# Prevalence of bovine viral diarrhoea in Scottish beef suckler herds

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**Abstract**

Bovine viral diarrhoea (BVD) is an endemic condition of cattle, inflicting substantial losses to both beef and dairy enterprises worldwide. Knowledge of the spread of BVD virus (BVDV) within the target population is crucial to the assessment of regional control options and the economic implications of infection in addition to the animal welfare issues. The goal of this study was to estimate the BVDV seroprevalence in young stock and from this derive the prevalence of active BVDV infection within the population of Scottish beef suckler herds. Data was collected from 301 beef suckler herds using a stratified random sampling design based on Scottish agricultural census data. Spot test serum samples were tested using BVDV antibody ELISAs.

Classification of herds with and without active BVDV infection was based on statistical analysis of within herd BVDV seroprevalence in young stock using Bayesian finite mixture modelling. This method accounted for within and between herd variability and allowed for classification error by the diagnostic tests. The observed sample data supports the discrimination of three distinct seroprevalence cohorts.

The study showed evidence for active BVDV infection on 16% of Scottish beef suckler herds (95 CI: 11.6, 19.7). Conversely, approximately two thirds (95 CI: 62.3, 74.2) of herds showed no evidence of recent exposure to BVDV. An additional 16% of herds (95 CI: 11.3, 21.3) have young stock with a BVDV seroprevalence between 26.3% and 38.5%. These results will provide support to the decision process on national BVD control.

## 1. Introduction

Bovine viral diarrhoea virus (BVDV) is a pathogen endemic to cattle populations worldwide. BVDV is a Pestivirus within the family *Flaviviridae*, related to classical swine fever virus and border disease virus (Vilcek et al., 2004). Infection can have detrimental effects on cattle health and welfare with severity depending on the viral strain causing infection and the background herd immunity (Duffell et al., 1986). Direct costs to the cattle industry in Great Britain (Bennett and Ijpelaar, 2005) and losses associated with BVD outbreaks in beef herds have been estimated to be significant (Gunn et al., 2004). In practice, the economic and welfare impact are likely to be underestimated, as endemic herd infection can be occult, thus remaining un-detected. Furthermore, due to the immunosuppressive characteristics of BVDV, losses might solely be attributed to co-infecting pathogens (Charleston et al., 2001). Overt clinical disease symptoms are mostly seen in young cattle where both the respiratory and enteric systems can be affected (Nettleton and Entrican, 1995). Epidemiologically and financially the infection of naïve breeding stock plays a major role. Depending on gestational stage of the dam at infection, embryonic death, abortion, congenital malformations, birth of weak calves and birth of persistently infected (PI) calves are likely outcomes (McGowan et al. (1993), Moennig and Liess (1995), Fray et al. (2000)). PI calves usually succumb to mucosal disease before reaching maturity, however they play the central role in disease propagation within and between herds, as they continually shed virus (Brownlie et al. (1984), Houe (1993), Bolin (1995)).

Reliable diagnostic tests at individual animal and herd levels as well as vac-

cines are available (Sandvik, 2005). Several European countries have established mandatory eradication programmes, whereas in other countries, including the UK, BVD control is voluntary through individual herd health plans and commercial health schemes (Synge et al. (1999), Lindberg and Alenius (1999), Valle et al. (2005)). Countries implementing systematic control measures have demonstrated that the economic impact of BVDV on the cattle industry can be reduced (Houe, 2003). However, the success and cost effectiveness of BVD control depends on agricultural structure, specifically livestock management, national disease prevalence and the protocol employed (Lindberg, 2004). A retrospective assessment of BVD eradication under Norwegian farming conditions indicates that the intervention was cost beneficial (Valle et al., 2005), whereas a prospective analysis of BVD eradication under farming conditions encountered in a French province came to the opposite conclusion (Dufour et al., 1999).

A reliable assessment of the current prevalence is an essential step to making an informed decision on appropriate control measures for BVD in a given country or region. In general, prevalence is estimated at herd and animal level using milk and blood samples (Sandvik, 2005); the financial resources available and the objective, e.g. detection of individual PI animals or of herds previously exposed to BVDV, determines the choice of sample and diagnostic test (Rüfenacht et al., 2000). When inferring infection status of a herd, it has to be considered that BVDV infection causes long-lasting immunity (Fredriksen et al., 1999) and therefore the interpretation of within herd seroprevalence depends on the age group sampled (Houe and Meyling, 1991). Furthermore, testing of calves with maternal antibodies, vaccinated animals or animals bought from sources of

unknown BVD status might mask the true infection status of a herd.

For the purpose of disease control, the detection of herds with active infection is the first step in stopping the spread of the causative agent. In the case of BVD, the detection of herds with one or more PI animals is of paramount importance; however at a national level the identification of individual PI animals and dams carrying PI calves requires large resources. A cost effective alternative is to employ spot test sampling, whereby a sentinel group is used as a proxy for a herd (Houe et al., 2006). Such an approach, however, relies on the use of an appropriate interpretation protocol for the spot test. In order to be independent of arbitrary cut-off values in the protocol we follow a novel approach to prevalence estimation: we do not ask how many farms show evidence for exposure to the virus (e.g. 1 or more seropositive animals), but rather ask how many distinct seroprevalence cohorts can be identified in the study population. The latter question is of greater epidemiological value as it makes better use of the data available and gives insight to how the agent is distributed in its host population.

The purpose of this study was to estimate the prevalence of farms with active BVDV infection within the population of Scottish beef suckler herds. To achieve this, a stratified random sampling design was employed to collect field data from young stock from Scottish beef suckler herds. Bayesian statistical inference techniques were utilised to develop and facilitate robust analysis.

## 2. Materials and Methods

### *2.1. Study Design*

A cross-sectional design targeting young stock was applied to 301 randomly selected farms. The survey was conducted between October 2006 and September 2007. A sampling frame of 2,145 suckler beef holdings with herds of at least 20 mature female cattle was randomly generated on the basis of 2004 census data from the Scottish Government containing a total of approximately 9,800 beef holdings. The actual number of study farms was based on statistical power calculations which took into account the precision of the desired prevalence estimate and the most effective use of available financial resources (see supplementary material for details).

The sample was cross-stratified by Scottish Animal Health Division and herd size proportionately to the number of beef holdings of different sizes containing each division (see Table 1).

A total of 552 farms were contacted, of which 137 eligible farms refused participation. Another 114 farms were not eligible: 82 could not be considered because they had fewer than 7 animals within the required age range; 15 had ceased farming beef; and 17 could not be contacted by telephone.

### *2.2. Data Collection*

Farm visits were arranged by five Scottish Agricultural College (SAC) Disease Surveillance Centres and one private veterinary practice; training and advice had been given to standardise both farm recruitment and sample collection. On study farms which ran more than one management group, each group was sampled.

For each management group between 7 and 10 animals in the age range 6 to 16 months inclusive were randomly blood sampled. During the farm visit investigators administered a standardised questionnaire on farm management practices and history of BVD including the BVD vaccination status of the herd (see supplementary material for details).

### *2.3. Diagnostics*

Blood samples were collected by venipuncture, maintained at ambient temperature and sent by overnight courier to the laboratory. A total of 2,984 blood samples from 293 farms were processed using a commercially available indirect ELISA test kit (Svanovir BVDV antibody ELISA, Svanova Biotech AB, Uppsala, Sweden). The tests were performed following the manufacturer's instructions. All sample and reference optical density (OD) values were corrected before interpretation by subtracting the OD values of the corresponding wells containing the control antigen. The antibody titre was interpreted on the basis of the percentage positivity (PP) by dividing the sample OD values by positive reference sample OD values. The cut off value was set to 14. A further 80 samples from 8 farms were tested with the Biobest BVD ELISA, following the in-house protocol. For this test, the percentage positivity of samples was calculated against an 8 point standard curve using a positive antiserum dilution series, with a value less than 9 specified as negative and a value greater than or equal to 9 specified as positive.

The impact of using two different BVD antibody ELISAs was assessed by comparison of results from study herds sharing similar characteristics (same region and member of the same health scheme) but tested by different tests.

### *2.4. Statistical Analysis*

The data comprised the observed number of animals sampled on each of 301 farms, and of those how many tested BVDV seropositive. Our objective was to infer the distribution of within herd seroprevalence across all young stock in beef suckler herds in Scotland. This distribution was used to define herds in which recent active infection with BVDV was very likely.

There were two distinct levels of sampling variability which had to be accounted for in order for such analysis to be robust: i) within herd sampling variability and; ii) between herd sampling variability. The former was to account for the effect of random selection of young stock from within a given herd, and the effect of random selection of herds from the population of all beef suckler herds. For example consider the 274 farms in which exactly 10 animals were sampled. In these 274 farms we could only observe a maximum of 11 distinct prevalence values (0 from 10, 1 from 10, ... , 10 from 10). Suppose that one farm had four antibody positive animals out of 10 and another had five antibody positive animals out of 10. The key statistical question was the extent to which an observed difference in the within herd seroprevalence supports the hypothesis that the within herd seroprevalence in young stock for each of these two farms is different. Clearly the greater the difference in observed seroprevalence the greater our statistical confidence that the two herds differed in their exposure level to BVDV. However it was not simply the distribution of within herd seroprevalence in our sample which we wished to estimate, but rather that in the population of all beef suckler herds in Scotland. A hierarchical Bayesian finite mixture modelling approach is ideally suited to this estimation problem.

Finite mixture modelling is a generic technique and was applied to our study data to identify the number of statistically distinct seroprevalence cohorts in the population of young stock in beef suckler herds. Finite mixture modelling is a widely used and well established statistical methodology (Diebolt and Robert, 1994), however, while ideally suited to many epidemiological studies, its use is not yet mainstream in veterinary epidemiology (though see Detilleux and Leroy (2000) and Boettcher et al. (2007) for its use in a different context in regard to mastitis in dairy cows). The supplementary material briefly describes some of the key statistical aspects of finite mixture modelling, including adjustment for clustering and test classification error.

Our finite mixture modelling approach incorporated sensitivity and specificity of the BVDV antibody ELISA into our analysis. For simplicity we did not discriminate between the two different ELISA used as the purpose of including test error was to ensure that the extra uncertainty due to misclassification was taken into account in our seroprevalence estimates rather than to provide a formal assessment of the accuracy of each test.

## 3. Results

A sample of the observed proportions of animals testing seropositive in each spot sample is shown in Figure 1 (note that for clarity the figure only includes the 274 herds where exactly 10 animals were sampled, a full breakdown of sample sizes and the corresponding figure for all 301 herds can be found in the supplementary material). The empirical distribution suggests that at least two distinct cohorts can be identified when considering the population of Scottish beef suckler herds

from which our data were drawn: a cohort with very high seroprevalence and a BVDV exposure free cohort. The formal statistical analysis concurs closely with this visual assessment. Our mixture model has optimal goodness of fit when $k$, the number of cohorts, equals 3 with the explicit inclusion of a disease free cohort, where the properties of these three components comprise (estimates given as 95% confidence intervals): a) 11.6% to 19.7% of herds have young stock with a seroprevalence between 91.9% and 99.8%; b) 11.3% to 21.3% of herds have young stock with a seroprevalence between 26.3% and 38.5%; c) 62.3% to 74.2% of herds have young stock which show no evidence of former exposure to BVDV. Details of the model selection process along with appropriate diagnostics can be found in the supplementary material. Table 1 and Figure 2 produce a detailed breakdown of seroprevalence estimates and the estimated proportion of herds with young stock in each cohort.

Twenty study herds from the Northern Isles were member of the same health scheme of which 12, respectively 8 were tested by the same test. Eleven of the twelve farms tested by Svanovir BVD ELISA and seven of the eight farms tested by Biobest BVD ELISA had only BVDV seronegative young stock. Hence, there was insufficient data to estimate sensitivity and specificity of the Biobest BVD ELISA, and assess whether the characteristics of the two tests are different.

Further by-products of the model fitting process are estimates of the characteristics for the diagnostic tests. However as only 8 farms were tested using the Biobest ELISA, and of these only a single test positive animal was reported there was insufficient data to estimate the accuracy of the Biobest ELISA. There was enough data to estimate the accuracy of the Svanovir ELISA, and we estimated

(using data from the 293 farms where this test was used) median values for $S_e$ and $S_p$ for this test of 96.3% and 98.8% respectively (see Table 2 and Figure 2 for more details). These estimates are constant for the three seroprevalence cohorts. For the purpose of this study the prevalence estimates given are corrected for the diagnostic test characteristics of the Svanovir ELISA.

## 4. Discussion

In a representative random sample of herds from Scottish census data, spot samples from young stock were tested for BVDV antibodies in 301 study herds. These samples were used to infer the distribution of within herd seroprevalence in young stock across Scottish beef suckler herds. In order to ensure representative national prevalence estimates the sample was stratified by location and size of study farms. We estimated that a median figure of 16% of Scottish beef suckler herds have undergone active BVDV infection in the months prior to testing and an average of 69% of herds have had no recent exposure to BVDV.

To help identify farms with evidence for recent active BVDV infection, the distribution of within herd seroprevalence in young stock was studied. In the current study using spot samples comprising 7-10 animals three distinct BVDV exposure cohorts were observed. Young stock with a within herd seroprevalence of 91.9% and 99.8% were most likely to have been in contact with a PI animal and therefore belong to herd with recent/ongoing active BVDV infection. The presence of only seronegative young stock, is characteristic of herds with no recent exposure to BVDV. Our statistical analysis suggests that the third, intermediate group, with a seroprevalence between 26.3% and 38.5% is a distinct

cohort but with more similarities to the exposure free cohort. Ad hoc evaluation of the distribution of spot samples results observed by Viltrop et al. (2002) and Rüfenacht et al. (2000) leads us to suspect that this intermediate exposure cohort can also be observed in other published surveys. One obvious explanation is that in these herds the spot sample provides only a snapshot for groups where BVDV is actively spreading among the calves and that a follow up sample would show high sero-prevalence. However, in this case one would expect this cohort to be less distinct with a mean value right between the two other cohorts. Alternatively this cohort might consist of herds where some pre-exposed, non PI calves have been bought in from exposed herds elsewhere or where individuals have been exposed to BVDV through lapses in biosecurity. The spread of infection within such herd must then remain under the epidemiologically critical threshold as transmission rates from transiently infected animals are low (Meyling et al., 1990). Even though maternal antibodies usually vanish after 6 months of age, anecdotal evidence suggests that in some animals passive immunity persists for longer. In any case, the role of this cohort in the spread of BVDV remains an important topic of further investigation.

In previous studies BVD prevalence has been estimated for various European countries. The methodology and target population of these studies differed substantially. For this reason it seem approbriate to quote prevalence estimates of similar studies, but not to make a direct comparison. A survey based on BVDV antibody titres in bulk milk samples concluded that 65% of 1,070 dairy herds in England and Wales were likely to have undergone recent BVDV infection (Paton et al., 1998). A Swiss prevalence study comprising 121 dairy farms using serum

samples from all animals on each study farm found PI animals on 15% of study farms (Rüfenacht et al., 2000). A study in Estonian cattle using spot test sampling observed a prevalence of herds potentially having PI animals of 46%, 16% and 18% for three consecutive time periods (Viltrop et al., 2002). The substantial change in prevalence observed in the later study leads us to stress that the results of the current study describe only a snapshot of the spread of an endemic disease which is likely to fluctuate over time.

Disease transmission depends on the livestock management system. In many countries so far the focus of BVD prevalence estimation has been on dairy herds. This study was designed to assess the spread of BVD in more extensively run cattle. Selection criteria included farm size and location. Vaccination protocols, however, were not considered in the selection process, as the goal of the study was to establish estimates representative of the majority of commercial beef suckler herds. Information on the vaccination regime of study herds was collected with one quarter of all farms routinely vaccinating the herd against BVDV. In the exposure free cohort 28% of farms vaccinated, in the intermediate cohort 26% with 24% in the cohort with the highest BVDV seroprevalence. A 3-sample test for equality of proportions was performed showing no statistical difference between the three cohorts (p-value = 0.83) (Newcombe, 1998). It was concluded that the influence of vaccination was not critical, but vaccination might have led to a slight overestimation of farms with active BVDV infection.

An inference for herd level exposure status based on a spot test result can only be valid where efficient transmission of virus within the tested management group can be assumed. For Scottish beef suckler herds, which have a large degree

of interaction between adult breeding animals and young stock, this assumption seems appropriate (Sowell et al., 1999). However, periods of closer contact would lead to faster circulation of BVDV, whereas extensive pasture management would cause a slower virus spread. Hence, the time of year when a herd was sampled might have influenced our reported outcome. The sampling period lasted for one year, with the majority of farms (275 farms) sampled between November 2006 and March 2007. This period closely corresponds to winter housing time for Scottish beef suckler herds. Given that seroconversion to BVDV can be detected within weeks (Fredriksen et al., 1999) and the majority of animals have been sampled during the period of close contact, we assume that the spot test results reliably reflected the recent BVDV exposure of the herd.

Test characteristics vary depending on the populations they are applied to, thus influencing appropriate interpretation of test results (Greiner and Gardner (2000a) Greiner and Gardner (2000b)). The Bayesian mixture model generated estimates of sensitivity and specificity for the Svanovir BVD ELISA, the diagnostic test used for most of the samples. On the Northern Isles 20 out of 21 study farms were member of a health scheme which employed the Biobest BVD ELISA. For the purpose of this study it had been planned that participating herds would all be tested with the Svanovir BVD ELISA. However, due to institutional inertia 8 herds were assayed by Biobest and a repeat sampling of these animals was not felt to be ethical because of management and animal welfare issues. Removing these 8 farms from the study would have been likely to introduce a bias in the prevalence estimates as these farms are members of a health scheme and therefore systematically more likely to have a low seroprevalence. However, including these

farms introduced a difficulty in interpretation, since the seroprevalence estimates were no longer interpretable as relating to a single test with specified properties. By comparing results from the Northern Isles where both tests have been used, no evidence could be found that the characteristics of the two diagnostic test would differ. Within this region, the selection of farms and sampling of the young stock was carried out by the same investigators. This is very important, as it seems appropriate to assume that between and within farm sampling variability accounts for the majority of the total sampling variability. The only drawback in assuming that the data generated by the two tests have the same provenance is that the high variability which should be associated with estimates related to the Biobest ELISA (due to the small sample) will not be fully accounted for in the model, so the confidence intervals will be slightly too narrow. However, since this relates to the contribution of only 8 farms out of the entire sample of 301, this effect should be negligible.

A robust estimate for the prevalence of active BVDV infection in Scottish beef suckler herds has been provided through serological spot sample testing of young stock. We believe that this was a cost effective approach and we discuss the effects of herd management system, vaccination and time of year of sampling in relation to our estimate. We also present the novel use of the Bayesian finite mixture modelling approach to evaluation of the disease prevalence results. The statistical approach employed allowed identification of a cohort of farms where active BVDV infection was most likely and also herds where there was little likelihood of active infection. This same method allowed us to calculate the test characteristics of sensitivity and specificity and so increase our confidence in our

estimate. Robust prevalence estimation is a necessary step in the process of defining appropriate control measures and establishment of regional costs associated with BVDV infection. The high percentage of herds without recent BVDV infection is very encouraging from an animal welfare point of view and provides a firm basis for further exploration of a strategies for national BVD control.

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Table 1: Cross-stratification of sample comprising 301 Scottish beef suckler herds by location and farm size

| | **20-44 animals** | **45-85 animals** | **over 85 animals** | **Total** |
|---|---|---|---|---|
| **Central Scotland** | 23 | 22 | 16 | 61 |
| **Northeast Scotland** | 16 | 19 | 21 | 56 |
| **Northern Islands** | 9 | 4 | 8 | 21 |
| **Scottish Highlands** | 12 | 16 | 19 | 39 |
| **Southeast Scotland** | 15 | 18 | 21 | 54 |
| **Southwest Scotland** | 20 | 23 | 27 | 70 |
| **Total** | 95 | 101 | 105 | 301 |

Table 2: Mixture model parameter estimates of BVDV seroprevalence in young stock. The estimated proportion of herds in each seroprevalence cohort are denoted by $\pi_1$, $\pi_2$ and $\pi_3$; seroprevalence in each cohort by $\mu_1, \mu_2$, and $\mu_3$; mean serum ELISA sensitivity by $S_e$ and specificity by $S_p$. The numerical estimates are identical (to one decimal place) when we consider either all 301 farms or the subset which was tested by the Svanovir BVDV Ab ELISA.

| **Parameter** | **Median** | **95% C.I.** |
|---|---|---|
| $\pi_1$ | 68.6 | (62.3,74.2) |
| $\pi_2$ | 15.8 | (11.3,21.3) |
| $\pi_3$ | 15.5 | (11.6,19.7) |
| $\mu_1$ | 0 | - |
| $\mu_2$ | 32.2 | (26.3,38.5) |
| $\mu_3$ | 96.4 | (91.9,99.8) |
| $S_e$ | 96.3 | (91.9,99.8) |
| $S_p$ | 98.8 | (98.0,99.3) |

Figure 1: Observed frequency distribution of BVDV seroprevalence among young stock for all herds with 10 animals sampled (274 herds in total).

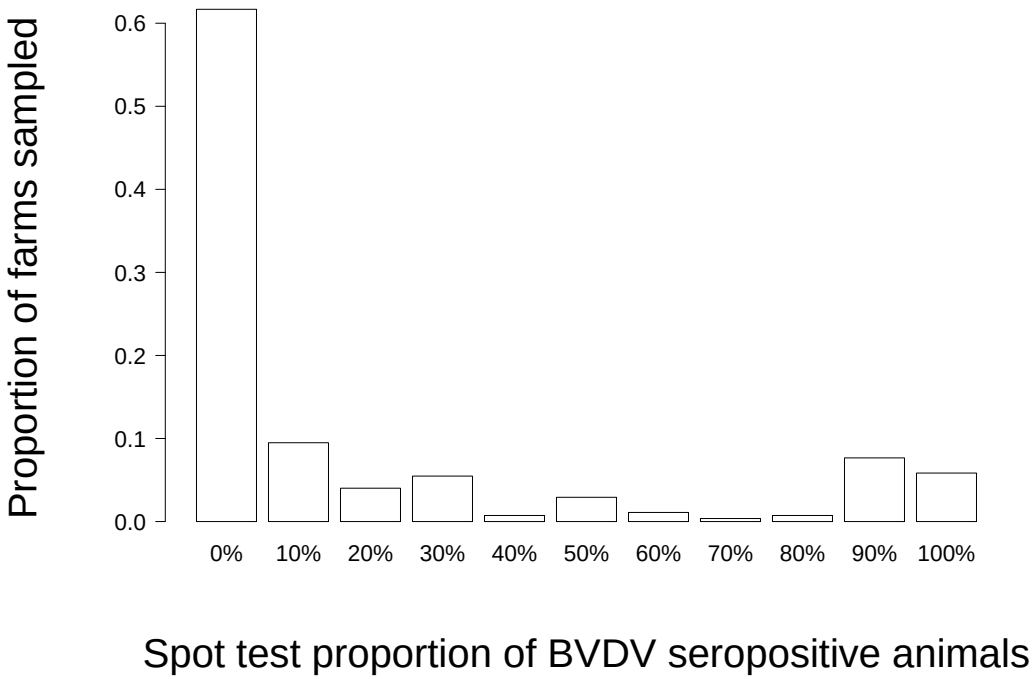

Figure 2: Posterior density estimates of mixture model parameters. (a) The estimated proportion of herds in each cohort, $\pi_1$ - exposure free, $\pi_2$ - medium seroprevalence, and $\pi_3$ - high seroprevalence; (b) Seroprevalence in each cohort, $\mu_2$ - Cohort 2, and $\mu_3$ - Cohort 3; (c) Serum ELISA accuracy.