. After arrival, swabs were transferred into tubes containing 10 ml Mueller Hinton Broth supplemented with 6.5% NaCl and incubated aerobically at 37°C for 24h under agitation. One ml from this pre-enrichment was inoculated into 9 ml tryptone soy broth containing 3.5 mg/L cefoxitin and 75 mg/L aztreonam and incubated further aerobically at 37°C for 24h. One loop-full was then spread onto MRSA selective agar plates (BBL ™ CHROMagar™ MRSA; Becton Dickinson, Franklin Lakes, NJ), which were incubated at 37°C for 24h. Pink to mauve-colored colonies were regarded as suspicious and up to five presumptive colonies were cultivated onto tryptone soy agar plates containing 5% sheep blood (TSA-SB) (Oxoid Ltd, Basingstoke, England) at 37°C for 24h. *S. aureus* colonies were identified using Vitek 2 GP cards (BioMérieux, Mary l'Etoile, France) following the manufacturer recommendations. The *mecA* resistance gene was detected by PCR [19].

## Results and Discussion

In 2009, MRSA was found in 9 of 406 pig nasal samples representing a prevalence of MRSA in Swiss slaughter pigs of 2.2% (95%CI 1.0-4.2). MRSA were detected in samples from January, March, August, September, October and December 2009 (Figure. 1).

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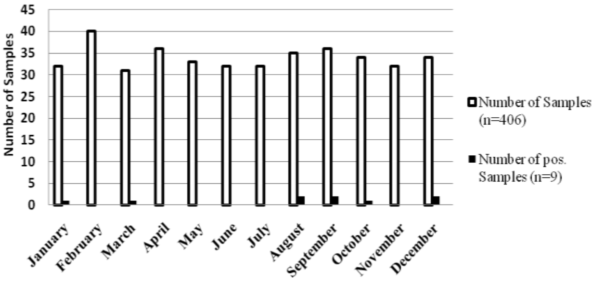
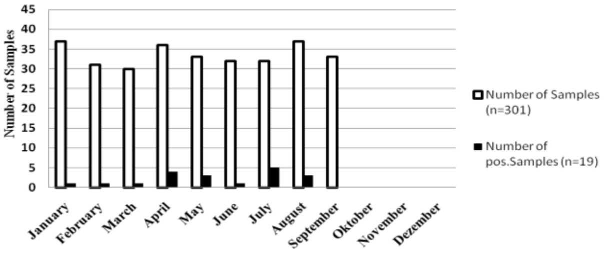
**Figure 1**: Detection of MRSA in nasal swabs from slaughter pigs in 2009



In contrast, a total of 19 MRSA were detected within 301 nasal swabs from slaughter pigs from January 2010 to September 2010. The prevalence increased to 6.3% (95%CI 3.8-9.7). In 2010, MRSA were detected every month, except in September, whereas MRSA were only sporadically detected over the year in 2009 (Figure 2).

**Figure 2**: Detection of MRSA in nasal swabs from slaughter pigs in 2010



MRSA isolates were found in pigs raised in cantons where the pig population is the highest. No geographical or saisonal associations could be observed.

Of note, the increase of MRSA prevalence from 2009 to 2010 reached nearly 300% in one year. Although the prevalence of MRSA in the Swiss pig population is still low compared to other European countries, the reasons for such an increase needs to be clarified. The importation rate from pigs is very low and consists only of breeding pigs and is therefore not likely to be the main source of contamination. Multi-locus sequence typing (MLST) and *spa* typing should help to determine whether such an increase is a consequence of the spread of formerly existing clones or is due to the emergence of new clones into the pig population from Switzerland.

MRSA has a zoonotic potential and monitoring of MRSA should help to control the spread of these pathogens into the community. Moreover, further studies are necessary to elucidate the role and potential spread of MRSA clones from livestock to humans and vice versa. Periodic monitoring of pigs, veal calves and poultry at slaughter house has now been established since 2009 at the ZOBA in Switzerland.

These results should also stress the nessecity of updating existing monitoring models since situations may rapidly change in unexpected ways.

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# Clinical diagnosis of West Nile Fever in equids by classification and regression tree analysis and comparative study of clinical appearance in three European countries

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## Abstract

This retrospective study describes risk/protection factors for the development of clinical West Nile Fever (WNF) in equids, compares clinical presentation in three European countries, France, Italy and Hungary, and creates classification and regression trees (CART) to facilitate clinical diagnosis to improve the passive surveillance of the disease. The peak of WNF occurrence was observed in September whatever the country and was linked with the vector activity in temperate climates. A significant difference between Italy and France was observed in the delay between initial clinical signs and veterinary consultation. This difference is thought to be due to a lack of awareness of disease at the time of the study and/or to the absence of a centralised epidemiological surveillance system. No clinical sign was significantly associated with WNF. Despite similar clinical presentations in the three countries, occurrence of hyperthermia was more frequently reported in France. French owners living in endemic areas could be more attentive and seek prodromic clinical signs. CART demonstrated the major importance of geographical locality and month to reach a diagnosis and emphasized differences in predominant clinical signs depending on the period of detection of the suspected case (epizootic or not). However, definite diagnosis requires specific serological tests. Centralized reporting surveillance system and time-space risk mapping should be promoted in every country.

**Keywords**: clinical epidemiology, data mining, arthropod-borne viral encephalomyelitis, West Nile Fever, equine.

## Introduction

West Nile fever (WNF) is a worldwide viral zoonotic infection caused by a mosquito-borne *Flavivirus* of the *Flaviviridae* family. Recently has WNF become a major veterinary public health concern. Horses are particularly sensitive to WNV with approximately 10% of infected animals presenting neurological disorders, as compared to 1% of humans; rendering its detection (surveillance) in equids highly pertinent in a public health perspective [1].

The purpose of the study was to describe risk/protection factors for the development of clinical WNF in equids (retrospective study), compare clinical presentation in France, Italy and Hungary (comparative study), and create classification and regression trees (CART) to facilitate clinical diagnosis by veterinarians (to render clinical impressions objective) and thus to improve the efficiency of the clinical (passive) surveillance of the disease. In addition, this collective effort ensures the reinforcement of the clinical detection in several dedicated epidemiological surveillance network such as the RESPE.

## Materials and methods

Horses presenting neurological clinical signs were classified as confirmed WNF cases when they tested positive to the ELISA IgM assay as performed by certified national laboratories. Control animals used in the retrospective study were French equids suspected of WNF but later proven free from infection by laboratory analyses.

For the comparative study of WNF, three previously published datasets were used, which included raw clinical data of 14 Italian confirmed cases from 1998 [2], 39 French confirmed cases from 2004 [3] and 20 Hungarian confirmed cases from 2007-2008 [4]. For the retrospective study, equine data from the French epizootic of 2004 were collected (39 cases and 61 controls). In addition, data from suspected but infirmed cases from 2008 (i.e., one year after a phase test of the French network) were also included (39 other controls) to investigate the possible differences in the recruitment of suspected cases in a non epizootic period. The French data were also analysed with classification and regression tree (CART, see below).

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The statistical data analysis was conducted using chi-square tests and the tendency of each parameter to become a risk/protector factor was evaluated by odds ratios. The frequency of occurrence of each clinical sign and the month of occurrence of WNF were compared in pairs with a Fisher's exact test. Comparisons of clinical scores between countries were performed using a two-sample Wilcoxon rank-sum test. The clinical score of each animal was also compared with its level of WNV-IgM and WNV-IgG antibodies using Spearman's rank correlation (called $r_s$ in the text). The statistical significance of the tests was set at a level of $p \leq 0.05$.

The CART analysis was conducted on the data set, where the presence or absence of WNF determined by laboratory analyses was used as the dependent variable and the animal description (breed, age, sex), and clinical (N=13 clinical signs), as well as spatio-temporal (month of occurrence and geographical location) data were used as independent or predictor variables. A CART analysis is a non-linear and non-parametric model that is fitted by binary recursive partitioning of multidimensional covariate space. Using CART 6.0 software (Salford Systems, San Diego, CA, USA), the analysis successively splits the dataset into increasingly homogeneous subsets until it is stratified meet specified criteria. The Gini index was used as the splitting method, and 10-fold cross-validation was used to test the predictive capacity of the obtained trees.

In this study, four different CART analyses were performed. The first (CART I) and third (CART III) analyses were performed on all the French data available without distinguishing the year of occurrence of the suspected cases (2004 and 2008; N=139). The second (CART II) and fourth (CART IV) analyses only took the French data from 2004 into account (N=100). CART analyses I and II only used the clinical signs for their tests, whereas CART analyses III and IV included all independent variables.

**Result and discussion**

The peak of WNF occurrence was observed in September whatever the country (Fisher's exact; *p*=0.83). This result is in accordance with previous studies on WNF and vector activity in temperate climates.

Considering equine data from the French epizootic of 2004 for which quantitative serological data were available, the clinical score seems to be inversely proportional to the level of WNV-IgM antibodies ($r_s$=-0.38; *P*=0.04) but not to the level of WNV-IgG ($r_s$=0.26; *P*=0.18). IgM antibodies occur earlier in the course of the disease. The clinical presentation of WNF is known to vary from one equid to another and during the course of illness. Indeed, some clinical signs are only observed at the initial stages and disappear as disease progresses, requiring great awareness of the owner/veterinarian during that period for their detection. Other clinical signs only appear during the final stages of the disease.

In addition, no clinical sign was found to be significantly associated with WNF renders the syndromic surveillance useful. However, considering the French dataset, living in the location "Saintes Maries de la Mer" significantly increased the risk of WNF (chi-square=14.85 with *p*<0.001; OR=10.67 with 95% CI: 2.76 – 41.17). In addition, during the non-epizootic period, the presence of cranial nerve deficit was found to significantly decrease the probability of the horses having WNF (chi-square = 4.55 with *P*=0.03; OR= 0.34 with 95% CI: 0.12-0.94). No breed, age or gender effects were observed as previously published.

A significant difference between Italy and France was observed in the delay between initial clinical signs and veterinary consultation (Wilcoxon rank sum test; *p*=0.006). This difference is thought to be due to a lack of awareness of disease by Italian horse owners and to the absence of a centralised epidemiological surveillance system at the time of the outbreak in 1998. Indeed, an efficient surveillance system could permit registration of suspected and confirmed cases of WNF in a standardised manner. Despite similar clinical presentations in the three countries, occurrence of hyperthermia was more frequently reported in France (Fisher's exact test; *p*=0.02), whereas weakness and recumbency were more frequently reported in Hungary (Fisher's exact test; *p*<0.04). Finally, ataxia, weakness and paralysis were significantly more frequently reported in Italian than in Hungarian cases (Fisher's exact test; *p*<0.04). This could be due to a closer observation and thus earlier detection in Hungary compared to Italy, as horses are in closer contact with humans in rural areas (working horses *versus* sport and leisure horses). Another hypothesis is that the differences observed in this comparative study are due to differences in viral strains, each viral strain potentially having a different pathogenicity.

The CART I (Table 1) and II (Table 2) analyses reported different clinical predictors depending on the inclusion (CART I) or not (CART II) of WNF suspected but not confirmed cases of 2008. Moreover, the sensitivity of the tree was more important for CART I in comparison with CART II and the reverse was observed for the specificity. This observation could suggest the influence of different recruitment of suspected cases by owners/veterinarians during epizootic (CART II) or non epizootic (CART I) periods. This observation stresses the necessity of regular awareness campaigns amongst owners and veterinarians. In accordance with the results of the retrospective study, the results of the CART analyses demonstrate that, in a case of emergence of WNF, clinical suspicions are the first and essential step towards diagnosis but confirmation by serology remains necessary.

The CART III (data not show) and CART IV (data not show) CART demonstrated the major importance of geographical locality and month to reach a diagnosis and emphasized differences in predominant clinical signs depending on the period of detection of the suspected case (epizootic or not). However, definite diagnosis requires specific serological tests. Centralized reporting system of WNF clinical suspects and time-space risk mapping should be promoted in every country.

**Table 1**: CART I analysis that takes into account all the French suspected cases from 2004 and 2008 (N=139; Sensitivity= 82.1%; Specificity=78.0%)

| *Predictor variable* | *Score** |
|---|---|
| Paresis | 100.00 |
| Weakness | 81.37 |
| Recumbency | 75.45 |
| Hyperthermia | 63.59 |
| Paralysis | 61.61 |
| Prostration | 60.27 |
| Cranial nerve deficit | 46.13 |
| Trembling, tetany, fasciculations | 42.15 |
| Change in behaviour | 40.81 |
| Anorexia | 34.64 |
| Hyperesthesia | 34.27 |
| Ataxia | 13.05 |

***** Power of the different clinical signs splitters obtained after CART analysis (maximum score=100)

**Table 2**: CART II analysis that takes into account French suspected cases from 2004 epizootic exclusively (N=100; Sensitivity=74.4%; Specificity=88.5%)

| *Predictor variable* | *Score** |
|---|---|
| Hyperesthesia | 100.00 |
| Recumbency | 93.60 |
| Anorexia | 81.41 |
| Prostration | 58.88 |
| Cranial nerve deficit | 57.87 |
| Hyperthermia | 52.13 |
| Trembling, tetany, fasciculations | 51.05 |
| Paralysis | 49.65 |
| Paresis | 39.14 |
| Change in behaviour | 32.60 |
| Weakness | 25.80 |
| Ataxia | 17.76 |

***** Power of the different clinical signs splitters obtained after CART analysis (maximum score=100)

Despite several previous studies on the subject, WNF remains a challenging disease and an important veterinary public health issue. Awareness of its potential emergence must be promoted. A centralised passive animal surveillance system reporting WNF and suspected WNF cases, in a standardised manner, must be organised in different countries to permit an early detection of its emergence. Communication between countries and between veterinary and public authorities is essential for an efficient control of WNF in the currently unstable epidemiological situation. Spatio-temporal modeling could be of significant help for assessing risk of emergence and detecting high risk areas to allow active surveillance.

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# Animal rabies surveillance in Bhutan, 1996-2009

**Tenzin**[1, 2], N.K. Dhand[1] and M.P. Ward[1*]

## Abstract

Rabies is endemic in Bhutan. Here we describe the spatio-temporal distribution of rabies in animals, identify rabies risk areas and classified sub-districts of Bhutan into rabies risk zones − high endemic, endemic, sporadic and absent areas based on some semi-quantitative criteria and epidemiologic occurrence. Anselin's local indicator spatial autocorrelation was also conducted to identify similar spatial patterns of cases at the sub-district level, and identified high clusters in south central and south west region. Fifty nine of the 205 sub-districts and 11 of the 20 districts reported rabies from 1996 to 2009. The trend of rabies cases was stable until 2005, but increased in 2006 and 2008 which is associated with major outbreaks in south west and eastern Bhutan. High risk areas were identified in south central, south west and south east region that border with India. This study demonstrates how available data in a resource-limited developing country can be used to delineate risk zones, facilitating the allocation of scarce resources for rabies prevention and control.

**Keywords**: rabies, animal, surveillance, rabies zone, Bhutan.

## Introduction

Bhutan is located between China and India in South Asia. Rabies is endemic in southern parts of Bhutan that border India [1]. Frequent outbreaks occur in these endemic areas affecting mostly domestic animals such as cattle and dogs. Sporadic human cases (~1.2/10000 population) are also reported after exposure to rabid dog bites [1]. In the recent past, rabies outbreaks in animals have been reported in eastern and south west Bhutan, where rabies had previously not been reported, resulting in a substantial cost to society and the government [1, 2]. This indicates that there is a risk of the disease re-emerging (increased distribution or incidence) in Bhutan, if proper surveillance and control programs are not implemented.

In this retrospective study we report the rabies situation in Bhutan over the period 1996−2009. The main objectives were 1) to describe the spatio-temporal distribution of rabies in animals, 2) to identify rabies risk areas and classify sub-districts into rabies risk zones (high endemic, endemic, sporadic and absent areas) based on some semi-quantitative criteria and epidemiology of rabies occurrence, and 3) to generate baseline information on the rabies situation in Bhutan. The information generated from this study will help policy makers in prioritizing the allocation of resources for rabies surveillance, prevention and control programs in Bhutan.

## Materials and methods

Bhutan is administratively divided into 20 districts and is further sub-divided into 205 sub-districts. Sub-districts are further sub-divided into villages. Rabies case data were retrieved from the Veterinary Information System (VIS) database for the period 1 January 1996 to 31 December 2009. The data contains the location of outbreak (village, sub-district, and district), date of occurrence, species and number of animals affected.

Descriptive statistical analysis was performed to examine the frequencies and animal patterns of occurrence. A Geographic Information System (ArcGIS 9.3 ESRI, Redlands, CA, USA) was used to visualize the spatio−temporal distribution of rabies in Bhutan. Owing to a lack of accurate estimates of animal population at risk (particularly dogs), calculation of incidence or prevalence could not be determined in this study. Therefore, the case data (cattle, dogs, cats, horses, pigs and goats) were aggregated at the sub-district level and thematic choropleth maps were produced. Proportional symbols were used to display reported cases by sub-district level.

To identify possible similar spatial patterns of cases, a univariate local indicator spatial analysis (LISA) was performed using the rook contiguity weight matrix (that included each sub-district and its neighbours sharing a common border) [3]. LISA statistics identify a cluster of sub-districts with high cases surrounded by sub-districts with high cases (high-high) or a cluster of sub-districts with low cases surrounded by sub-districts with low cases (low-low), as well as outlier (high-low and low-high) cases [3]. The analysis was performed in GeoDa software version 0.9.5.i.

The sub-districts in Bhutan were then categorized into four areas: high endemic; endemic; sporadic; and absent areas. The categorization was done based on a set of semi-quantitative criteria developed to allow disease status classification of individual sub-districts in Bhutan [4].

The sub-districts that fulfill all or any one of the four criteria were classified accordingly. The identified rabies risk areas/zone were mapped for visualization and displayed as a graduated colour surface of four classified areas.

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**Table1**: Rabies risk zone classification criteria

| *Classification* | *A** | *B** | *C** | *D** |
|---|---|---|---|---|
| High endemic | >20 | 10 to 14 | >40 | >20 |
| Endemic | 8 to 20 | 5 to 9 | 11 to 40 | 4 to 19 |
| Sporadic | 1 to 7 | 1 to 4 | 1 to 10 | 1 to 3 |
| Absent | 0 | 0 | 0 | 0 |

Note:

***A.*** *Number of months with one or more reports of rabies in any species of animals between 1996 and 2009*

***B.*** *Number of years with one or more years of report of rabies in any species of animals between 1996 and 2009*

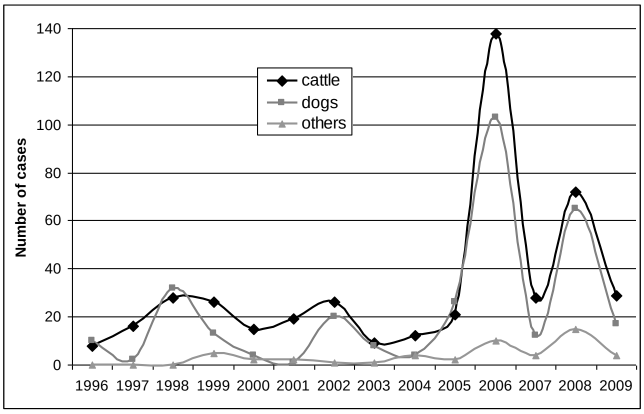
***C.*** *Number of rabies cases in any species of animals reported between 1996 and 2009*

***D.*** *Number of outbreaks between 1996 and 2009*

## Results

A total of 814 rabies cases in animals were reported from 1996 to 2009. The majority of cases were reported in cattle (55%, 447/814) and dogs (39%, 317/814), with only a few cases in other species (horses 17/814; cats 14/814; pigs 13/814; and goats 6/814). Figure 1 shows the trend of rabies in cattle, dogs and other animals.

**Figure 1**: Annual trend of rabies cases in Bhutan



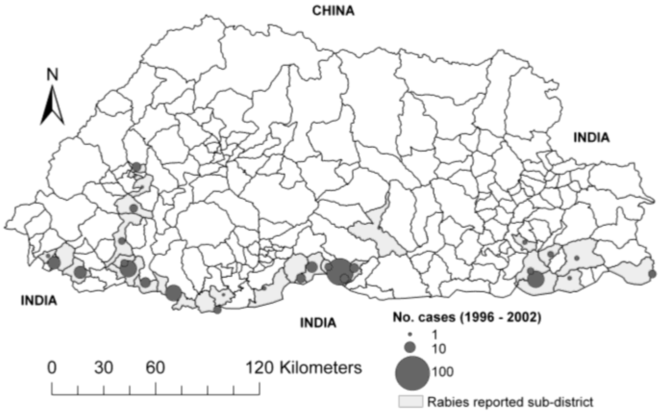
Fifty nine of the 205 sub-districts (29%) reported rabies from 1 January 1996 to 31 December 2009. Rabies incidences were commonly reported in sub-districts located in southern Bhutan (particularly in south west, south central and south east Bhutan) that shares a border with India. Figures 2 and 3 show reported cases of rabies in animals (dogs, cattle) by sub-district from 1996 to 2009. Figure 4 shows the rabies risk zone by sub-district in Bhutan.

## Discussion

Rabies cases were reported in dogs and other domestic animals, but the number of cases in cattle exceeded that of dogs and other animals. This could be due to either under reporting of cases in stray dogs or a single rabid dog might have transmitted infection to many cattle during some outbreaks. It is often difficult to trace the infected stray dogs, unlike cattle or other domestic animals, particularly in border areas. In most instances, the rabid dog carcasses would not be available for examination. Nevertheless, there was high correlation between the number of cases reported in dogs and cattle in each sub-district.

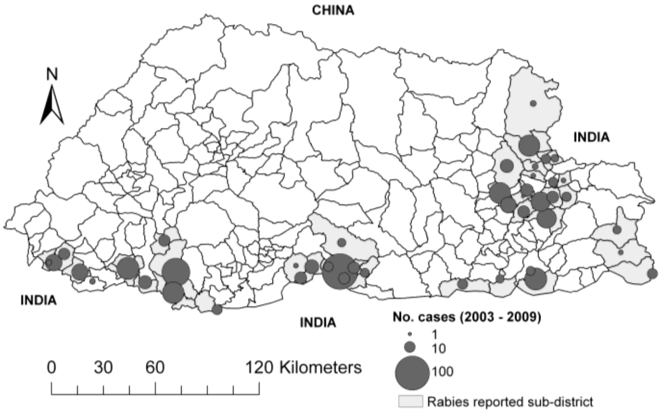
The trend of rabies cases was stable until 2005, but increased in 2006 and 2008 (see Figure1), due to major outbreaks in eastern and south west Bhutan [1, 2].

**Figure 2**: Reported cases of rabies in animals, 1996-2002



The results show that 59 of the 205 (29%) sub-districts reported rabies from 1996 to 2009. The frequency of report was variable among those affected sub-districts. For instances, three sub-districts (Gelephu, Phuentsholing and Deothang) located in south central, south west and south east, respectively have reported frequent rabies incidences compared to other sub-districts. Some sub-districts reported only once and did not report later, which may be due to control program – vaccination of dogs and selective elimination of in-contact dogs.

**Figure 3**: Reported cases of rabies in animals, 2003-2009

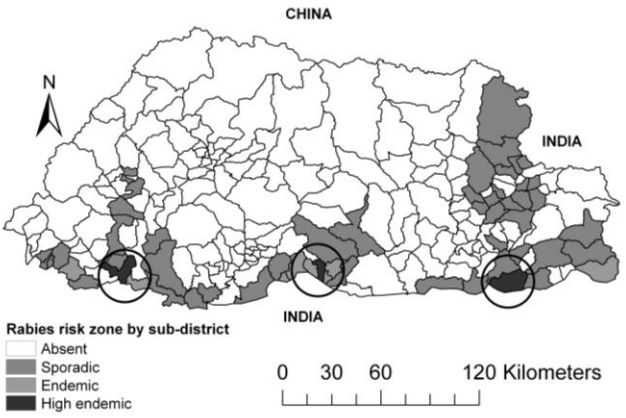


The spatial distribution of rabies occurrence in Bhutan was clustered; suggesting that the presence of rabies in one sub-district could predict an increased likelihood of similar rabies incidences in neighbouring sub-district or places. It is likely that one sub-district's proximity to its neighbours might be linked to the diffusion or spread of rabies between neighbouring sub-districts. Infected stray dogs would move from one place to another and spread the disease by biting other dogs or domestic animals.

The high-high clusters of rabies incidences were identified in south central and south western Bhutan (data/map not shown). Identification of areas with significantly high incidence of rabies cases will be helpful for investigating the underlying causes of increased risk and also for instituting effective control program. However, increased rabies incidences were reported in the border towns. It is likely that rabies virus endemicity had been maintained among stray dog population in these border areas or could be the result of trans-border movement of infected stray dogs. Molecular epidemiological study suggests that the rabies virus strain circulating in southern Bhutan area is closely related to Indian rabies virus strain [5]. However, further detailed epidemiological investigations are needed to discover the reasons for increased risk of rabies incidences in these areas.

We have categorized the sub-district into four classes: high endemic, endemic, sporadic, and absent areas based on some semi-quantitative sets of criteria (Figure 4).

**Figure 4**: Risk zone of rabies by sub-district



High endemic areas are located surrounding the three main border towns of Bhutan – south east, south central and south west (see Figure 4, circle areas). These border towns serve as entry points into Bhutan. However, this categorization should not mislead the policy makers to neglect rabies prevention programs in other adjacent areas. The existing practice of mass vaccination of stray dogs in all districts of Bhutan irrespective of local epidemiology of rabies have not controlled rabies in endemic southern Bhutan. Therefore, we recommend that the rabies prevention program (mass vaccination of dogs with >70% coverage) should be focused and prioritised in these high endemic and endemic areas in south Bhutan. Prevention of rabies in endemic areas would eventually eliminate the main source of rabies virus in the country.

In conclusion, this GIS based spatial analysis clearly indicates that the risk of rabies incidences in dogs and other domestic animals exhibits strong regional trends towards south east, south central and south west Bhutan. The identification of high risk areas and their spatial visualization as a risk map in this paper would be useful for prioritization of disease surveillance and control activities for rabies in Bhutan. Furthermore, the existing passive surveillance system for rabies may be strengthened by laboratory diagnosis of all cases, particularly rabies incidence in stray dogs. Continued surveillance will be required for early detection of cases and to prevent rabies spread into other areas.

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# First outputs of an endemic Foot and Mouth Disease risk model to inform a Space-Time information System for enhanced surveillance & control

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## Abstract

Foot and Mouth Disease is endemic in southern Africa and is associated with wild African buffalo populations that act as carriers of the disease. A study to determine the grazing patterns of both cattle and buffaloes was conducted to better predict their occurrence and hence possible interspecies contact to affect disease transmission. Moreover, disease control measures, such as fencing and vaccination was included in a Bayesian probabilistic framework to estimate the spatio-temporal risk of contact and possible transmission between these two species. The model employs multi-dimensional Kriging and random walk predictions in conjunction with static and dynamic covariates, including remotely sensed data. The model has given realistic outputs and will be further developed to inform a space-time information system to aid decision makers in prioritizing surveillance and control measures.

**Keywords**: FMD, endemic, Bayesian, control, risk.

## Introduction

Foot and Mouth Disease (FMD), and in particular the Southern African Territories (SAT) type viruses, are all endemic to African buffaloes (*Syncerus caffer*) of southern Africa [1, 2]. These SAT viruses are also unique in that they have discrete geographic areas of spread and are adapted to persist in a multi-species wildlife environment with significant African buffalo populations [3, 4]. This complicates the epidemiology of FMD in the region greatly [2, 5] since, to achieve eradication, all infected buffalo populations would have to be exterminated - something indefensible from an aesthetic, ecological as well as economic point of view [6, 7].

The logistical and economical burden that eradication of this disease poses, has made those countries who have achieved eradication very wary of re-importing it, resulting in trade embargoes on agricultural products from countries not adequately controlling the disease [2, 6]. This, in turn, has had the effect that many developing countries harbouring the disease, have to spend great amounts of resources to control FMD in an attempt to gain access to the lucrative international agricultural market [5, 8]. The significance of FMD in wildlife, therefore, lies in its potential transmission to livestock and the presence of the disease, rather than direct clinical effect.

Many developing countries do not have the resources to sustain a FMD control programme that would grant them access to export markets. Finding ways of applying risk-based surveillance and targeted controls through a better understanding of the risk factors and their spatio-temporal distribution could make such programmes more feasible. Especially applying limited human resources and vaccines more effectively in those areas at highest risk, could already improve these control efforts.

Our goal was to develop a FMD risk model that could take into account the spatio-temporal heterogeneity of this wildlife-livestock interface and point out priority risk and intervention areas.

## Materials and methods

We constructed a Bayesian probability framework for disease transmission risk, based on contact between buffaloes and cattle.

*Study area*: Whilst the model looks at the entire region surrounding the Kruger National Park in South Africa, three more intensive study sites were also identified based on their landscape and population heterogeneity to study communal cattle demographics as well as grazing patterns.

*Cattle distribution, density & demographics:* Monthly veterinary livestock inspection records for the five year period 2003 to 2007 were collected. These contained information on cattle numbers, births, mortalities, sales and slaughter.

For grazing analysis, two cattle tracking studies were completed. In the southern study area, handheld GPS devices were used by 24 herdsmen to record their grazing patterns over 14 months, whilst in the central and northern study areas, GPS/GSM collars were put on 14 cattle to record their movements for one year.

*Buffalo distribution, density & demographics:* Annual buffalo census data were obtained from the South African National Parks Board (SANParks) for the period 1998-2008. These data differentiated between bull groups and family herds.

Tracking data were obtained from various studies conducted in the KNP over the last 10 years as well as presence records from field rangers (in a routine recording system).

*Fence permeability:* Data regarding the permeability of the fence were obtained from literature and expert inputs. This focused mainly on:

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River crossings - A hydrological dataset was used to determine which river crossings had the highest risk of flooding.

Elephant density - Elephant density, based on annual KNP census data was used to determine which sections on the fence were most likely to be damaged by them

Human density - Proximity to populated areas was used to quantify this influence

Fence type and maintenance - These are important factors influencing the type of damage (ie to allow buffaloes to escape) as well as the repair delays.

*Stray buffalo records:* Records of buffaloes controlled outside the park was collected for the period 1998-2008, to determine the frequency, extent and group size/makeup these stray animals. These data were used to refine the model parameters with regard to buffalo movements outside the park/reserve.

*Bayesian probabilistic framework:* This comprises three steps.

The first is only done at the beginning of the model and is an individual fitting model for cattle, buffalo herds and buffalo bulls using a multidimensional kriging fitting procedure. This allows consideration of a multidimensional spatio-temporal autocorrelation function (with 2 spatial dimension + 1 temporal dimension + g static dimensions) to correct the residual term obtained from the fixed part of the model (animal density ~ v dynamic covariates).

The kriging surface estimation is done using the dynamic covariates (*e.g.* NDVI, temperature) as regressors of the response variables .the cattle, buffalo herd and bull densities) in the polynomial term; and the static covariates (*e.g.* land cover, human population, *etc.*) for the definition of the Gaussian random field. In practice it is a general additive model with multidimensional autocovariance function. Cattle, buffalo herds and bulls can be modelled in different times (fitting time series).

Secondly, a partially constrained random walk prediction of buffaloes is done considering the strength and the position of the fence which they need to cross before getting into the area grazed by cattle. In practice, the buffalo herd/bull moves from one point (pixel) to another according to an environmental gradient (kriging surface) and a Boltzmann probability. When the new point is reached, the walk path is reconstructed to create the surface of buffalo presence outside the park.

Finally, the Bayesian theorem allows the calculation of the risk of transmission using the probabilities of cattle and buffalo herd/bull presence. This formula is the ratio between the probability of infection and the sum of probability of infected and non-infected animals.

The probability of infection is based on the probability of presence of cattle, the probability of presence buffalo herds/bulls, group size, infection rate, and age distribution. The probability of non-infection is based on the cattle presence probability, infection rate and age distribution.

The final risk is corrected for the vaccination value.

The model runs on a timestep of one week and a 250m pixel resolution.

## Results

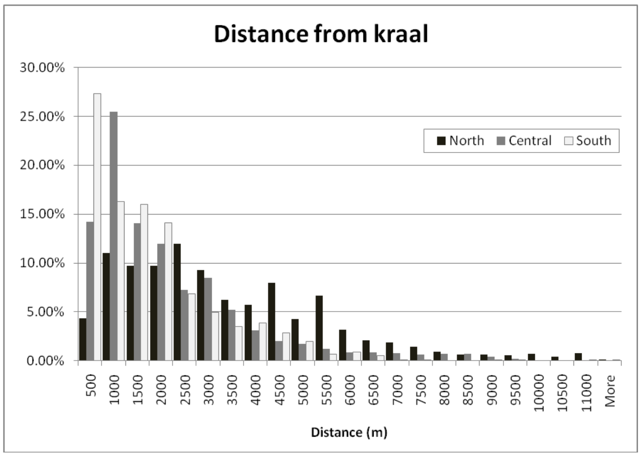
A summary of the key difference between the three study areas is shown in Table 1.

**Table 1**: Study areas key differences

| | *North* | *Central* | *South* |
|---|---|---|---|
| **Average Rainfall (mm)** | 300 | 500 | 700 |
| **Area (ha)** | 40 449 | 142 991 | 34 844 |
| **Households** | 2 321 | 30 762 | 40 023 |
| **Population density (people/ha)** | 0.23 | 0.94 | 5.31 |
| **Households keeping cattle** | 13.01% | 6.73% | 3.22% |
| **Cattle** | 4 461 | 27 120 | 12 073 |
| **Cattle per owner** | 19.6 | 13.1 | 7.8 |
| **Cattle density (cattle/ha)** | 0.11 | 0.19 | 0.35 |

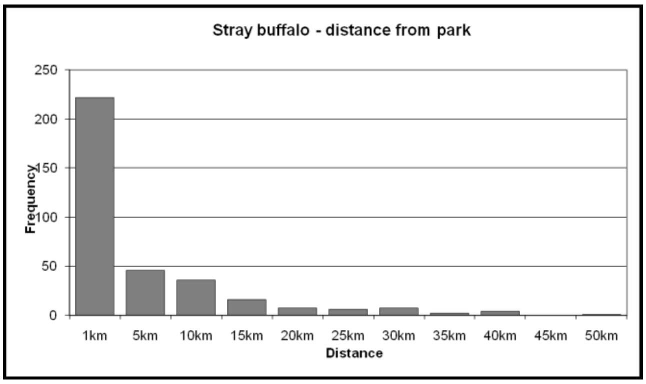
A total of 3525 tracking days were recorded with 719, 1935, 871 in the Northern, Central and Southern areas respectively.

**Figure 1**: Distance cattle grazed from kraals during tracking study



A fence permeability layer one pixel wide was created with a single permeability factor, weighted according to the risk factors affecting it.

**Figure 2**: Distance stray buffaloes were found from the KNP



A total of 398 stray buffalo records were analysed. Figure 2 shows the distance these animals were found from the fence.

Whilst sensitivity analyses are still underway, initial outputs of the model have been realistic, emphasizing known problem areas.

Internal validation of the model was within acceptable limits.

## Discussion

Cattle demographic data have given useful insights into the varied coping/economic strategies of the different communal farming areas adjoining the KNP. It has also shown clear seasonal patterns that could potensially influence risk patterns.

Cattle tracking studies have revealed important information on the distance livestock graze from their 'kraals' (pens) (Figure 1) as well as the frequency at which they sleep over at their 'kraals', effectively removing them from risk. These differences were related to the population density, NDVI and land cover of each study area.

Stray buffalo data gave the first empirical picture of frequency and extent of buffalo movements outside the KNP. The data show important trends in time, group composition and distance moved from the fence.

Due to the limited number of FMD outbreaks experienced over the last 25 years (<10), external validation of this model will be very difficult.

The matter of undetected spread among cattle after the initial wildlife-livestock transmission has been raised by some decision makers and is an important component to be added to the model to more realistically look at where clinical disease would be most likely to appear among cattle, given the risk of contact with buffaloes and their current vaccination status.

Future and related ongoing work includes the development of a Space-Time Information System (STIS) that would inform decision makers on priority surveillance and intervention areas as well as provide them with a set of tools to manipulate large datasets, prepare data for import into the STIS, as well as have scenario analysis capabilities. An example would be to enter the real-time coordinates of a reported stray buffalo and assess the immediate risk posed by it based on susceptible densities and vaccination status or by drawing up vaccination schedules based on risk prioritization.

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