ESTIMATION OF THE FORCE OF INFECTION AND RELATED PARAMETERS

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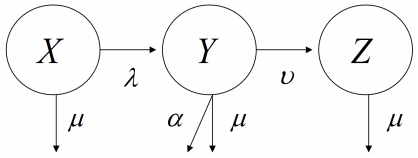
ABSTRACT

Within the framework of compartmental models for infectious diseases, we discuss the crucial role of the force of infection (FOI) and its relation to other infectious disease parameters. It is shown how these parameters can be estimated from serological data. As an example of how to deal with typical complications in such data and how to apply flexible estimation techniques, we illustrate the use of some recent statistical techniques on Bovine Herpes Virus-1 (BoHV-1) serological data from Belgian cattle. Finally we briefly review some extensions.

1. INTRODUCTION

In so-called compartmental models, the total host population is subdivided in different classes and differential equations describe the way in which the number or the proportion of individuals in each class changes with time and/or age. A basic model is the so-called SIR (Susceptible – Infected - Recovered) model, of which the dynamical behaviour can be described by a set of partial differential equations (Anderson and May 1991):



$$\partial X/\partial t + \partial X/\partial a = -(\lambda(a,t) + \mu)X(a,t)$$
$$\partial Y/\partial t + \partial Y/\partial a = \lambda(a,t)X(a,t) - (\alpha + \mu + \upsilon)Y(a,t)$$
$$\partial Z/\partial t + \partial Z/\partial a = \upsilon Y(a,t) - \mu Z(a,t)$$

with $X(a,t), Y(a,t)$ and $Z(a,t)$ the number susceptible, infected and respectively immune, of age *a*, at time *t* and with, per capita, μ the death rate, υ the recovery rate, α the disease-induced death rate and λ the force of infection (FOI), as demographic and epidemiological parameters. Initial conditions like $X(0,t) = B(t)$, where $B(t)$ is the net birth rate of the population at time *t*, complete the system. Many features, omitted in this basic SIR model, can be added: incorporation of other classes like an initial class of hosts protected by maternal antibodies, a latent class and/or a carriers class; inclusion of vertical transmission by modifying the boundary conditions; differentiation with distinct equations for different subpopulations like males and females; inclusion of loss of immunity by adding terms representing the transition from immune to the susceptible class; etc.

Muench (1959) proposed the use of the FOI as the instantaneous per capita rate at which susceptible individuals acquire infection. The FOI reflects changes in rates of infection independent of changes in the proportion of susceptibles in each age-class. Before, analyses were mainly based on age-specific incidence rates or attack rates, defined as the number of reported cases per time unit in a given age class, divided by the total number of subjects in that age class. But, since it takes no account of the number of susceptible subjects in an age group, incidence rates may just reflect the low proportions of susceptible subjects (like in older age groups). So, age-related changes in incidence rates are confounded by changes in the proportion susceptible subjects.

In general, under the `mass action' assumption, the age-specific FOI $\lambda(a,t)$ can be written as

$$\lambda(a,t) = \int \beta(a,a')Y(a',t)da',$$

where the transmission rate $\beta(a,a')$ describes the probability that an infective at age *a'* will have contact with, and successfully infect, a susceptible at age *a*, per unit time. The transmission rate $\beta(a,a')$ is a time-independent function, characterizing the infection. It combines a multitude of epidemiological, environmental, and social factors that affect transmission rates. It is not changed by programmes of immunization etc. It can though be altered by changes in personal or public hygiene. Knowing these transmission rates is crucial to use the mathematical model to make predictions about programmes of control or eradication. But the problem is that $\beta(a,a')$ is usually not known, and must be deduced from data about $\lambda(a)$ in the equilibrium state (in which there is no dependency on time) before any immunization and from knowledge on $Y(a')$ at equilibrium. Constraints on $\beta(a,a')$ are needed to turn the equation $\lambda(a) = \int \beta(a,a')Y(a')da'$ into an equation which can be solved for $\beta(a,a')$. The assumption of homogeneous mixing amounts to taking $\beta(a,a')$ to be constant (implying that the FOI is the same for all ages). Another assumption is that the transmission rate only depends on the age of the infectee $\beta(a,a') = \beta(a)$. A common assumption is that of symmetry, $\beta(a,a') = \beta(a',a)$, with the special case of proportional mixing $\beta(a,a') = u(a)u(a')$.
In practice the host population is usually divided into discrete age classes and the function $\beta(a,a')$ turns into a matrix, the so-called WAIFW matrix β_{ij} ('who acquires infection from whom' matrix). Once $\beta(a,a')$ or β_{ij} has been specified, solving the set of differential equations leads to $X(a,t), Y(a,t)$ and $Z(a,t)$ (at each step updating $\lambda(a,t)$).

2. ESTIMATION OF AN AGE-SPECIFIC FORCE OF INFECTION

At equilibrium $\partial X/\partial t = \partial Y/\partial t = \partial Z/\partial t = 0$ and assuming infection does not influence mortality and induces life-long immunity, assuming a population of constant size N and age distribution (a closed population), the proportion of susceptibles $x(a) = X(a)/N$ of age a is determined by $dx/da = -\lambda(a)x(a)$, which can be expressed in terms of the cumulative distribution (the prevalence) $\pi(a) = P(A \le a)$ of the age A at infection as (since $\pi(a) = 1 - x(a)$)

$$\lambda(a) = \pi'(a)/(1-\pi(a)).$$

The parameters of a model for $\lambda(a)$ can be estimated from cumulative case reports and/or from serological data (Grenfell and Anderson, 1985). In general, age-stratified serological data, if based on random samples, correspond directly to the cumulative distribution infected by age *a*, and provide a better source of information than case notifications (given the age-related biases and inaccuracies of case notification systems). Additionally, notifications often have to be corrected for asymptomatic cases . Of course, serological data are not perfect, the distinction between seropositive and seronegative is somewhat arbitrary and the ability to detect low antibody titres in the older age-groups can be uncertain. Here we focus on the estimation of the FOI using serological data.

But other complications can arise in the data. The design of the serological survey can be more complex than just a random sample of individual animals, like e.g. sampling herds introducing intra-herd correlation. Missing data frequently occur in surveys and ignoring this might introduce bias. Next to data issues, the estimation of the FOI poses additional modeling challenges from a statistical point of view: the estimated FOI should be positive or equivalently the estimate for $P(A \le a)$ should be a non-decreasing function of age *a*; the model should be flexible enough to avoid misspecification bias, etc. We next illustrate some possible statistical approaches to these complications on Bovine Herpes Virus-1 (BoHV-1) data from Belgian cattle.

The BoHV-1 seroprevalence (apparent prevalence) in the Belgian cattle population was determined by a large serological survey, conducted from December 1997 to March 1998 (Boelaert et al., 2005; Speybroeck et al., 2003). The sample taken was stratified for province. Within each province, 1% of the total number of herds was sampled. The blood samples, which were taken from all animals in the selected herds, were tested for antibodies against BoHV-1 by using an ELISA-test, specific for the BoHV-1 glycoprotein B (gB). Additional characteristics as gender, type of the herd (dairy, mixed or beef), purchased or homebred and size of the herd

were recorded. In total 11,284 cattle were investigated. In the left panel of Figure 1, the age-specific prevalence of gB-antibodies is displayed. Animals younger than 6 months typically have high seroprevalence of gB-antibodies because of acquired maternal antibodies and were therefore excluded.

Hens et al. (2005) show the disadvantageous effect of ignoring missing data on modelling the force of infection of BoHV-1 and propose the use of weighted generalized estimating equations (WGEE) to correct for bias. Faes et al. (2005) use a random effects approach to account for the correlation in the data, allowing to study both, a population averaged and a herd-specific force of infection. To obtain enough flexibility while retaining monotonicity, Hens et al. (2005) and Faes et al. (2005) apply constrained fractional polynomials to estimate $P(A \leq a)$ as a function of age (see Figure 1). Related methods and fully nonparametric estimation of the FOI is discussed by Shkedy et al. (2003, 2005). Bollaerts et al. (2005) used a Bayesian model to estimate the FOI, while incorporating expert opinions on sensitivity and specificity characteristics of the ELISA-test.

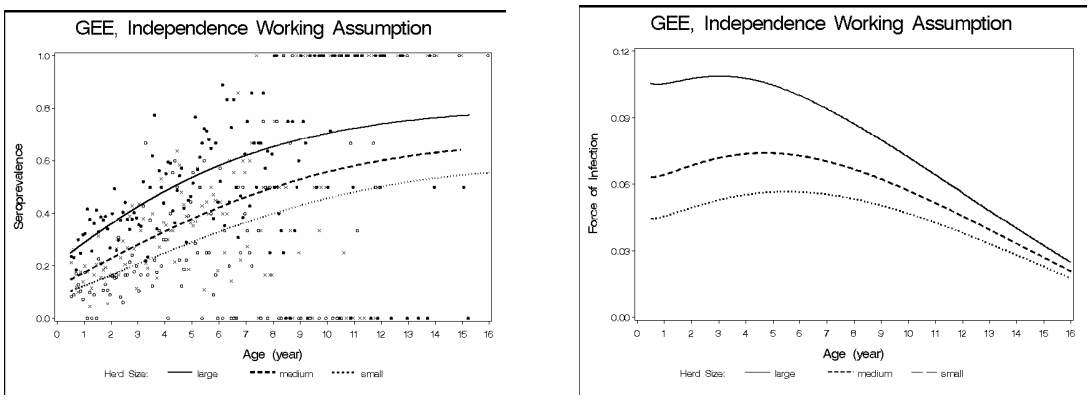


Fig. 1. The fitted prevalence (left) and FOI (right) according to the generalized estimating equations model, corrected for herd size (large, medium and small herd size).

3. THE BASIC REPRODUCTION NUMBER AND OTHER PARAMETERS

3.1 The Average Age at Infection

The average age at infection, the mean of the distribution of the age of infection, is given by $E(A) = \int_0^L (1-\pi(a))da$, where L is life expectancy. Farrington (1990) showed that if the data are observed up to a certain age U, $U \leq L$, then the average age at infection is given by

$$E(A) = \int_0^L (1-\pi(a))da + f(L-U),$$

where f is the fraction of individuals that remain uninfected (which can be estimated by $f = 1-\pi(U)$).
Using the GEE model to fit the seroprevalence BoHV-1 data (see Figure 1) and taking L=U=16, the estimated average age at BoHV-1 infection ranges from 3.45 to 9.46 (depending on herdsize).

3.2 The Basic Reproduction Number

The basic reproduction number R_0 in a given population is the average number of secondary infections which one infected individual would generate if the population were completely susceptible. So R_0 is essentially the average number of successful offspring that a parasite is intrinsically capable of reproducing. In large population $R_0 > 1$ implies the infection may become endemic, whereas if $R_0 \leq 1$, it will die out with probability 1. Knowledge of R_0 is important in guiding policy decisions (see Section 3.3). R_0 can be estimated i) directly as a function of other parameters describing the contacts in a population and which can be estimated from data on the number of contacts; ii) based on a deterministic or stochastic model, which can be fitted on data of time series of cases and susceptible individuals; iii) based on quantities that may be estimated from the endemic equilibrium of the infection, which requires data on the FOI. Here we concentrate on this last approach, as discussed in Farrington et al. (2001).

Suppose that a single individual who becomes infected at age y is introduced into a totally susceptible population. The average number of individuals of age x infected by this person during his or her infectious period is

$$\beta^*(x,y) = \frac{N}{L}\exp\left\{-\int_0^x \mu(t)dt\right\}\underbrace{\int_0^\infty \beta(x,y+u)\{1-F(u)\}\exp\left\{-\int_y^{y+u}\mu(t)dt\right\}du}_{D\beta^+(x,y)},$$

where N is the population size, L is the life expectancy, $\mu(a)$ the age-specific mortality rate, D the mean of the infectious period and $F(t)$ its cumulative distribution function. If the infectious period is in comparison with the timescale on which $\beta(x,y)$ and $\mu(a)$ vary, then $\beta^+(x,y) \approx \beta(x,y)$. After "averaging" over individuals of different ages and after some calculus, one arrives at the following expression for R_0

$$R_0 = \frac{\int_0^\infty l(a)\lambda(a)\exp\left\{-\int_0^a \mu(t)dt\right\}da}{\int_0^\infty l(a)\lambda(a)x(a)\exp\left\{-\int_0^a \mu(t)dt\right\}da},$$

where $l(x)$ is the leading left eigenfunction of $\beta^*(x,y)$.

In case of homogeneous mixing (implying a constant FOI), this expression reduces to $R_0 \approx L/A^*$ with A^* the mean time to removal by infection or death. For proportional mixing, the above integral expression reduces to

$$R_0 \approx \frac{\int_0^\infty \lambda^s(a)\exp\left\{-\int_0^a \mu(t)dt\right\}da}{\int_0^\infty \lambda^s(a)x(a)\exp\left\{-\int_0^a \mu(t)dt\right\}da},$$

with s=2. The same expression but now with s=1 holds for the case that the contact function $\beta(x,y)$ only depends on the ages x of the susceptible individuals.

Using the GEE model (Figure 1) and taking *L=16* (and type I mortality), the BoHV-1 basic reproduction number estimates vary (depending on herdsize) from 1.49 to 3.40 for s=1, and from 1.41 to 2.88 for s=2.

3.3 Effective Reproduction Rate, Herd Immunity and Critical Proportion for Eradication

At equilibrium, each infection will on average produce one secondary infection; that is, the effective reproductive rate is $R=1$. Assuming the host population is homogeneously mixed, the effective reproduction rate $R = R_0 x$ with x the fraction of the host population that is susceptible. This leads to the important relation between R_0 and the fraction x^* at equilibrium: $R_0 x^* = 1$. It is not necessary to vaccinate every subject to eradicate a disease; by a process known as herd immunity. If a proportion p is successfully immunized, the proportion remaining susceptible is at most $x^* = 1-p$ and the critical proportion to be immunized for eradication is $p_c = 1-1/R_0$.

4. SOME EXTENSIONS

In literature one has studied several modifications and extensions. For example, Farrington et al. (2001) discuss how methods can be modified for SIS models and how they can be extended to incorporate the effects of individual heterogeneity, including spatial heterogeneity. Whitaker and Farrington (2004) conclude that standard methods (assuming the infection is in endemic equilibrium) that do not adjust for regular epidemics can still be valid.

An extension in a different direction is the use of stochastic epidemic models. Deterministic models cannot accommodate all variation and uncertainty that characterizes the development of many epidemics. The importance of stochastic modelling has been recognized by many authors (see for example, Andersson and Britton 2000, Mode and Sleeman 2000). For example, in Höhle et al. (2005), data from a Belgian classical swine fever virus transmission experiment are used to study, within the Bayesian framework, an extension of the stochastic SEIR model to disease transmission experiments. Thanks to fast computing facilities, stochastic

modelling based on Bayesian inference using Markov chain Monte Carlo methods, can deal with a lot of complications in an elegant way and is therefore a promising avenue for further research.

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CHALLENGES IN THE DESIGN AND ANALYSIS OF INFECTIOUS DISEASE SURVEYS IN LIVESTOCK

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1. INTRODUCTION

Politicians, marketing departments of companies, and many others, constantly need information on characteristics of populations. Survey research methodology can also be applied to obtain information about a health characteristic in an animal population. The investigation of a subset instead of the complete population is attractive because it is less labour-intensive, quicker and less costly [21]. Also, the laboratory capacity needed is reduced.

More formally, a *sample survey* may be defined as a study involving a *sample* (subset) of individuals (elements) selected from a larger, well-delineated *population*. The population is the entire set of individuals to which findings of the survey are to be extrapolated. Most sample surveys can be put in the class of observational studies known as 'cross-sectional studies'. The essence of a cross-sectional study is the contemporaneous classification of individuals with respect to both exposure and disease [24]. Their sampling protocol used implies that individuals are sampled without considering health and exposure status beforehand. Individuals are tested for the presence of the disease under study and for the exposure status at the same time. Technically, cross-sectional studies thus provide a snapshot of events at a particular time.

This type of study is often known as a *descriptive survey*. Its main objective is that of *estimating* the mean level of some characteristics in a defined population [17], including a measure of the precision for those estimates. A secondary objective of surveys often is the measurement of the relationship between two or more variables measured at the same point in time. The main focus of such research is to test a hypothesis [21] concerning the association between a set of independent variables (e.g., exposure to suspected causal risk factors) and a dependent variable (e.g., disease prevalence). These are *analytical surveys*. In veterinary epidemiology, analytical surveys are frequently used to screen for potential *risk factors*. Risk factors are factors that are associated with an increased likelihood of an event occurring (e.g. disease).

2. RANDOM AND SYSTEMATIC ERROR IN INFECTIOUS DISEASE SURVEYS IN LIVESTOCK

Every epidemiological study should be viewed as a measurement exercise. Its overall goal is estimating the true value of the parameter that is the object of measurement with little error [24]. Errors in estimation of population values are arising from both sampling design and measurement problems. Both sources of error may be classified as either random or systematic. The principles of survey design emerge from consideration of approaches to reducing both random and systematic errors. Any interpretation of survey findings should take both types of errors into consideration [17].

2.1. Random error

The *reliability* (lack of random error, lack of variance) of an estimated population characteristic refers to how reproducible the estimator is over repetitions of the process yielding the estimator. It depends on how well the measurements were made (quality of the data). If we assume that there is no measurement error in the survey, then the reliability of an estimator can be stated in terms of its sample variance or, equivalently, its standard error, which is the standard deviation of the sampling distribution. The sampling distribution is the distribution of sample estimates that would be formed if an infinite number of samples of a given size were drawn. The smaller the standard error of an estimator, the greater is its reliability. Appreciation of the variance (spread,

dispersion, noise) is one of the most important concepts in statistics. Random error can be reduced (controlled) by increasing the sample size. More samples means more information, and thus a higher precision: it increases the statistical power of the study. To allow calculation of the sample size, basically three important choices have to be made: the expected (*a priori*) prevalence of disease, the allowable error in estimate of prevalence, and a confidence level.

The choice of a sampling strategy rests in part on feasibility and costs, but it also involves the precision of sample estimates, since all different sampling plans need appropriate methods of estimating population characteristics. That is, the design of the sampling plan (simple random sampling, systematic sampling, stratified sampling, cluster sampling and multi-stage sample) affects the estimates of sampling error for a sample of a given size.

2.2. Systematic error

The *validity* of an estimator refers to how the mean of the estimator over repetitions of the process yielding the estimate, differs from the true value of the parameter being estimated. It depends on how well the sample was chosen. If we assume that there is no measurement error, the *validity* of an estimator can be evaluated by examining the *bias* of the estimator. The smaller the bias, the greater is the validity. An unbiased estimator yields, on average, estimates without systematic errors. Bias is of major concern in observational studies. It is the consequence of flawed research design, leading to selection of a non-representative sample of the population and to a systematically wrong (biased) estimate. All sources of bias can be eliminated (controlled) through randomisation, except accidental bias. Thus, without proper randomisation, we cannot validly extrapolate from the sample to make inferences about the population it is supposed to represent.

Dozens of possible types of biases can detract from internal validity [25]. The distinction among these biases is occasionally difficult to make, but three general types are classically identified: selection bias, information bias, and confounding. A fourth systematic error is test misclassification bias. Indeed, one of the major challenges in the interpretation of observed (measured, apparent) prevalence data is the need to 'adjust' test results for misclassification. Once an analytically optimal test is available, it should undergo field (epidemiological) evaluation to demonstrate its relative merits in terms of its diagnostic performance characteristics [14]. Two of these attributes, the diagnostic sensitivity and specificity are population parameters describing the diagnostic characteristics of the test [13, 18] and, by consequence can differ if the test is used in another population (country, species, …). If an individual has a specific disease, *diagnostic sensitivity* indicates the conditional probability that the patient will be positive for the particular test. If an individual is free of a specific disease, *diagnostic specificity* indicates the conditional probability that the subject will be negative for a certain test.

3. INFECTIOUS DISEASE SURVEYS IN LIVESTOCK: ACCOUNTING FOR THE INFLATION OF RANDOM ERROR OF ESTIMATORS

Intermezzo: veterinary infectious disease surveys: prevalence at herd, animal or within-herd level

A complexing aspect is the clustering of the animal population, a key feature of modern intensive husbandry. As a consequence, veterinary surveys typically aim at estimating the herd prevalence (P_H) (herds are the units of concern), the within-herd prevalence (P_{WH}) (e.g. for herd certification), or the (animal) prevalence across herds (P). No standard sampling design exists that accommodates these three different objectives. Thus, one must concentrate on one.

When dealing with infectious diseases, the group of animals which is of importance in terms of the transmission and maintenance of infection - and therefore of disease control and eradication - is the herd [27]. Thus P_H is a relevant parameter to study in case of infectious diseases. Analytical surveys with the dichotomised serological status at herd-level as the response variable provide economically important information. The drawback however is that animal-level covariates have to be aggregated (collapsed) into herd-level summary statistics, with the inherent danger of not controlling for important covariates. Also herd-level risk factors are primarily related to the introduction or presence of infections, or seropositive animals, into the herd [15]. These associations detected at the herd level do not necessarily correspond to those existing at the animal level. There might be some confusion between aggregate and individual effects, an issue that is often referred to as the ecological fallacy [23].

From an epidemiological viewpoint, also *P* is relevant. Animal-level analyses offer a complementary and vital insight into the understanding of the epidemiology, since it allows proper consideration of factors measured on individual animals. The motif for such analysis is to unravel the phenomena that emerge when animals, the individual (elementary) units of the usual analytic currency of epidemiology [26], are assembled into larger units (clusters). Animal prevalence is a more suitable indicator for the spread, on an annual basis, of highly contagious infections. Animal-level Risk factors are not only related to the introduction or presence of infection into the herd, but also to the maintenance and transmission of these infections within an infected herd [15]. But, the interpretation of animal-level risk factors, however, must consider the biology the infection. It must be 'post epidemic', since seroprevalence figures are non-informative with respect to the infection chronology and to the within-herd (pre-epidemic) index case [3].

3.1. Accounting for clustering in the design phase

The sampling procedure affects the estimate of the precision of the sampling error, and thus systematic sampling, stratified sampling, unequal probability sampling, cluster and multistage sampling are all schemes that need adapted sample sizes. In the case of cluster sampling, infectious disease prevalence investigation involves *larger* number of animals, compared to the standard calculations. The sample size has to take account of the clustering effect via the intra-cluster correlation coefficient that must be obtained via pilot projects [7, 10, 19, 22]. There are a host of publications on the topic of cluster randomization and sample size (e.g. Int J Epidemiol, 1999, 28, 319-326). Initially, sample-size calculations referred to formulas and tables based on the assumption of an infinite population and - more importantly - the presumption of using perfect tests (i.e. tests with a sensitivity and specificity of 100%) [6]. Later, Cameron and Baldock [4, 5] presented a new method for the calculation of sample sizes (having developed the computer program FreeCalc). However, sample size calculations seem largely meaningless in the case where the diagnostic test characteristics of the survey test used are unknown [1, 2].

3.2. Accounting for clustering in the analysis

The data analysis should match the design. An analysis of sample survey data that takes into consideration the features involved in the sample design can be defined as a 'design-based analysis' [17]. In veterinary infectious disease surveys, the contagion, measured by the intra-herd correlation coefficient, means that the animals' responses tend to look alike. The calculation of the standard errors of the parameter estimates should then be based on the number of herds rather than on the number of animals since the information provided by a single animal would more amount to the total information provided by the whole herd to which the animal belongs. This de facto sample size reduction attenuates risk factor effect estimates – they become non-significant - and increases standard errors relative to estimates from analyses with standard errors of the parameter estimates calculated based on the number of animals.

4. INFECTIOUS DISEASE SURVEYS IN LIVESTOCK: ACCOUNTING FOR THE TEST MISCLASSIFICATION BIAS

It appears in literature that the impact of test misclassification bias can be assessed on the interpretation of herd seroprevalence figures, via herd testing – rules (aggregate testing, herd-level interpretation of tests). First, a herd test has to be defined, which is a test at the aggregate level. Herd will be classified as test positive or negative, based on decision rules to summarise the individual tests (taking the test misclassification bias into account) and on rules concerning the uncertainty due to sampling of animals from the herd. A threshold value (*c*) is required that denotes the maximum number of test positive animals that are accepted for the diagnosis of "no infection" on the herd-level.

Herd sensitivity (Se_H) is defined as the probability of a truly diseased herd to be classified as diseased by the test [5]. Thus, Se_H is the proportion of diseased herds in which the number of reactors meets or exceeds the specified cut-point number of reactors [18]. A herd is usually considered as non-diseased if all animals in that herd are disease free. Herd specificity (Sp_H) is the probability of a truly non-diseased herd to be classified as non-diseased with the test (only negative results). But again if the health status is defined in relation with cut-point number of reactors, Sp_H is also the proportion of non-diseased herds in which the number of positive reactors is below the specified cut-point [18]. P_H is calculated using the formula for P in which number of herds replaces the number of animals.

The following comments can be made. First, Se_H and Sp_H not only depend on the Se and the Sp of the test but also on the sample size and the critical number c. A bigger within-herd sample size, results in improved Se_H and lowered Sp_H values [18]. Second, the theoretical deductions of Se_H and Sp_H re based on the binomial model. They are only valid for (small) sample sizes from large herds (infinite populations), i.e. a sample fraction less than 0.05 [6, 20]. Alternatives for small herds and relatively more important sample sizes (finite populations) are based on the hyper-geometric distribution [4]. Thirdly, the estimations of the Se_H, Sp_H, P_H and the herd predictive values (PV_H) are problematic when disease is not distributed at random in the population, since those estimations assume a constant P_{WH} [16] and constant individual test Se and Sp. For most diseases, it is reasonable to assume that P_{WH} varies, since it is merely a reflection of the probability that a randomly selected animal is diseased which itself varies between herds due to intra-cluster correlating factors that may be specific for each herd. For the same reason, individual test *Se* and *Sp* may vary from cluster to cluster [8]. So it is important to know how these varying parameters are distributed in order to get a central representative value. A beta-binomial model that takes into account the clustering of Se, Sp and P within herds, has been proposed [8, 9]. This model enables calculation of Se_H, Sp_H and thereby P_H and PV_H from parameters such as Se, Sp and P that can be assumed to have a beta-binomial distribution. However Donald's model works under the assumption that all herds have the same size. This constitutes a limit because in reality herds are often of different size.

Lastly, often data are lacking to determine the true herd status (P_{WH} >0). Assumptions are then made regarding the minimum P_{WH}, once the infection is imported into the herd. This minimum prevalence may be drawn from the knowledge of disease epidemiology or from results of previous surveys carried out in other similar populations. For most contagious diseases a minimal P_{WH} of 10 to 20% is hypothesised. However, in the case of slow spreading infections like paratuberculosis, with low P_{WH}, herd testing can prove to be senseless [1]. Also, when estimating the bovine herpesvirus-1 prevalence in Belgian cattle via a large serosurvey, P_H, the predictability of the level seems rather difficult, not only from a statistical viewpoint, but also from the viewpoint of biological relevance. Indeed arguing the irrelevance of differences in bovine herpesvirus-1 P_H of 65% to 28% seems untenable [2].

5. DECISION AT THE END OF THE DAY: EXECUTE THE INFECTIOUS DISEASE SURVEY IN LIVESTOCK, OR NOT

If there are major unknowns then it is cardinal to conduct a pilot project, before carrying out a nation-wide survey regarding infectious diseases. Such a pilot project could involve some 30 randomly selected herds. It has to initially estimate the different prevalence parameters and intra-cluster correlation coefficients. In general, it is advisable to always begin with such a small survey.

If the intended survey test was not locally validated, a pilot diagnostic validation study is of cardinal importance, with special reference to the local positive (diseased) and negative (disease-free) animal population. Diagnostic test validation studies regarding the immunological infection status should appreciate the test misclassification bias. These studies are more difficult compared to diagnostic validation studies regarding clinical disease status, because the gold standard is defined less easy. The latent class approach provides a unified framework for various methods [28]. It is probably the future way to go into diagnostic test validation, but one consistently needs big sample sizes.

6. PERSPECTIVES FOR CONTROL AND ERADICATION

In absence of a 'gold standard test' a misclassification bias reduction strategy is needed. This means the use of several (at least 4) tests, or minimal follow-up by repeated sampling. When qualifying the health status of animals, it must be emphasised that repeated and multiple testing generally does not yield the expected improvement in the detection of infected animals if test sensitivities are conditionally dependent [11, 12].

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ASSOCIATIONS BETWEEN ANTIBODY LEVELS AGAINST *FASCIOLA HEPATICA* AND PRODUCTION PARAMETERS IN DAIRY HERDS

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ABSTRACT

The objective of this study was to determine the relationships between *Fasciola*-specific antibody levels (ODRf) in bulk tank milk and measures of productivity in dairy herds. A bulk tank milk sample was collected in March 2004 on 1105 dairy herds from which production data were available for n= 463. The effect of ODRf on four production parameters (milk yield, milk protein%, milk fat% and inter-calving interval) was assessed by multivariable linear regression models. An increase in ODRf over the interquartile range (0.428 – 1.064) was associated with a decrease in the annual average milk yield of 0.7 kg/cow/day (P=0.002), with a decrease in the average milk fat% of 0.06% (P<0.001) and with an increase of the mean inter-calving interval with 4.7 days (P=0.03). No significant relationship was found with the average milk protein%.

SAMENVATTING

Het doel van deze studie was om de verbanden te onderzoeken tussen *Fasciola*-specifieke antistoffenniveaus (ODRf) in tankmelk en productiviteitsparameters op melkveebedrijven. In maart 2004 werd een tankmelkstaal verzameld op 1105 bedrijven waarvan er voor 463 bedrijven productiedata beschikbaar waren. De verbanden tussen ODRf en vier productieparameters (melkproductie, melkproteïne%, melkvet%, tussenkalftijd) werden onderzocht aan de hand van multivariabele lineaire regressie. Een toename in ODRf over de interkwartielafstand (0,428 – 1,064) was geassocieerd met een daling van de jaarlijkse gemiddelde melkgift met 0,7 kg/koe/dag (P=0,002), met een daling in het gemiddelde melkvet% van 0,06% (P<0,001) en met een toename van de tussenkalftijd met 4,7 dagen (P=0,03). Er werd geen significant verband gevonden met het gemiddelde melkeiwit%.

1. INTRODUCTION

Fasciola hepatica is a parasite of cattle and sheep with a world-wide distribution. In cattle, fasciolosis occurs generally under the form of a subclinical infection, but is considered to produce marked economic effects (Torgerson and Claxton, 1999). However, until now surprisingly little studies have been conducted to estimate the effect of fasciolosis on productivity (Vercruysse and Claerebout, 2001) and these studies were mostly improperly controlled (Dargie, 1987). The objective of this study was to determine the relationships between *Fasciola*-specific antibody levels in bulk tank milk and productivity parameters (milk yield, milk solids content and inter-calving interval) in order (1) to estimate economical losses, associated with *Fasciola* infections and (2) to investigate if a bulk tank milk ELISA is a promising tool to detect dairy herds with production losses due to *F. hepatica*.

2. MATERIALS AND METHODS

Thousand hundred and five herds were selected by convenience from the Flemish dairy herd population (n≈ 8400). A bulk tank milk sample was collected from these herds in cooperation with the Milk Control Centre Flanders (MCC Flanders) in March 2004.

Farm and production data were obtained from the milk production recording programme of the Flemish Cattle Breeding Association (V.R.V., Vlaamse Rundveeteelt Vereniging). The data were collected from April 2003 until March 2004. Following monthly herd-level data were substracted or computed based on the individual test-day production data: average milk yield (kg milk/cow/day), average protein%, average fat%, average lactation number, average days in milk, average log transformed somatic cell count, number of milk producing cows, calving distribution, main breed and province. The average inter-calving interval in March 2004 was computed based on the inter-calving intervals of all the lactating animals in this month that had a previous lactation.

The antibody levels against *F. hepatica* were determined as described by Salimi-Bejestani et al. (2005) with modifications. The test results were expressed as an optical density ratio (ODRf) with the formula ODRf = (OD – NC) / (PC - NC), where OD is the optical density of the sample and NC and PC are the OD of the negative and positive control respectively.

The effect of ODRf on four different production parameters (milk yield, protein%, fat% and inter-calving interval) was assessed by multivariable linear regression models including covariates that were considered as possible confounders. The milk production parameters (milk yield, protein%, fat%) were averaged over the period of one year before sample collection and their relationship with ODRf was investigated with covariates also averaged over this period. These covariates were average number of producing animals, average lactation number, average days in milk, average log somatic cell count, calving distribution, province and main breed. Since there existed a negative correlation between annual average milk yield and fat% presumably due to a dilution effect, milk yield was also included as a covariate in the analysis to investigate the effect of ODRf on fat%. The relationship between ODRf and inter-calving interval was investigated with a model containing calving distribution, main breed, province and annual averages of number of producing animals and lactation number as covariates.

3. RESULTS

The average ODRf of the 1105 sampled dairy herds was 0.762 with a range from 0.177 to 2.313 and a standard deviation of 0.420. The interquartile range of the ODRf values was 0.428-1.064. The average ODRf of the herds for which production data were available was 0.706 with a range from 0.251 to 1.867. The standard deviation of these herds was 0.402 and the interquartile range 0.407 to 0.944. Milk production data and average inter-calving intervals were available for respectively 463 and 449 herds out of the 1105 sampled herds.

The regression coefficients of the multivariable linear models to determine the relationship of ODRf with annual average milk yield, protein%, fat% and the inter-calving interval are presented in table 1. An increase in ODRf over the interquartile range (0.428 – 1.064) was associated with a decrease in the annual average milk yield of 0.7 kg/cow/day (P=0.002), with a decrease in the average milk fat% of 0.06% (P<0.001) and with an increase of the mean inter-calving interval with 4.7 days (P=0.03). No significant relationship was found with the average milk protein%.

Table 1. Regression coefficient (with 95% confidence interval) and *P*-value of the *Fasciola*-specific antibody level (ODRf) in multivariable regression models to determine the relationship of ODRf with annual average milk yield (kg/cow/day), milk protein%, milk fat% and inter-calving interval (days).

Model	Regression coefficient (95% CI)	*P*-value
Milk yield	-1.10 (-1.79;-0.40)	0.002
Protein%	-0.015 (-0.037; 0.007)	0.193
Fat%	-0.096 (-0.146; -0.047)	<0.001
Inter-calving interval	7.454 (0.846; 14.062)	0.028

4. DISCUSSION

The study demonstrates that there exist significant negative associations between *Fasciola*-specific antibody levels in the bulk tank milk and production parameters. The estimated losses in herds with high antibody levels are considerable and suggest that control of liver fluke infections can be economically profitable. However, the obtained estimates may be biased by the association of *F. hepatica* infections with management factors that negatively affect production. The results suggest that liver fluke infections do not affect the protein content of the milk, whereas there is a negative effect on the fat content. This is in contrast with gastrointestinal nematode infections for which there are minimal or no effects on milk fat and milk protein%, (Mc Pherson et al., 2001; Charlier et al., 2005). The effect of helminth parasites on reproductive performance is a poorly studied area

(Dargie, 1987). The longer inter-calving intervals for high antibody level herds in the present study indicate that studies investigating the economical losses due to *F. hepatica* infections should take into account reproduction effects.

The negative relationship between *F. hepatica* bulk tank milk antibody levels and production parameters suggests that an "economical" threshold can be determined that identifies the herds where the infection interferes with productivity. Antibody detection tests are already commercially available and could be re-evaluated by investigating if there are threshold levels that can predict positive production responses after control of *Fasciola*-infections.

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GUIDELINE FOR THE DESIGN OF FIELD SURVEYS IN THE EUROPEAN UNION

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Article 4 of Directive 2003/99 states that for the monitoring of zoonoses details rules concerning the following elements for the monitoring may be laid down to harmonize systems in place in the Member States. These requirements may in particular specify the population to be sampled, data to be collected, case definitions, sampling scheme, laboratory methods, and reporting. It should be noted that, according to the Directive, this is not an exhaustive list.

When protocols for specific E.U. wide studies have been drafted (e.g. to study the prevalence of salmonella in broilers), they recommend uniformity for some study elements. However, thusfar the elements that are uniform may vary between studies.

When the monitoring of zoonoses will be further harmonised, it is important to have agreement on elements to covered in a harmonised monitoring schemes.

EFSA working groups often review published literature and unpublished reports, including surveys. In order to ascertain which studies to include in such reviews, the scientific adequacy of such studies needs to be ascertained and this in a transparent and consistent manner

In order to make sure that a study meets expectations of scientific quality it is important that guidance be available on the conduct of surveys. As indicated, such guidelines can be of use on the one hand in the design of future surveys and on the other hand to assess whether past studies meet these proposed guidelines.

The purpose of the Guideline on Good Survey Practices is to identify and discuss key elements that need to be addressed in a study protocol to help guarantee scientific soundness of surveys. This includes pre-established procedures for the organization, conduct, data collection, documentation, and verification of survey studies.

The scope of this guideline includes zoonotic and non-zoonotic diseases in animals, food and food products of animal origin, and feed. It will only address issues that are of relevance for all these different subject areas.

ESTIMATION OF PREVALENCE AND DIAGNOSTIC TEST EVALUATION THROUGH BAYESIAN MODELING.

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ABSTRACT

The influence of both the data at hand and the choice of prior information on the parameter estimation in a Bayesian model is illustrated, based on prevalence studies for *Giardia* and *Cryptosporidium* in calves and based on diagnostic test evaluation in epidemiological and clinical studies. The prevalence of *Giardia* and the sensitivity and specificity of three diagnostic tests for the detection of *Giardia* in fecal samples were estimated with only an informative prior on the prevalence. For *Cryptosporidium* however a four test model was developed and the outcome varied depending on the choice of dataset, i.e. clinical or epidemiological. Furthermore the addition of a dataset of 2 PCR tests resulted in the development of a six test model necessitating extensive prior information for model converging.

SAMENVATTING

De invloed van zowel de dataset als de keuze van prior informatie op het schatten van parameters in een Bayesiaans model wordt uiteengezet op basis van prevalentiestudies naar het voorkomen van *Cryptosporidium* en *Giardia* bij kalveren en op basis van test evaluatie voor klinische of epidemiologische studies. De prevalentie van *Giardia* en de test karakteristieken van drie diagnostische testen werden geschat enkel met behulp van een informatieve prior op de prevalentie. In de *Cryptosporidium* studies werd een vier-test model ontwikkeld. De bekomen schattingen van de test karakteristieken varieerde tussen epidemiologische en klinische studies. Het bijvoegen van de resultaten van 2 bijkomende diagnostische PCR reacties aan de data resulteerde in de ontwikkeling van een zes-testen model , waarbij uitgebreide prior informatie nodig was om het aantal te schatten parameters terug te dringen.

1. INTRODUCTION

The intestinal protozoan parasites *Cryptosporidium* and *Giardia* are commonly identified in the faeces of dairy calves and should be considered in young animals with diarrhea and failure to thrive (12; 5). The wide variation in prevalence described for both parasites is not only due to differences in farm management practice or climate (12), but also to the differences in study design, such as choice of diagnostic test (8). Differences in sensitivity and specificity between diagnostic techniques can result in a substantial variation in prevalence estimates (1). However, diagnostic test characteristics are not known in a bovine population, yet reliable estimates of these parameters are needed to adjust prevalence estimates. Furthermore, the estimates of these test characteristics vary among published validation studies which are generally performed in human medicine. General scepticism is appropriate against the extrapolation of test parameters validated on human samples to bovine samples, because these test characteristics would not apply in populations with a different prevalence (8). Since there is no gold standard for the diagnosis of both parasites in dairy calves, the infection status of the population under study is uncertain and the accuracy assessment of a new test can be seriously biased by the use of an imperfect reference test as gold standard (1; 3).

A relatively new approach used to circumvent this gold standard problem is the Bayesian approach, which has proven its potential in validating diagnostic techniques and providing a reliable estimate of the disease prevalence, when at least three independent diagnostic test results are available (2; 4; 7; 9). In this study, the prevalence of both *Giardia* and *Cryptosporidium* in calves in the province of East-Flanders was estimated using a Bayesian approach. For the evaluation of the diagnostic tests, different approaches were used, illustrating the benefits and potential pitfalls of a Bayesian analysis.

2. MATERIALS AND METHODS

Fifty dairy farms in the province of East-Flanders, Belgium, were randomly selected and visited on a single occasion. Faecal specimens were collected rectally from Holstein or Holstein cross calves aged from newborn to 10 weeks. For the detection of *Giardia* a sucrose flotation technique followed by iodine staining (ME), the combined MERIFLUOR *Cryptosporidum/Giardia* kit (IFA) and the TechLab *Giardia* test (Techlab Elsia) was performed. For *Cryptosporidium* a carbolfuchsine smear method followed by microscopical examination (ME), the IFA, the TechLab *Cryptosporidium* test (Techlab Elisa), the Bio-X Digestive ELISA Kit (Tetra) and two PCR reactions targetting an unknown sequence (C-PCR) and the sequence for the *Cryptosporidium* Oocyst Wall Protein (COWP-PCR), was performed. Furthermore 186 samples from calves suspected to have a *Cryptosporidium* infection based on clinical symptoms, were processed in a clinical study using the IFA, Techlab, Tetra and a Dip-stick (Bio-X).

A Bayesian analysis framework was used to draw inferences about the prevalence of *Cryptosporidium* and *Giardia* and the test properties (sensitivity and specificity) of the different tests. Different models were constructed in WinBUGS 1.4 (10). For *Giardia*, a three-test model was constructed, requiring 15 parameters to be estimated. For *Cryptosporidium*, different approaches were used: a four-test approach, requiring 31 parameters to be estimated, and a new six-test approach, requiring 127 parameters to be estimated. These models are in fact not identifiable, since the data provide not enough degrees of freedom to allow all parameters to be estimated. Therefore, the model building strategy consists of incorporating extraneous prior information in a dependence model, such as expert opinion (11). For some parameters no objective prior information can be formulated. Therefore it is necessary to leave prior information on these parameters non-informative, if one wants to maintain a minimum degree of honesty (6). Prior information can also be applied to reduce the possible range of values for a specific parameter. This reduction may affect the possible range of values for other parameters as well.

3. RESULTS

In the *Giardia* three-test only prior knowledge on the prevalence had to be included to make the model converge. The sensitivity and specificity of each test is presented in table 1. In the *Cryptosporidium* four-test approach prior information on prevalence and on the specificity of the IFA, Techlab and Tetra had to be included into the epidemiological model to reach convergence. Adding information on the sensitivity of the IFA further improved the model. Additional constraints did however not improve the model. In the clinical study, the same model was used although only prior information on the specificity of the IFA, Techlab and Tetra had to be included to optimise the model. The estimated sensitivity and specificity of each test in these models is presented in table 1. In the *Cryptosporidium* six-test model, prior information on the specificity of both PCR assays (sp=1) was included. The specificity of both PCR assays was confirmed by the sequencing of amplification products of randomly chosen samples throughout the study. The specificity constraints made the model converge and made estimates of test characteristics possible (table 1).

4. DISCUSSION

These are the first known studies to use a Bayesian approach to estimate the prevalence of both parasites in dairy calves and to evaluate several diagnostic assays. Since there is no gold standard for the diagnosis of infection, a prevalence estimation based on the results of a single diagnostic assay would have been unreliable. In this study for example, the calf prevalence ranged from 13% to 46 % for *Cryptosporidium* and from 17% to 25% for *Giardia* depending on the technique being used. A Bayesian approach has proven its potential to circumvent this gold standard problem when 3 (9) or 4 tests (4) were used to diagnose infection. Both approaches were also used in these studies.

The results of the present studies illustrate, next to the benefits, the pitfalls of test evaluation and prevalence estimation using Bayesian analysis. Similar to diagnostic test evaluation in a frequentist approach, extrapolation of results on test characteristics beyond the limits of the study cannot be made in a Bayesian approach and results

should always be considered within the limits of the study. In a Bayesian analysis the posterior estimates are the result of both prior information and the data at hand (4). Prior information or constraints are essential in the present analysis in order to reduce the number of parameters to be estimated. In all models the use of prior information was needed to constrain the parameter space, allowing estimation of all remaining parameters. Expert opinion or published results from previous studies can be used as valuable prior information (11). In the *Giardia* three test approach and in the *Cryptosporidium* four-test approach the use of wide range constraints, based on previously published estimates of prevalence and test characteristics or on information provided by the manufacturer of the diagnostic assays, was sufficient to reduce the number of parameters to be estimated. In the *Cryptosporidium* six-test approach however, the use of more stringent constraints was necessary to reduce the high number of parameters. Although the validity of this prior information used in the six-test approach was previously described and further confirmed by sequencing PCR amplification products of randomly selected samples throughout the study, the posterior estimates of both the prevalence and the test characteristics are not stringent parameters, but should be considered as best-possible estimates based on the observations and the prior information. Since the data at hand in the present studies resulted mainly from epidemiological studies including both calves with and without clinical symptoms, and greatly depend on the choice of test for diagnosis, the posterior estimates of the test characteristics must be interpreted taking these limits into account. The rather low sensitivity estimates of some techniques is mostly due to the low excretion in healthy or subclinically infected calves. It does however not imply that these techniques are not reliable for clinical diagnosis, since clinical symptoms correlate with an increased excretion of oocysts, as was confirmed in our clinical analysis estimating higher sensitivity and specificity values were found for the IFA, Techlab and Tetra (see table 1).

Table 1: sensitivity (Se%) and specificity (Sp%) estimates obtained by the different models

	3 test approach *Giardia* epidemiological		**4 test approach *Crypto* Clinical**		**4 test approach *Crypto* epidemiological**		**6 test approach *Crypto* epidemiological**	
	Se (%)	**Sp (%)**	**Se (%)**	**Sp (%)**	**Se (%)**	**Sp (%)**	**Se (%)**	**Sp (%)**
C-PCR	NA	NA	NA	NA	NA	NA	**79 (69-87)**	**100**
COWP PCR	NA	NA	NA	NA	NA	NA	**59 (50-67)**	**100**
ME	**56 (39-73)**	**87 (81-91)**	NA	NA	**78 (56-95)**	**79 (72-87)**	**40 (31-49)**	**84 (75-97)**
IFA	**77 (53-97)**	**95 (91-99)**	**86 (78-94)**	**91 (81-99)**	**78 (54-95)**	**95 (91-99)**	**26 (19-34)**	**94 (88-99)**
Techlab Elisa	**89 (70-99)**	**90 (83-97)**	**88 (81-95)**	**89 (82-97)**	**76 (54-92)**	**89 (84-94)**	**37 (28-46)**	**84 (76-92)**
Tetra Elisa	NA	NA	**89 (81-95)**	**89 (81-97)**	**59 (39-77)**	**93 (89-96)**	**30 (21-42)**	**88 (81-94)**
Dip-stick	NA	NA	**79 (71-88)**	**74 (19-96)**	NA	NA	NA	NA

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MODEL-BASED INFERENCES FROM SURVEYS TO DOCUMENT DISEASE FREEDOM

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ABSTRACT

This paper will review current methodology for documenting freedom from disease. The different data sources and analysis methods will be discussed at the example of applications in the control of infectious animal diseases.

1. INTRODUCTION

The Sanitary and Phytosanitary (SPS) agreement of the World Trade Organisation (WTO) requires that, in international trade, measures taken to protect animal, plant or human health should be based on scientific principles and not maintained in the absence of sufficient evidence. The World Organisation for Animal Health (OIE) as competent international authority and national animal health organisations have accepted the use of both quantitative and qualitative assessments for this purpose. The data sources in both cases may include non-representative data such as laboratory records, abattoir sampling, notifiable disease databases, etc., as well as the results of structured surveys. However, qualitative assessments are subjective, and generally provide a dichotomous outcome with an unmeasured level of uncertainty. Therefore, this review will focus on quantitative methods.

"Freedom from disease" (referred to as FFD hereafter) is a technical term that describes methods used for substantiating an exporting country's claim of the absence of a particular disease that may pose risks to human, animal or plant health. "Disease" is a generic term and in most cases, the presence of infection or infectious individuals rather than clinical disease is of concern. The absence of disease from a population cannot be proven with absolute certainty. This would require testing of all individuals from the population with a perfect diagnostic test. FFD methods provide specialised tools for the analysis of surveillance data for epidemic diseases such as "exotic" transboundary animal diseases, zoonoses or emerging diseases, which are meant to be absent from the population of interest at the time of the investigation. Although animal disease will be used for illustrations below, the principles described in this paper are generic and applicable to human and plant diseases after appropriate modification to account for the nature of diseases and available data. These application-specific features can be summarised in a conceptual model for the sampling and testing procedure and is reflected in a corresponding data model. "Model-based inference" then refers to the application of statistical methods that make appropriate use of the information provided by the models for the conclusions about the target population.

The overarching objective of FFD methods is to contribute to a country's substantiation of a claim of the absence of a defined disease from a defined population. Some typical data sources and their characteristics are described in section 2. Different approaches, all with different strengths and limitations, can be used to tackle this goal and are described in section 3. Further research issues are outlined in section 4. The purpose of this text is to review the main issues involved in FFD methods. A comprehensive coverage of the subject is beyond the scope of the paper.

2. DATA SOURCES

Data that can be used for documenting FFD in animal populations arise either through routine health care or preventive systems or through studies specifically conducted for the purpose. There is a clear need for methods to substantiate claims of freedom including evidence from both types of data (6,17,18). Routine data collections include for example slaughterhouse data or productivity monitoring data. The great advantage of such data is that, because it was collected for different purposes, it is very cost-efficient to re-use them for FFD purposes. Moreover, the data often covers substantial parts of the target population. The down-side is that the process of data generation (particularly the selection procedure) is out of control for the investigator and principally non-random. The selection probability is likely correlated with certain characteristics of the animals, which gives rise to biases. Moreover, the disease indicators and case definitions in data bases used for collection of routine data may be such that a later use for documenting FFD is difficult; a problem that currently has attracted some attention (Hans Houe, personal communication). On the other hand, structured surveys are expensive and a randomisation of samples is difficult to implement in practice. Reliance solely on the results of structured surveys ignores the potential value of all other sources of evidence. The data required for FFD methods include those that describe the selection of herds and animals for a surveillance system. Moreover, data is required that describe the fate of animals in the diagnostic cascade and that characterise the diagnostic performance of all procedures that are used for early detection, screening or confirmation.

3. OVERVIEW OF APPROACHES TO DOCUMENT DISEASE FREEDOM

3.1 Classical survey statistics

Standard survey statistics are applicable for estimation of population parameters and provide powerful tools to account for the stratification of the sample, auto-correlation phenomena (cluster effect) and unequal sampling probabilities (9). An adjustment for diagnostic misclassification bias can be incorporated (13,15). However, these methods are not directly applicable to the FFD problem, simply because their focus is on quantification of a non-zero population parameter. One of the strengths of these methods is actually to account for the empirical occurrence patterns of the trait of interest (11), which is not applicable in a situation where the complete absence of the disease is the plausible true state of nature. Specialised survey analysis methods that are specifically designed for FFD problems will be described in section 3.3.

3.2 Bayesian methods for prevalence estimation

An alternative approach for the estimation of a prevalence in the population with allowance for misclassification is to use latent class models. The principles of the methods have been reviewed elsewhere (7). Briefly, the approach requires that paired data from more than two diagnostic tests are available. A potential correlation of test errors must be considered. The method has been used to investigate whether classical swine fever is present in a wild boar population (12).

3.3 Surveys to document disease freedom

The classical method for FFD documentation is the structured, representative survey of the relevant population and has been described elsewhere (2-4). The theory behind statistically based surveys is that each animal in the source population can be assigned a known, non-zero probability of being selected for testing (probability sampling). Furthermore, each animal sampled has a known non-zero probability of being tested positive given the disease status. Survey statistics as described above provide tools for handling unequal selection probabilities, which arise in many practical situations. However, in contrast to standard survey methods, the primary goal is now to establish the probability to detect the disease in the population, given that it occurs at a specified level of prevalence, the so-called design prevalence. The probability of detecting the disease in the population is also referred to as “confidence”, “power” or simply as sensitivity of the survey. The design prevalence is usually a low value, say 2% of herds and 5% of animals within infected herds. In the absence of international agreements, epidemiologically appropriate levels of the design prevalence must be chosen.

It is noted that the confidence or survey sensitivity is not the same as the probability of the country being free from disease. However, in a Bayesian framework, the sensitivity of the survey can be combined with a prior probability of the disease status of the country to derive a post-survey probability of the disease status. How this can be achieved may be debated. Koen et al. estimate the prior probability from the data and achieve this through incorporation of the prior into the likelihood function for the observed data in a latent class approach (12). One may argue that this undermines the independence assumption for the likelihood and the prior probability. It can be considered to derive the prior from an empirical Poisson parameter, estimated from the number of observed

outbreaks in the past. One practical solution could be to restrict the analysis to the likelihood (established using agreed design prevalence values), which is equal to the survey sensitivity or confidence.
The design prevalence plays a central role in the FFD survey concept. It is a kind of bench mark or fixed value and cannot be estimated from the survey data (which should provide no evidence of disease anyway). The choice of the design prevalence for a given application may be disputed. The working definition of "an epidemiologically expected prevalence at the time point of an outbreak, at which its detection in the population is mandatory under cost-efficiency considerations" gives an impression of the number of parameters that should be considered: prevalence-time function, potential of spread, survey costs and consequences of late detection.

Routine data collections, for example at slaughterhouses, lead to a purposive sampling and do not meet the requirements of (quasi) randomness. For this reason, methods for the analysis of structured surveys should not, or only with reservations, be applied to data generated by non-random procedures.

3.4 Documenting disease freedom based on surveillance
The methods used for documenting FFD using surveillance data are similar to those described under 3.3. However, the data generation process is continuous and the stream of data must be aggregated over time periods to allow the application of survey analysis methods. Aspects that have been addressed in the analysis of surveillance data for infectious bovine rhinotracheitis include the choice of the time windows (a whole year or shorter time periods of 4, 8 and 12 weeks) and different levels (also within the country) of design prevalence (16). In the context of surveillance for bovine spongiform encephalopathy (BSE), the longitudinal aspect of data collection was exploited to allow calculation of the cumulative evidence for disease freedom (1). The approach uses similar assumptions as the official surveillance model for BSE (14), but provides a stopping rule for the testing of candidate free birth cohorts, which are suggested as epidemiologic entities similar to "compartments".

3.5 Statistical hypothesis testing
The approach of estimating the sensitivity of a surveillance system can be reformulated in a hypothesis testing framework. The hypotheses are given in terms of the population prevalence parameter. It is impossible to test the null hypothesis of a prevalence of zero against the alternative non-zero prevalence, simply because of the lack of statistical power in situations where the prevalence (under the alternative hypothesis) is close to zero. The pragmatic solution is again to define a partial alternative hypothesis using the design prevalence. The situation is not trivial because the observation of positive cases (which would preclude the application of the approach) might be consistent with a true prevalence below the level of the design prevalence. The uncertainty in the model parameter sensitivity and specificity can be expressed using prior distributions (8). Statistical methods of hypothesis testing provide objective rules to reject the hypothesis that a population is free. If the results of FFD methods are to be used in risk assessment models, it will be more straightforward to work with probability estimates, such as the sensitivity of a surveillance system.

3.6 Scenario tree model
A scenario tree model has been found useful for incorporating data sources other than those arising from structured surveys (10) and has been applied to case studies on classical swine fever and avian influenza. A typical surveillance component, which can contribute to the evidence for the absence of a disease, is clinical surveillance. Clinical signs or a drop in productivity may be notified by the farmer, who eventually consults a veterinarian, who, in turn eventually triggers a diagnostic follow-up, which could lead to the detection of the disease in question. Each of the events that lead to detection and all factors that influence the probability of these events can be represented as branches in a scenario tree. The probabilities assigned to the branches are based on empirical data or expert opinion. The scenario tree is populated with the empirical number of animals "processed" in the respective limbs of the tree. The total probability of all terminal nodes that result in the detection of a single infected animal is used to derive the population estimate of the probability to detect at least one diseased animal. One challenge inherent to the scenario tree approach, when applied to multiple data sources (i.e. surveillance components), is to account for the lack of independence. In the cited paper, Bayesian methods are used to achieve this.

3.7 Simulation modelling
A direct estimate of the confidence can be obtained by computer simulation of a surveillance procedure. In this case, the detection of disease in the population is the outcome of one virtual representation of the surveillance procedure. The probability of detection (confidence) is the proportion of successes (at least one diseased animal detected) out of all iterations. A simulation model has been used to demonstrate freedom from classical swine fever in a vaccinated swine population (5). The authors emphasised that the herd-level diagnostic performance should be improved. This can theoretically be achieved by designing a testing strategy (parallel or sequential testing) to optimise sensitivity (or specificity). A more practical solution may be to optimise the sample size

(within herd) and cutpoint (number of positive results required to score the herd positive) with regard to some optimality criterion (typically a combination of herd-level sensitivity and specificity). Current work of the author in this direction will be presented during the meeting. Briefly, an algorithm was used to select, for any given herd size, a sample size and cutpoint such that herd specificity is maximised and a specified minimum herd specificity is maintained. Optionally, one can also set a constraint on the absolute or relative sample size. Such optimised, herd-size specific testing schemes can be applied to simulate the performance of the surveillance in the population.

4. RESEARCH ISSUES

Mathematical models and simulation models share a lot of problems, even when this is not obvious at the first glance. In fact, both approaches are truly model-based and the choice of models and their interpretation is an area that requires more research. Below, some examples are given.

On what basis should one choose between the binomial and the hypergeometric model for diagnostic performance on herd level? The well-known difference is the underlying sampling model, which is usually chosen with regard to the (herd) population size and sampling fraction. The issue that needs further clarification is the subtle difference in the interpretation. Under the hypergeometric model, we assume that the number of diseased animals is known and fixed, whereas we assume only the binomial parameter and sample size as known in the other case. The hypergeometric model may be preferred in the context of FFD because here we assume some fixed threshold level of design prevalence. If we were more interested in the behaviour of a surveillance programme for some endemic diseases, for which we have empirical prevalence values, a binomial model, or an appropriate over-dispersion model (beta-binomial) could be more appropriate. The important practical difference becomes evident in the simulation approach. Assume the extreme example of a herd with size N=1. Under the hypergeometric model, we randomly assign this herd to as infected and the animal-level probability of disease is 100%. Under the binomial model, this probability would just be the design prevalence.

The use of stochastic values (rather than point estimates) for sensitivity and specificity is another interesting issue. How do we really interpret uncertainty of the parameter estimate versus biological variability? Assume we have k and n as source data for the estimate of sensitivity Se=k/n. The usual beta model for variability in Se reflects the stochastic properties of the population estimate rather than any variability of Se among infected animals. Consequently, the way to introduce stochasticity into the model is to draw a random number for Se (and Sp) for each iteration of the simulation. The source data k/n is not sufficient to parameterise any over-dispersion (random effects) model for Se, i.e. random values for each animal. Following the parsimony principle in modelling, one can argue for using fixed values for the diagnostic parameters for the whole simulation or (for comparative purposes) random numbers for each iteration. It is also unrealistic to assume that estimates of the extra-binomial variability of the diagnostic parameters are available in practice.

Another, challenge is to account for the specific structure of data collected in multiple surveillance components. The statistical inference will be biased towards high values for confidence when observations (i.e. negative test results) within and among different surveillance components are not independent. Dependence of observations within one component may arise through clustering of disease within herds and, if multiple diagnostic tests are used for final classification of the animals, through conditional dependence of the test results. Dependence among surveillance components is plausible if one herd can be included in more than one component. To model the total dependence structure is a complex task or even untraceable. Some solutions for dealing with the lack of independence have been described for the scenario tree models as outlined above.

5. CONCLUSIONS

Various quantitative methods are described for substantiating freedom from disease in a population of animals. All these methods are based on a model for the data generation process. They also have in common that they provide probability statements (chance to detect the disease if present or chance that the population is free) rather than any proof for the absolute absence of the disease. No single method exists that can be applied or recommended under all circumstances. The flexibility and intuitively understandable representation of the data generation process by the scenarios tree and simulation make these approaches most promising, though. The issue of documenting disease freedom is still an important research area.

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DEVELOPMENT OF A SANITARY RISK INDEX FOR *LAWSONIA INTRACELLULARIS* SEROPREVALENCE IN PIG FARMS

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ABSTRACT

Risk factors for *L. intracellularis* seroprevalence in slaughter pigs were investigated in a cross-sectional survey on 60 Belgian farrow-to-finish herds belonging to one slaughterhouse co-operation. Herd data were collected using a questionnaire. The blood samples were serologically analysed. Significant risk factors, associated with the average PI-value of a herd were identified by a general linear mixed model with herd and herd*season as random effects. The herd prevalence was 97% in summer and 98% in winter when using cut-off 30. The average within-herd prevalence was respectively 52% and 43% for summer and winter. Sampling in winter, having a hygienic-lock facility, applying the strict all in/all out principle, worming pigs less than 2 times and having slots wider than 1.4 cm were all associated with lower average PI-values. Lower copper dose in feed, decreasing space allowance per pig, lower cleaning frequency as well as increasing ph of drinking water were related with higher average PI-values.

SAMENVATTING

In een cross-sectionele studie van gesloten varkensbedrijven werd onderzoek verricht naar risicofactoren voor *L. intracellularis* seroprevalentie bij slachtvarkens. Alle 60 bedrijven behoren tot eenzelfde integratie werkend op basis van een kwaliteitscontrolesysteem. Via een enquête werden bedrijfsgegevens verzameld en het bloed werd serologisch geanalyseerd. Er werd gebruik gemaakt van een general linear mixed model met bedrijf en bedrijf*seizoen als random effecten om significante risicofactoren, geassocieerd met de gemiddelde PI-waarde van een bedrijf te identificeren. De bedrijfsprevalentie bedroeg 97% in de zomer en 98% in de winter bij een afkapwaarde 30. De gemiddelde binnen-bedrijfsprevalenties waren 52% en 43% voor respectievelijk de zomer en de winter. Stalen nemen in de winter, het gebruik van een hygiënesluis, het toepassen van het all in/all out principe, minder dan 2 keer ontwormen en het hebben van roosterspleten breder dan 1.4 cm zijn geassocieerd met lagere gemiddelde PI-waarden. Lagere koperdosissen, een minder volume beschikbaar per big, een lagere reinigingsfrequentie en een stijgende ph van het drinkwater is gerelateerd met hogere gemiddelde PI-waarden.

1. INTRODUCTION

Proliferative enteropathy, caused by *L. intracellularis*, is classified as a global disease persisting in pigs widespread throughout the world. The economic impact to the swine industry was estimated to be very high (3). By the end of 2005 all use of antibiotic growth promoters will be banned in Belgium. After the ban, an increase in Lawsonia infections is to be expected as seen in Denmark. Therefore, risk factor studies are required to have a scientific basis to control proliferative enteropathy in Belgian pigs.
This paper presents the results of a risk factor analysis based on the seroprevalence of *L. intracellularis* in Belgian slaughter pigs. These risk factors are combined in a scientifically based Sanitary Risk Index (SRI), defined as an objective measure of the *L. intracellularis* seroprevalence of a pig farm.

2. MATERIAL AND METHODS

A total of 60 Belgian farrow-to-finish pig herds were included in the cross-sectional study. The herds were all located in Flanders and were members of the same slaughterhouse cooperation. Herd data were collected using a questionnaire, consisting of 2 major parts, in order to identify potential risk factors. Following topics were included: housing and ventilation, management, hygiene and biosecurity and production parameters. The serological herd status was determined by blood sampling of finishing pigs at slaughter. From each of the herds, 33 pigs from an average slaughterhouse delivery of 77 pigs were sampled. The pigs were selected systematically with randomization of the first animal. To take into account seasonal variation in seroprevalence, each herd was sampled two times i.e. in summer (July-October) and in winter (December-March). Serological examination for specific antibodies against *L. intracellularis* was performed by means of an ELISA (Bioscreen, Germany).
For the determination of risk factors, a general linear mixed model was used (SAS®) with the Percentage Inhibition (PI) value on pig level as dependent variable and with herd and herd*season as random effects. In a first step each of the factors obtained from the questionnaire were separately introduced in the model to assess whether any of these were univariate associated with the PI-value. Risk factors with a significant value ($P<0.05$) and minimum 85% of the observations present, were subjected to further analysis. In a second step a backwards elimination of variables was performed to analyse explanatory variables simultaneously. Factors found to be significant at the 0.05 level were combined to fit the final Sanitary Risk Index. The inferred SRI was validated concerning repeatability and reliability. Pearson correlation coefficients, 2x2 tables, a Differential Positivity Rate graph and a Receiver Operating Characteristics curve have been used for validation purposes. The final model was developed based on 70% of all the observations. The validation dataset consist of the remaining 30%, selected at random.

3. RESULTS

Results of 3974 samples were available. In summer, 97% of the herds were positive with a minimum of 1 positive animal. The herd prevalence in winter was 98%. The average within-herd prevalences were respectively 52% (stdev: 29%, min: 0%, max: 100%) and 43% (stdev: 27%, min: 0%, max: 100%) in summer and in winter. The cut-off value PI=30 has been used as recommended by the manufacturer. Samples with PI% larger than the cut-off were considered as positive.
A Pearson correlation coefficients of 0.95 ($P<0.0001$) was found between the average PI-value of a herd and the within-herd *L. intracellularis* prevalence (%positive animals) of a herd.
The sanitary risk index consists of nine explanatory variables i.e. 9 risk factors were significantly ($P<0.05$) associated with the average PI-value of a herd (Table 1). First, the analysis showed a seasonal effect on average PI-values. Average PI-values were higher in summer compared with the winter. Secondly, herds with a hygienic-lock facility had lower average PI-values compared with herds without hygienic-lock room. Thirdly, herds that produce pigs according to the strict all in/all out principle had a significant lower average PI-value than herds that make use of continuous production or semi-all in/all out. Fourthly, herds were pigs are wormed less than 2 times had lower average PI-values compared with herds were worming took place 2 or more times. Fifthly, the width of slots in the nursery is important towards the average PI-value of a herd. Herds with slots wider than 1.4 cm, had a lower average PI-value. Sixthly, a higher copper dose in the feed of growing pigs is related to lower average PI-values. Seventhly, increasing volume per pig in the nursery is associated with lower average PI-values. Last but one, a higher ph of drinking water is linked with higher average PI-values. Finally, the yearly frequency of wet cleaning in the nursery is a significant risk factor i.e. the higher cleaning frequency, the lower average PI-values.
The objective of the validation is to evaluate how accurate the SRI can estimate the within-herd prevalence, by means of an average PI-value, of a new herd. Therefore, an average herd dependent PI-value for every herd in the validation dataset was calculated according to the obtained SRI. This value can be determined by using the estimates in Table 1. A Pearson correlation coefficient of 0.21 ($P<0.0001$) between the estimated average PI-values and the serological average PI-values was found. Next, the serological average PI-values of the herds were classified positive or negative according to cut-off 30 to evaluate the usefulness of the SRI in categorizing herds according to their status. By plotting the sensitivity+specificity-1 as a function of the SRI PI-values, the cut-off value at which both sensitivity and specificity is maximal can be found. At a SRI cut-off 29, the sensitivity is 0.50 and the specificity 0.72. The area under the ROC curve is 0.62, reflecting the accuracy of the SRI.

4. DISCUSSION

The study showed a high *L. intracellularis* herd prevalence and within-herd prevalence. Such high prevalences were also reported in other studies (3,6)

To our knowledge, this is the first time that the relation between average PI-values of a herd and within-herd prevalences was investigated. The high Pearson correlation coefficient showed that the average PI-value is a good predictor for the within-herd prevalence. Nine risk factors were found to be associated with the *L. intracellularis* seroprevalence in pig herds, reflected in an average PI-value. Until now, no literature has been produced concerning the relation between risk factors and the average PI-value. Nevertheless, all the risk factors found in this study are consistent with other studies or are biologically logical. Because Lawsonia can survive for at least several days on the surfaces of material, the Lawsonia problem is basically a hygienic one. It's reflected in the many risk factors in the SRI related to hygienic management on the herd. This is also supported in literature (2,5,7). Furthermore, it was shown that herds were pigs are wormed less than 2 times had lower average PI-values compared with herds were worming took place 2 or more times. Although, Pearce *et al*. (1999) suggested a synergism between infections with endo-parasites and infection with enteric bacteria such as *L. intracellularis*. But, in this study, a strong negative relationship could be demonstrated between frequency of worming and white spot problems at slaughter, suggesting that only herds with a high frequency of white spots are worming more than once. This supports the findings of *Pearce et al*.

Antibiotic growth promoters have a protective effect on proliferative enteropathy (8), but those are forbidden in the near future. The pig feeding practice shows an interest in excessive doses of copper. This obviously reflects the search for substitutes to antibiotic feed additives. A negative relation between copper dose in the feed and average PI-values has been seen in the present study. It's general accepted that more space leads to higher welfare and less stress. The relation between higher volume per pig and lower average PI-values, in this study, supports the hypothesis that pigs shed *L. intracellularis* in the faeces, particularly after stress, causing transmission of the bacteria among pen mates. The ph in the stomach can be lowered by organic acids. This provides a hostile microbiological ecosystem for *L. intracellularis* (1). This has been confirmed in our study.

As seen in the results, the sensitivity and specificity can be used to determine the appropriate cut-off level. But, the maximization of both may not always point out the optimal cut-off value. Sometimes, it's more important to consider the percentage false positives and false negatives herds for economical or logistic reasons.

5. ACKNOWLEDGEMENTS

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Table 1 Summary of the risk factors (P<0.05) making up the sanitary risk index for *L. intracellularis* in finishing pigs. The dependent variable is the average PI-value.

Parameter	Level of parameter	Estimate
Intercept		32.03
Season	summer	4.67
	winter	0
Hygienic-lock	isolated hygienic-lock facility	-3.40
	no hygienic-lock facility	0
All in/all out	continuous production or semi all in/all out	3.40
	using strict all in/all out principles	0
Worming schedule	pigs are wormed less than 2 times	-1.68
	pigs are wormed 2 or more times	0
Width of slots in nursery	slots > 1.4 cm	-4.33
	slots <= 1.4 cm	0
Copper dose in feed of growing pigs	continuous variable	-0.03
Volume per pig in nursery	continuous variable	-4.35
Ph of drinking water	continuous variable	1.41
Yearly frequency of wet cleaning in nursery	continuous variable	-0.53

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ESTIMATING THE FORCE OF INFECTION FROM SEROLOGICAL DATA: THE IMPACT OF CLUSTERING, INCOMPLETE DATA AND DIAGNOSTIC UNCERTAINTY

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ABSTRACT

Epidemiology deals with the study of infectious diseases and their determinants within a given natural population. Infectious diseases data are complicated and statistical modelling should account for these burdens. In this paper, the impact of (1) clustering, (2) missing data and (3) diagnostic uncertainty on the estimation of the force of infection of the bovine herpesvirus-1 in Belgian cattle is established and several methods to deal with these complications are proposed.

SAMENVATTING

Epidemiologie is de studie van infectieuze ziektes en hun determinanten in een gegeven natuurlijke populatie. Infectieziekte data zijn ingewikkeld en de verschillende moeilijkheden dienen die in rekening gebracht te worden bij het statistisch modelleren. In dit artikel, bestuderen we de impact van (1) clustering, (2) ontbrekende gegevens en (3) diagnostische onzekerheid op het schatten van de infectiedruk voor het boviene herpesvirus-1 in Belgisch vee. Er worden verschillende methoden voorgesteld om met deze moeilijkheden om te gaan.

1. INTRODUCTION

The seroprevalence survey of the bovine herpesvirus-1 (BoHV-1) in Belgian cattle [1], conducted in 1998, is a study of a transmissible disease in cattle, which is of economic importance and significance to international trade. A central characteristic of infectious disease dynamics is the transmission of the infection from infectious to susceptible subjects. The force of infection (FOI) is the rate of acquisition of the infection for a susceptible host. Empirical data show that, in general, the FOI is age-dependent. Like many other infectious diseases data, the BoHV-1 data suffer from several complications and thus statistical modelling has to deal with these.

A first complication is clustering. Indeed, animals within clusters (herds) have a higher chance of becoming infected once the infection is introduced into the herd. Thus, individual responses are more homogeneously distributed within herds than in the whole population. There exist several methods to deal with clustering [2].

A second complication is the not unlikely occurrence that some subjects have one or more missing values. If the missingness is ignorable as defined by [2], the analysis can be based on the so called complete cases, i.e. all

observations for which all values are observed. If, however the missingness is non-ignorable, analyses can be affected by merely using the complete cases. Several methods to handle missing data are known [3]. None of them are without limitations.

A third complication is the diagnostic uncertainty when modelling the seroprevalence (apparent prevalence) instead of the true prevalence. The true prevalence of a disease is the proportion of a given population that is affected with that disease, whereas the seroprevalence is the proportion of test-positives for that disease. Evidently, main interest lies in this true but unobserved prevalence rather than in the observed test-specific seroprevalence. However, often serological data are analyzed such that only conclusions with respect to the observed seroprevalence are justified.

2. MATERIAL AND METHODS

In the present dataset, from a Belgian 1998 sero-survey, the response variable is the gB-test result for the presence of antibodies to BoHV-1. Additionally, age and origin (purchased yes/no) of the cows were recorded. Unfortunately, there was a considerable amount of animals for which the origin was not recorded. The FOI as a function of age was derived from the sero-prevalence function. Let us now present the different methods to deal with the complications of clustering, missing data and diagnostic uncertainty, respectively.

There are several ways to deal with clustering, some of which estimate population-averaged measures of effect and some of which estimate herd-specific (cluster-specific) measures of effect. Generalized estimating equations (GEE) can be used to estimate population-averaged measures while accounting for the clustering. Herd-specific profiles can be studied by using a generalized linear mixed model (GLMM) [4]. A more detailed study can be found elsewhere [5].

One of the techniques to deal with missing values, which gained a lot of attention recently, is the use of weighted estimating equations [6], where each contribution of a case is weighted with the inverse of the probability that this case is observed. In this way cases with a low probability to be observed gain more influence in the analysis and thus represent the missing values. One can look at this approach as an implicit imputation of missing values. Dealing with both missing values and clustering can be done using a weighted GEE, where the weights are those inverse probability weights [7], which are preferably estimated nonparametrically.

The diagnostic uncertainty related to modelling the seroprevalence instead of the true prevalence is determined by two test characteristics, i.e., diagnostic sensitivity and specificity. True prevalence is then modelled using serological data and prior information on diagnostic sensitivity and specificity. We propose the use of Bayesian techniques to study the impact of this uncertainty [8].

3. RESULTS

To show the impact of clustering on the estimation of the seroprevalence, the estimated FOI curves, based on a logistic model (ignoring clustering), on GEE (population averaged model) and on a random intercepts model (herd-specific), are shown in the next figures. The logistic regression model clearly underestimates the variability. The random effects model shows the large differences between herds.

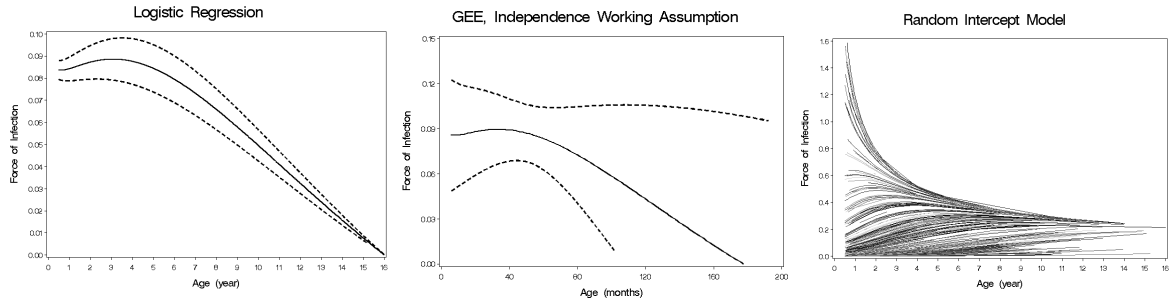

Figure 1: Estimated age-specific force of infection based on logistic regression (left panel), GEE (middle panel) and GLMM (right panel).

To illustrate the effect of ignoring missing data, Figure 2 describes the results of modelling the FOI as a function of age, based on all cows, termed as 'all cases' (AC) on the one hand, and on the other hand based on those cows for which origin is observed, termed as 'complete cases' (CC). Next to showing the effect of merely using the complete cases, the use of weighted estimating equations (WCC) is illustrated to correct the CC-analysis.

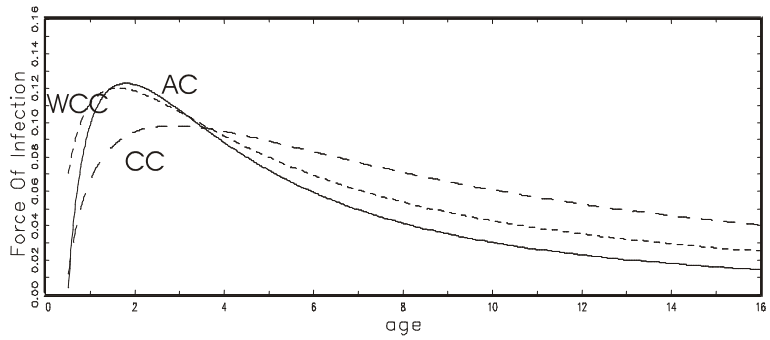

Figure 2: The force of infection from a population averaged point of view as a function of age based on 'all cases' (AC), on the 'complete cases' (CC) and using weighted generalized estimating equations (WCC).

The effect of test misclassification is illustrated in the next two graphs that compare a model assuming a perfect test (Se = Sp = 1) with a model accounting for test misclassification with sensitivity and specificity as high as 0.995 and 0.990, respectively. Although virtually no differences are observed with respect to the estimated prevalence (left panel), larger differences are observed on the scale of the force of infection (right panel)

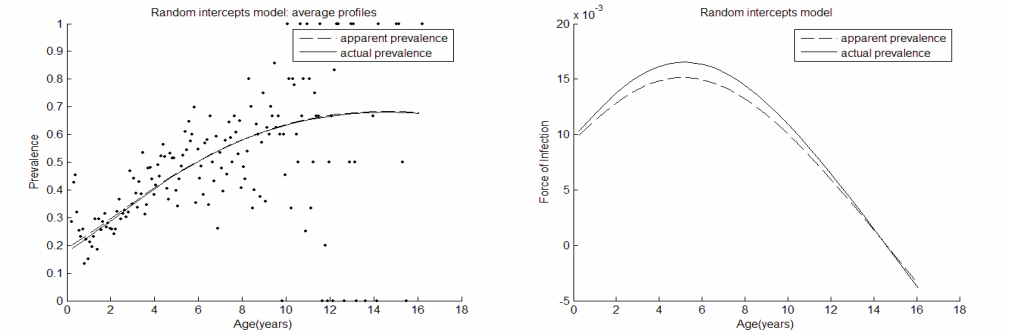

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Figure 3: The prevalence (left panel) and the force of infection (right panel) as a function of age for a model assuming perfect test (dashed lines) and a model accounting for test misclassification (full lines).

4. DISCUSSION

In this paper, it is shown that specific models are needed to estimate the force of infection while dealing with clustering and missing values, both complications epidemiological data often suffer from. Moreover, main interest lies in the true but unobserved prevalence rather than in the observed test-specific seroprevalence. However, often serological data, as in this case, are analyzed such that only conclusions with respect to the observed seroprevalence can be drawn.

To deal with correlated data both generalized estimating equations and generalized linear mixed models have been proposed while inverse probability weights have been shown to be able to handle missing values. Bayesian methodology was used to assess the impact of diagnostic uncertainty. The proposed methods deal with two

additional complications, the constraint that the force of infection has to be positive and an informative cluster size, for which the details were omitted from this paper.

In practice, policy makers base their decisions on conducted surveys. Therefore, correct modelling of the epidemiological quantities of interest and correct interpretation of model parameters are crucial.

5. ACKNOWLEDGEMENTS

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EVALUATION AND VALIDATION OF A QUESTIONNAIRE REGARDING BIOSECURITY AND CONTACT STRUCTURE OF PIG HERDS IN FLANDERS

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SUMMARY

A postal survey was set up to measure biosecurity and contact structure between pig herds in Flanders. This paper focuses on the methodology and validity of the survey. It discusses the structure and design of the questionnaire, sampling of pig herds and validity of the survey. Non-responding herds were analysed and the reliability and validity of the questionnaire were controlled. It was concluded that the postal survey was a good tool to collect data on biosecurity and contact structure of pig herds in Flanders.

SAMENVATTING

Via een schriftelijke enquête werd de graad van bioveiligheid en contactstructuur van varkensbeslagen in Vlaanderen onderzocht. Dit artikel gaat de methodologie en validiteit van de survey na. Verschillende onderwerpen die worden belicht zijn: het ontwerp van de vragenlijst, het bemonsteren van de varkensbeslagen en de validiteit van de enquête. Niet deelnemende varkensbeslagen worden geanalyseerd. Als conclusie kan gesteld worden dat de schriftelijke enquête een geschikte methode is om gegevens te verzamelen over de graad van bioveiligheid en contactstructuur van Vlaamse varkensbeslagen.

1. INTRODUCTION

In Belgium, intensive pig production is mainly concentrated in Flanders (northern part of Belgium) and is characterised by densely populated livestock areas (DPLAs). In these DPLAs, good biosecurity measures are crucial to prevent introduction or spreading of pig diseases. Biosecurity can be defined as the application of health controls and measures to prevent the introduction of new infectious diseases into herds and to avoid them from spreading within the herd and towards other herds (Barceló and Marco, 1998). Farms with a low level of biosecurity and frequent contacts with other farms have a higher risk for introduction and spreading of infectious diseases.

In this paper, the set up of a postal survey and its methodology are described, with focus on the validity of the survey. Objective of this questionnaire was to measure present external and internal biosecurity and contact structure between pig herds in Flanders. The questionnaire interrogated both professional and non-professional swine farmers and aimed at describing a number of characteristics that may enable us to classify swine holdings according to their risk of spreading swine diseases. With the gathered data, a model to simulate disease spread between herds will be developed in the near future.

2. MATERIALS AND METHODS

In May 2005, 509 owners of pig herds were administered a written questionnaire by post. Pig herds were randomly selected from an I-and R database (Sanitel-Pigs, 2005). Selection was stratified by province. Selection criteria were that at least one pig was present at a geographical location based on a recent visit of the herd veterinarian (after September 2004). One month later (June 2005) a reminder was sent by post. Finally, non-participating farmers were contacted by telephone and asked whether they were yet willing to cooperate or informed for their reasons of non-response. The survey was closed in the month of August 2005.

The number of herds contacted was calculated based on an expected prevalence of 50% (considering no a priori knowledge of prevalence and a 95% confidence level and desired accuracy of 5%) (Thrusfield, 1995).

The questionnaire consisted of 10 pages with 86 questions and was semi-closed. It covered several aspects regarding the level of biosecurity and different types of contact between farms. Before the questionnaire was finalised and posted, questions were pre-tested on seven pig farms regarding content, interpretation of questions and responses. The questionnaire form was accompanied with a covering letter and a return envelope. An incentive was promised to all participants. It was emphasised that questionnaires were processed anonymously (except coding for response control) and validity of the survey depended on the honesty of the questionnaire's respondents. Collected data was entered by double entry to ensure accuracy and was coded into a database (Access, Microsoft Cooperation).

Analysis of (non)-responders was done in SPSS 11 (SPSS Inc. Illinois, USA, 2001). It was controlled if statistical differences were found between responders and non-responders. Validation of the survey was done using both internal control (reliability: the ability of a question to give consistent results on repeated trials) and external control (validity: degree to which the answers reflect the true state of nature) (Vaillancourt et al., 1991). As internal validation (reliability), Cronbach's Alpha (CA) was calculated as a measure for internal consistency to questions that were issued twice. The widely-accepted cut-off is that CA should be 0.70 or higher. Also, the CA statistic was calculated for questions organized into certain domains (internal biosecurity, …) (consistency within the questionnaire, the degree to which a subject answers similar questions in a similar manner). External validation was done by comparing answers with independent data sources (e.g. Sanitel-Pigs, 2005). This was achieved using paired statistical tests and the CA statistic. The practicability of the questions was controlled by counting the number of incomplete or incorrect answers.

3. RESULTS

7402 swine holdings fulfilled the selection criteria. Given a total population of 7402, the required sample size was 385. A priori cut-off value for response rate was set to 60% to be valid. Therefore the questionnaire should have been posted to 642 holdings. For logistic reasons, this number was reduced to $\approx$ 500. In total, 369 questionnaire forms were successfully returned by post. Total response rate was 75.25%. 53.83% responded on the first invitation, 15.91% after a reminder and 5.50% were persuaded to participate by telephone. Another 15 swine farmers responded by telephone but did not return the questionnaire (mostly swine holdings with only a limited number of pigs present, non-professional holdings and farms that recently had stopped or have the intention to stop production). Total number of pigs present at the location was significantly lower for responders which received a reminder (931.31) compared to responders without reminder (1264.62) (independent t-test, P=0.01).

As a measure of internal consistency, Cronbach's Alpha was calculated between items that questioned the same topic (own transport truck: α= 0.794; internal biosecurity measures: α= 0.822).

To evaluate the external validity, data available of all herds (responders and non-responders) were compared. It was found that the mean of total pigs present on the non-response holdings (689.84) was significantly lower then on the responding holdings (897.96) (independent t-test, P<0.01). Monthly on-and-of movements were also significantly lower on non-responding holdings (2.58 compared to 3.47). No differences were found between type of swine holding and location (province). Analysis of the answers of responders regarding species classification and supply of piglets were compared with external data (Sanitel-Pigs, 2005) and showed no statistical differences except the presence of poultry on the location. (Supply of piglets: α= 0.936; cattle: α= 0.933; sheep: α= 0.761; deer: α=0.635; poultry: α=0574).

The average number of missing values per question was 8.58 (min=6, max=33). Certain questions had a higher number of missing values: location of disinfection baths (n=33), location of hygiene entry (n=14), showering before entrance compulsory (n=13), location of separated material (internal biosecurity) (n=12).

4. DISCUSSION

The main advantage of a written questionnaire is that it is cheap, fast and makes a survey in a large population manageable. Its disadvantage is that the clarity of the questions is crucial and that the validity of the responses is difficult to assess, particularly if the answer is based on a subjective opinion (Stärk et al., 1998). Another weakness is the frequently limited or low response rate compared to other survey methods (telephone survey or interview). In this study we tried to overcome this using incentives, covering letter, questionnaire design

(structure, length, lay-out, …) and follow-up procedures (reminders and telephone calls). Especially the three first measures were successful as over 70% of the questionnaires of responders were sent before any reminders were started.

Stratification per province ensured that enough herds located in less densely populated regions were included. The high sample size guaranteed that all different types of swine farms were represented. More than the sample size, the acquired response rate is essential as non-responders can systematically represent a certain type of holding, resulting in a survey which is unrepresentative. In literature, a minimum response rate of 70 % is put forward in order to avoid bias (Thrusfield, 1995). This criterion was fulfilled and in addition, non-responders were analysed. Analysis indicated no differences between responders and non-responders in type of holding and province of location, although non-responders tended to be smaller holdings.

Cronbach's alpha ('the reliability coefficient') measures the extent to which responses on highly comparable questions obtained at the same time, correlate with each other. This was the case for the question that was issued twice (α=0.794) (own transport truck). High alpha scores were also obtained for item that measured internal biosecurity. These high results for the different Cronbach's alpha calculated suggested most respondents were reliable. Ideally, the reliability of certain answers could be validated by reissuing the questionnaire to a sample of responders. However this was not done in this study.

Validation of the survey using external data sources could not attribute differences that could not be explained. Validation is difficult and if possible, validity should be done by performing a farm visit in random respondents. Low CA value on the existence of poultry at the location was explained by the fact that the I-and R data does not register small stock poultry holders. Comparison of the number of pigs present with available data in the I-and R database showed statistical differences, but in general magnitudes of herd size were correct. Questions allowing continuous variables to be entered, such as 'number of pigs', are more difficult to answer and more variable and therefore probably less reliable (Stärk et al., 1998). Also, estimation of the number of pigs is sensible to responder bias (perhaps not all pigs are reported) and the total number may vary substantially in time.

Extensive evaluation of answers and comments revealed that some questions (use of shower before entry and quarantine period for persons) were misunderstood by some of the respondents (e.g. 27.1 % did not precise the duration of a quarantine period). This will be taken into account in further analysis. Also missing values on certain questions indicated certain options were missing for the respondent (place of material and hygiene entry). Respondents with missing data tended to be small, non-professional holdings, for which the suggested options were not applicable. This demonstrates that most missing data is due to the fact that the survey was conducted in a heterogeneous population (professional and non-professional holdings) and compromises in the questionnaires design had to be made to achieve a questionnaire applicable for all holdings.

The authors acknowledge the fact that the subject of this survey is sensible to an overestimation of the level of employed biosecurity by the farmer who wants to disguise the true level of sanitation measures at his herd ('socially desired response'). The questions that require a subjective assessment of a process or situation have to be interpreted with care as they may be biased. Nevertheless, because most characteristics of the management of the pig farm are static and rather straightforward, answers given by the respondent should be reliable, particularly if only 'yes' or 'no' answers were required (Stärk et al., 1998).

In conclusion, the postal survey was a good tool to collect sufficient data in different areas and this in only a limited period of time. With certain limitations, data can be used to rank farms according to their risk of spreading disease.

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SURVEY OF MARBOFLOXACIN ACTIVITY ON PATHOGENIC BACTERIA ISOLATED FROM PORCINE DISEASES IN EUROPE (1998-2004)

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ABSTRACT

Vétoquinol has set up a European surveillance program to collect field pathogenic strains from clinical cases of porcine respiratory diseases or meningitis infections. After what, marbofloxacin MICs were determined by the broth microdilution method (CLSI, M31-A).
1168 strains were collected between 1998 and 2004: 466 *Pasteurella multocida*, 349 *Streptococcus suis*, 201 *Actinobacillus pleuropneumoniae*, 88 *Bordetella bronchiseptica* and 64 *Haemophilus parasuis*. The prevalence result could not be interpreted country by country. The marbofloxacin MIC_{50}, MIC_{90} as the percentage of susceptibility were the same from the start of survey. *Pasteurellaceae* strains were the most susceptible strains and *Streptococcus suis* were the less susceptible strains. Some variability has been observed mainly due to technical difficulties in isolation (for *A. pleuropneumoniae or H. somnus*). Since 1998, only 9 strains (1%) have been considered as intermediate. This type of collection could be used to evaluate susceptibility to anti-infectious in Europe and to obtain some information on bacterial prevalence.

1. INTRODUCTION

Marbofloxacin is a third generation fluoroquinolone. This anti-infectious drug is used since 1997 in Europe for porcine injectable treatment of respiratory infections and mastitis-metritis-agalactiae syndrome. Fluoroquinolone use is currently being monitored through numerous surveys to look for resistance development, (DANMAP, SWARM, DEFRA, and others). A surveillance network was set up by Vétoquinol S.A. from 1998 with participation of European countries where marbofloxacin is marketed. The aims of this survey are, to follow on bacterial collection the bacterial prevalence of the main pathogens isolated, to identify the technical problems of identification and to survey the marbofloxacin susceptibility as for others anti-infectious.

2. MATERIALS AND METHODS

2.1. European collection

European surveillance program was set up in 1998 in France, Germany, Italy, Belgium, Netherlands, Spain, Ireland and United Kingdom. This methodology is the same as for the VETPATH collection (performed by the European pharmaceutical firm union).

2.2. Sampling

Samples performed by veterinarians on clinical cases of porcine respiratory disease infections and on meningitis were sent to the nearest laboratory of microbiology. Samples were performed “before initiation of any treatment and without recent treatment”, by transtracheal aspiration (not easy), sterile swabbing (AMI swab), biological samples at autopsy (lung, meninges). One sample by farm and or by outbreak was taken as reference to avoid the isolation of the same bacteria.

2.3. Isolation

The isolation is performed according the internal procedures of each laboratory of microbiology. The methodologies used could be summarized as follow:

The main aerobic pathogenic strains were isolated on selective and conventional medium, in Petri dishes and incubated for about 24 hours in an appropriate atmosphere and temperature:

- *Pasteurella spp.* on Columbia blood agar at 35 ± 2°C with 6 % CO_2,
- *Actinobacillus pleuropneumoniae* on chocolate polyvitex agar at 36 ± 2°C with 6 % of CO_2,
- *Haemophilus parasuis* on chocolate polyvitex agar or Columbia cooked blood agar at 36 ± 2°C with 6 % of CO_2,
- *Bordetella spp.* on Columbia blood agar, both at 35 ± 2°C
- *Streptococcus* and other Gram-positive bacteria on Columbia blood agar or on TKT agar at 35 ± 2 °C.

After Gram coloration and oxydase (Gram negative bacilli) or catalase (Gram positive cocci) tests, the bacteria were characterised by biochemical API® identification systems.

No anaerobic bacteria were searched neither viruses.

Not all the laboratories were able to isolate the *Mycoplasma* strains. When it has been tested, the methodology is on a Mycoplasma agar base or Mycoplasma sterile broth (for lung samples) supplemented with agar or broth Mycoplasma supplement, incubated 48 to 72 hours at 36 ± 2°C with 6% CO_2 or during about 7 days at 36 ± 2°C with 6% CO_2 for broth.

After identification, the bacterial strains were stored on cryobeads at about -70/-80°C and sent to a referenced laboratory.

2.4. Susceptibility determination

The MICs (minimal inhibitory concentration) were obtained by broth microdilution method according to the CLSI M31-A2 guideline [1] as antibiograms. The marbofloxacin breakpoints have been established on aerobic pathogenic strains according to the CLSI M37-A1 [2] including pharmacodynamics, pharmacokinetics and clinical data: susceptible strains ≤ 1µg/ml, intermediate strains = 2 µg/ml and resistant strains ≥ 4 µg/ml.

3. RESULTS

3.1. Prevalence

1168 pathogenic strains were collected between 1998 and 2004: 466 *Pasteurella multocida* (39.9%), 349 *Streptococcus suis* (29.9%), 201 *Actinobacillus pleuropneumoniae* (17.2%), 88 *Bordetella bronchiseptica* (7.5%) and 64 *Haemophilus parasuis* (5.5%). Others strains have been isolated but in too low number to be considered.

Nevertheless, this type of collection showed that the bacteria which are very difficult to grow are clearly not isolated by most of the laboratories (few laboratories used a molecular method to detect the Mycoplasma strains).

Moreover, obtaining strains before any treatment is very difficult (or impossible) in some countries. Consequently, the prevalence of bacteria isolated by pathologies could be considered as slightly partial. This is a general situation in Europe, in each national survey.

Pathogenic bacteria searched and isolated depend of each laboratory. This prevalence was similar over years but was not be relevant by country.

According to our results, one strain by sample is isolated for a respiratory disease as for meningitis. It's rare to observe two bacteria (no virus were searched).

3.2. Anti-infectious activity

No change in susceptibility to marbofloxacin occurred during these seven years (Table 1). Some variabilities have been observed over years (1998-2004) for *A. pleuropneumoniae* and *H. parasuis* strains mainly due to the very low number of strains collected and to technical difficulties in isolation. Nevertheless this variability was also observed for the other antibiotics tested.

The marbofloxacin MICs distribution for each species was comparable over years. It was unimodal for *B. bronchiseptica* and *S. suis* strains and was bimodal for the other bacteria species. The marbofloxacin MIC_{50}, MIC_{90} and the percentage of susceptibility were the same over years from the start of survey.

Table 1: Marbofloxacin activity on the main pathogenic bacteria isolated from porcine respiratory and meningitis infections.

Strain (1998-2004)	Modal class (µg/ml)	MIC_{90} Range (µg/ml)	% of susceptibility
P. multocida	0.015	0.047-0.061	98-100
S. suis	0.5	0.468-0.786	99-100
A. pleuropneumoniae	0.015	0.026-0.330	94-100
H. parasuis	0.008	0.026-0.812	93-100
B. bronchiseptica	0.5	0.410-0.707	90-100

All these results are similar to these presented in 2002 in this meeting [3].
The marbofloxacin MIC for *P. multocida* strains is clearly lower than for *S. suis* strains and shows that marbofloxacin, as all fluoroquinolones, is more active on *Pasteurellaceae* strains than on Streptococci strains.

4. CONCLUSION

This European survey has given partial epidemiological prevalence information on pathogenic strains isolated from porcine respiratory diseases and meningitis infections. The number of collected strains was not sufficient to obtain significant results in each country. This observation is the main reason for variability of anti-infectious susceptibility. Due to technical difficulties in culture, Mycoplasma spp. strains were not isolated in this survey. The main pathogenic bacteria were Pasteurellaceae strains. During 1998, no strain has been resistant to marbofloxacin, and only 9 strains (1%) have been considered as intermediate. The contribution of marbofloxacin individual treatment to the increase and spread of bacterial resistance has been limited from its launch in 1997. This type of collection could be used to evaluate antimicrobial susceptibility in Europe with a minimum of investment.

5. ACKOWLEDGEMENTS

Vétoquinol S.A. survey has involved several people who in various ways have made this programme possible. We would like to express our gratitude to all those who have contributed and in particular to veterinarians and laboratories involved for their assistance.

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ATTEMPT OF MYCOPLASMA ISOLATION RATE DETERMINATION FROM BOVINE RESPIRATORY DISEASES IN EUROPE FROM 2002

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ABSTRACT

Vétoquinol has set up a European surveillance program to collect *Mycoplasma* strains from clinical cases of bovine respiratory diseases. After what, marbofloxacin MICs were determined by the broth microdilution method.
The main bacterial species isolated (823 strains) were *P. multocida* (54%), *M. haemolytica* (30%), *Mycoplasma spp* (11.3%) and *H. somnus* (4.8%). 86% of *Mycoplasma* strains belong to the *bovis* species and 14 % to the *bovirhinis* species. Many laboratories are still technically unqualified to diagnose *Mycoplasma* strains and others detect presence of *Mycoplasma* strains with PCR method. The determination of a true prevalence of *Mycoplasma* strains involved in cattle pneumonia is uneasy and consequently the MIC determination in order to establish a fully adapted treatment is very difficult.
The marbofloxacin MIC distribution of *Mycoplasma* strains was unimodal with a modal class at 1 µg/ml. The marbofloxacin MIC_{90} was below 1 µg/ml since 2002 without evolution of susceptibility to marbofloxacin.

1. INTRODUCTION

Mycoplasma bovis strains affect ruminants and generate some of the most economically important diseases in Europe: for example calve pneumonia, mastitis, arthritis. However, no European survey program to assess *Mycoplasma* isolation rates has been performed for different reasons: difficulties in sampling, isolation or detection and difficulties in MIC determination. Consequently no survey of susceptibility is performed by the different national programs (DANMAP, SWARM, DEFRA and others). Vétoquinol has attempted to set up and combine a European survey program for the assessment of *Mycoplasma* isolation rates together with an epidemiological survey program. This survey covers pathogenic bacteria isolated from bovine respiratory infections, combined with marbofloxacin MIC.

2. MATERIALS AND METHODS

2.1. European collection

European surveillance program was set up in 2002 in France, Germany, Italy, Belgium, The Netherlands, Spain, Ireland and United Kingdom. This methodology is the same as for the VETPATH collection (performed by the European pharmaceutical firm union).

2.2. Sampling

Samples performed by veterinarians on clinical cases of bovine respiratory disease infections were sent to the nearest laboratory of microbiology. Samples were performed “before initiation of any treatment and without recent treatment”, by transtracheal aspiration (not easy), sterile swabbing, biological samples at autopsy (samples were eventually frozen). One sample by farm and or by outbreak was taken as reference to avoid the isolation of the same bacteria.

2.3. Isolation

The isolation is performed according to the internal procedures of each laboratory of microbiology. The methodologies used could be summarized as follow:
The main aerobic pathogenic strains were isolated on selective and conventional medium, in Petri dishes and incubated for about 24 hours in an appropriate atmosphere and temperature:

Pasteurella and Mannheimia strains on Columbia blood agar at 35 ± 2°C with 6 % CO_2,
Haemophilus somnus on chocolate agar supplemented with polyvitex at 36 ± 2°C with 6 % of CO_2,
Not all the laboratories were able to isolate the Mycoplasma strains. When it has been tested, the methodology is on a Mycoplasma agar base or Mycoplasma sterile broth (for lung samples) supplemented with agar or broth Mycoplasma supplement, incubated 48 to 72 hours at 36 ± 2°C with 6% CO_2 or during about 7 days at 36 ± 2°C with 6% CO_2 for broth

After Gram coloration and oxydase (Gram negative bacilli) or catalase (Gram positive cocci) tests, the bacteria were characterised by biochemical API® identification systems. For Mycoplasma strains, the species were determined by serotyping method.

No anaerobic bacteria were searched neither viruses.

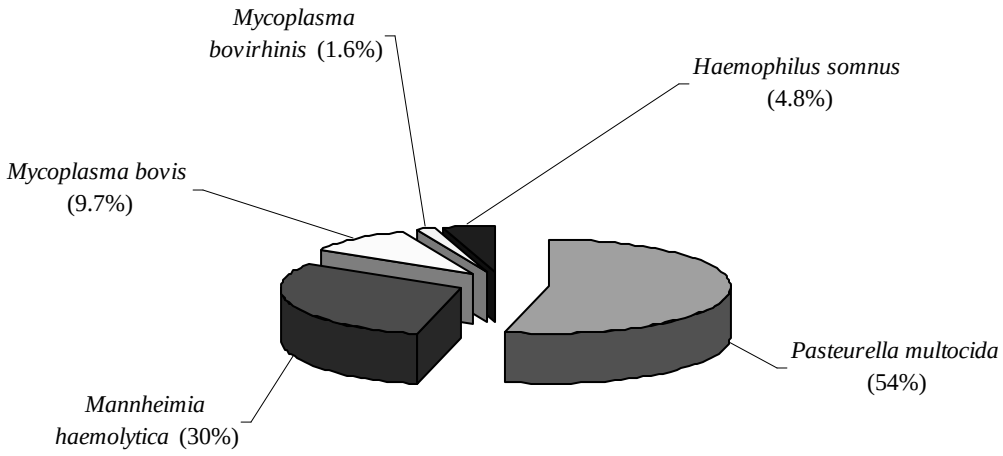
After identification, the bacterial strains were stored on cryobeads at about -70/-80°C and sent to a referenced laboratory.

2.4. Susceptibility determination
The MICs (minimal inhibitory concentration) were obtained by broth microdilution method inspired from the the CLSI M31-A2 guideline [1] and from publication of F. Poumarat and J.L. Martel (1989). The marbofloxacin MICs were determined in the Vetoquinol laboratory of microbiology.

3. RESULTS

3.1. Prevalence
Between 2002 and 2004, the main bacterial species isolated from bovine respiratory diseases (823 strains) were *Pasteurella multocida* (54%), *Mannheimia haemolytica* (30%), *Mycoplasma spp* (11.8%) and *Haemophilus somnus* (4.8%). Others strains have been isolated but in too low number to be considered.
Among *Mycoplasma* isolates, 86% belong to the *Mycoplasma bovis* species and 14% to the *Mycoplasma bovirhinis* species (Figure 1).

Figure 1**:** Prevalence of pathogenic bacteria isolated in Europe from bovine respiratory infections.



Mycoplasma strains isolation was technically optimised after the 2002 epidemiosurvey, thanks to better methodologies in sampling, transportation, storage and laboratory isolation, leading to better viability of strains. This allowed to increase the isolation rate from 4.4% in 2002 to 17% in 2004.

The rate of isolation results must be evaluated critically. Indeed, many laboratories are still technically unqualified to diagnose them (neither identification nor isolation of *Mycoplasma* strains). Only some laboratories in Europe are able to carry out an isolation of *Mycoplasma* strains (and even less are able to run MIC determination).

Moreover, more and more laboratories detect the presence of *Mycoplasma* strains with PCR method (more specific and without isolation of bacteria), directly on biological material.

According to all these technical problems observed no relevant prevalence could be determined for each country.

3.2. Marbofloxacin activity

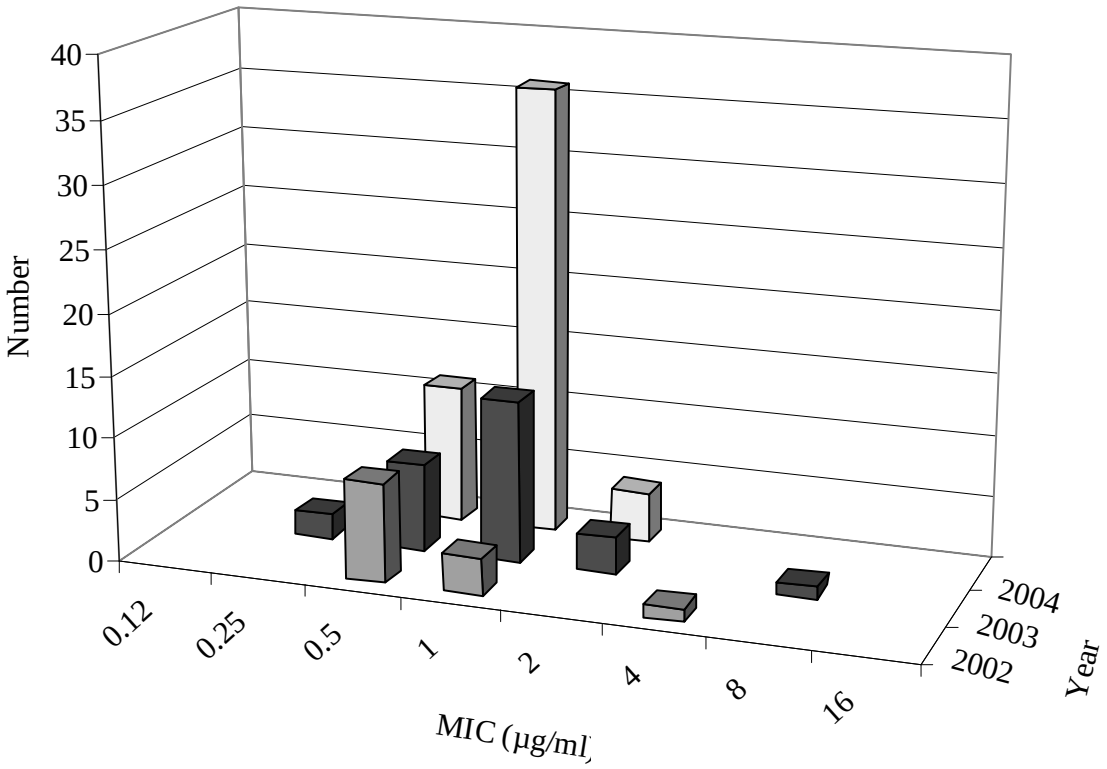
No change in susceptibility to marbofloxacin occurred during these three years (Table 1). The marbofloxacin MIC_{90} of *Mycoplasma bovis* strains was just below 1 µg/ml since 2002.
These results are similar to those from Belgium [3] against *M. bovis* strains obtained from strains isolated between November 1997 and December 2000.

Table 1**:** Marbofloxacin MIC distribution, MIC_{50}, MIC_{90} (in µg/ml) against *Mycoplasma spp.* strains isolated in Europe from clinical cases of bovine respiratory pathologies.

	Mycoplasma bovis				All *Mycoplasma* strains isolated			
MIC/year	2002	2003	2004	Total	2002	2003	2004	Total
0.25						2		2
0.5	8	5	10	23	8	7	11	26
1	3	13	33	49	3	13	36	52
2		2	3	5		3	4	7
4	1			1	1			1
8						1		1
Number	12	20	46	78	12	26	51	89
MIC_{50}	0.420	0.653	0.657	0.627	0.420	0.619	0.661	0.623
MIC_{90}	0.955	0.912	0.967	0.975	0.955	1.382	0.979	1.010

The marbofloxacin MICs distribution (**Figure 2**) of *Mycoplasma* strains was unimodal with a modal class at 1µg/ml.

Figure 2: Marbofloxacin MIC distribution against *Mycoplasma spp.* strains isolated from bovine respiratory infections.



4. CONCLUSION

This European survey has given partial epidemiological prevalence information on Mycoplasma strains isolated from bovine respiratory diseases.
Indeed, according to the results, the Mycoplasma prevalence was between 4.4% and 17% in Europe. This range was higher between countries. A variability was observed and due to different technical problems:

- Technical difficulties in Mycoplasma strains culture.
- Some laboratories in Europe can evaluate the presence of Mycoplasma strains using PCR method directly on biological material.
- Only a few microbiological laboratories in Europe can perform both, an isolation of Mycoplasma strains and a MIC determination.
- No antibiogram could be performed to determine the Mycoplasma susceptibility.

For this reason, the determination of a true prevalence of Mycoplasma strains involved in cattle pneumoniae in Europe is not possible (at this time) and consequently the MIC determination in order to establish a fully adapted treatment is very difficult.

Nevertheless, this work shown on the isolated Mycoplasma bovis strains that marbofloxacin remains an active anti-infectious agent with a MIC_{90} lower than 1 µg/ml.

5. ACKOWLEDGEMENTS

Vétoquinol S.A. survey has involved several people who in various ways have made this programme possible. We would like to express our gratitude to all those who have contributed and in particular to veterinarians and laboratories involved for their assistance.

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