Bayesian assessment of two competitive enzyme-linked immunosorbent assays for the detection of bovine viral diarrhoea virus antibodies in bovine sera

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C. Saegerman [(1)*], C. Quinet [(2)], G. Czaplicki [(2)] & F. Dal Pozzo [(1)]

(1) Research Unit in Epidemiology and Risk Analysis applied to Veterinary Sciences (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine, University of Liege, Liege, Belgium

(2) Association Régionale de Santé et d'Identification Animales (ARSIA), Animal Health Department, Ciney, Belgium

*Corresponding author: claude.saegerman@uliege.be

Summary

Infections due to bovine viral diarrhoea virus (BVDV) are endemic in most cattle-producing countries throughout the world and bovine viral diarrhoea is considered as a transboundary disease. The key elements of a BVDV control programme are vaccination, biosecurity, elimination of persistently infected (PI) animals and surveillance. The aim of this study was to assess the sensitivity (Se) and the specificity (Sp) of two commercial competitive enzyme-linked immunosorbent assays (ELISAs) based on selected immunodominant BVDV proteins, respectively the non-structural protein NS3 (p80) and the recombinant envelope glycoprotein E0 (Erns). Both tests were used on individual serum samples from randomly sampled young bovines in southern Belgium in order to detect specific BVDV antibodies. The Se and Sp were assessed using a Bayesian approach and were estimated, respectively, at 97.2% (with 95% credibility interval [Cr I]): 95.1–99.8) and 98.7% (95% Cr I: 96.6–99.9) for the first test and 95.8%

(95% Cr I: 91.1–99.7) and 96.1% (95% Cr I: 95.1–97.7) for the second test. The results obtained with the two tests were not significantly different. In addition, using both ELISAs, the current BVDV exposure among young bovines in southern Belgium was estimated at 23.3% (95% Cr I: 20.6–26.2). Combining virological testing of all newborns to detect PI animals with regular serological testing of young stock using ELISAs is recommended in the surveillance of BVDV.

Keywords

Assessment – Bayesian framework – Bovine viral diarrhoea virus – Cattle – Diagnosis – ELISA – Enzyme-linked immunosorbent assay – Sensitivity – Specificity.

Introduction

Infections with bovine viral diarrhoea virus (BVDV) are endemic in most cattle-producing countries throughout the world (1). The pathogenesis of BVDV is complex, with pre-, mid- and post-gestation infections leading to different outcomes. Infection of the dam during pregnancy can result in foetal infection, which may lead to embryonic death, teratogenic effects, or the birth of persistently infected (PI) calves. These PI animals shed BVDV throughout their lives and are of primary importance in the maintenance of the virus in the environment or on the farm (2).

Bovine viral diarrhoea virus infection causes financial losses estimated at between EUR 21 and 135 per cow, mostly associated with reproductive disorders and the occurrence of PI animals (3). The virus is endemically present in numerous countries in addition to Belgium. Consequently, a number of BVDV mitigation and eradication schemes are implemented around the world. These schemes are based on four major elements: vaccination, biosecurity, the elimination of PI animals and surveillance. Surveillance is mainly based on the verification of the absence of BVDV circulation at the individual level or the herd level using antigen and/or antibody enzyme-linked immunosorbent assays (ELISAs) (1, 4, 5).

Since bovine virus diarrhoea (BVD) was recognised as a unique disease complex, many different diagnostic methods have been used to detect BVDV itself, or the immune response to BVDV (6). The reference test for detection of antibodies against BVDV is the virus neutralisation test (VNT) (7). This is a sensitive and specific assay but is cell culture dependent, and labour intensive in comparison with ELISA. Therefore, the latter is usually preferred when a large sample throughput is required (8). Conventional ELISAs based on BVDV-infected cells have disadvantages because BVDV produces low levels of proteins in tissue culture and it is difficult to purify because of its cell association. Recombinant antigens provide an alternative for the reliable detection of BVDV antibodies in bovine sera (9). The most immunogenic proteins of BVDV are the envelope glycoproteins E0 (Erns) and E2, and the non-structural protein NS3 (previously named p80) (9). However, the E0 and NS3 proteins are more conserved among BVDV strains and the NS3 protein is essential for viral replication (in wild-type BVDV or modified-live BVDV vaccines) (10, 11). In addition, the agreement between ELISAs for the detection of pestivirus antibodies directed against conserved epitopes on NS3, E0 or E2 and the VNT [the World Organisation for Animal Health (OIE) reference test (12)] is considered to be excellent (6, 13, 14, 15).

The aim of this study was to assess the sensitivity (Se) and the specificity (Sp) of two commercial competitive ELISAs using a Bayesian approach. Individual serum samples from cattle in southern Belgium were used to detect antibodies produced against the recombinant envelope glycoprotein E0 and the non-structural protein NS3. In addition, the current seroprevalence of BVDV in southern Belgium was estimated among young bovines.

Materials and methods

Study area and sample collection

The study was conducted in the southern part of Belgium primarily for assessment of the Se and Sp of the ELISAs, in January and February 2014. This area is characterised by the presence of dairy and beef cattle (n = 11,160 herds). A cross-sectional study was performed using

serum samples randomly collected by herd veterinarians during a Belgian monitoring programme. This monitoring programme aimed to detect the circulation of different pathogens in bovines aged between 6 and 12 months (regardless of gender and breed) in 450 randomly selected herds. This age category was selected to avoid the detection of maternal antibodies. A maximum of ten randomly selected animals was sampled per herd in this specific age category. In some herds, no animals between six and 12 months of age were present at the time of sampling. In southern Belgium, the total number of bovines included in this age category is 140,000. In order to evaluate the current exposure to BVDV in young animals in this area and with an expected BVDV seroprevalence of 35% (due to natural infection and/or vaccination), an accepted error of 3% and a level of confidence of 95% (16, 17), the necessary sample size was calculated to be $n = 972$. In total, 988 blood sera derived from 164 herds in southern Belgium were randomly sampled and used in the current study.

Competitive enzyme-linked immunosorbent assays

Two commercial competitive ELISAs were used according to the producer's instructions (Bio-X Diagnostic, Rochefort, Belgium). In the first ELISA, 96-well microtitration plates were coated with BVDV NS3 non-structural protein (BIO K 230, Bio-X Diagnostic, Rochefort, Belgium). In the second test, 96-well microtitration plates were coated with the recombinant BVDV E0 protein (BIO K 283, Bio-X Diagnostic, Rochefort, Belgium).

The optical densities (ODs) of the positive and negative control sera (OD pos and OD neg) and those of all the tested samples (OD samples) were measured. The percentage of inhibition for each tested sample and the positive serum was calculated by means of the following formulas:

$$\% \text{ inhibition sample} = [(\text{OD neg} - \text{OD sample})/\text{OD neg}] \times 100 \quad \text{(Equation 1)}$$

$$\% \text{ inhibition positive} = [(\text{OD neg} - \text{OD pos})/\text{OD neg}] \times 100 \quad \text{(Equation 2)}$$

According to the manufacturer, each test was validated if the OD neg – OD pos was > 0.7. The cut-off value was fixed as a % inhibition positive of > 50%.

Estimation of true prevalence, test sensitivity and test specificity

A Bayesian approach was used to evaluate the performance of the NS3- and E0-competitive ELISAs by estimating Se and Sp as previously described (18, 19, 20). The results obtained for a given animal using the two tests were considered conditionally dependent. The Bayesian model was developed by taking into account this correlation in exposed animals (positive for BVDV-specific antibodies) as well as in unexposed animals (negative for BVDV-specific antibodies) (20). Furthermore, in the applied Bayesian model, field data (obtained from the results of both ELISAs on the collected bovine sera) and prior information obtained from the literature were included. In particular, the following available prior information was used: BVDV seroprevalence in Belgium (0.20–0.40) (16), Se and Sp of the NS3-competitive ELISA [Se: (0.95–1); Sp: (0.95–1)], Se and Sp of the E0-competitive ELISA [Se: (0.60–1); Sp: (0.95–1)] (2, 9, 21), with covariance as previously described (19). The model was run in Winbugs® (22) (see Appendix 1). Additional calculations during the Bayesian analysis were done in R software/environment (R-3.0.1, R Foundation for Statistical Computing, http://www.r-project.org/) (23). Three parameters were monitored during the analysis: the deviance information criterion (DIC), the effective number of estimated parameters (p_D) and the Bayesian *P*-value. As previously described (20), the DIC and the Bayesian *P*-value were used to check conflicts between prior information and current ELISA results. The impact of the constraints was assessed using the p_D of the model. The model used three parallel Markov chains, with a burn-in of 10,000 iterations and an additional 90,000 iterations to obtain the posterior distributions. In order to explore the convergence of the model, trace plots were simultaneously combined with autocorrelation plots. If the trace plot showed good mixing and the autocorrelation plot showed very little or no correlation among samples, convergence could be

claimed. In situations where autocorrelations were still high after the first few lags in the chains, thinning was applied. The thinning coefficient was determined by the number of lags at which the autocorrelations significantly dropped to zero. Additionally, the Brooks–Gelman–Rubin (BGR) statistical test for convergence was used. A good fit of the model was shown by a Bayesian *P*-value around 0.5 and going towards zero under strict constraints (18, 20).

Assessment of agreement between the tests

A concordance analysis was performed in order to assess the agreement of the two tests. The level of agreement was expressed in terms of indices of positive and negative agreement (24), respectively the observed agreement proportion for positive and negative test results. Confidence intervals were calculated according to the method described by Graham and Bull (25). Calculations of the different parameters were done using R. Using a two-by-two contingency table (Table I), the indices of positive agreement (P_{pos}) and negative agreement (P_{neg}) were, respectively:

$$p_{pos} = \frac{2a}{2a+b+c}$$

(Equation 3)

and $$p_{neg} = \frac{2d}{2d+b+c}$$

(Equation 4)

where P_{pos} and P_{neg} are, respectively, the indices of positive agreement and negative agreement (24); *a*, *b*, *c* and *d* are given in Table I.

Please insert Table I here

Prior sensitivity analysis

Using the Bayesian model with conditional dependence between tests, the parameter estimates were found to be varying together with the prior distributions (19). Therefore, in order to assess the influence of

the proposed prior distributions on the estimated parameters, a sensitivity analysis was performed using non-informative priors (19, 26). In addition, for each set of alternative prior distributions considered for the parameters, the model was run with the same number of chains and similar diagnostics were performed.

Results

Serological results

The two-by-two contingency table (Table IA) cross-classified the test results of the 988 samples based on both the NS3-competitive ELISA (test 1, T1) and the E0-competitive ELISA (test 2, T2), resulting in the following classes: a = 221, b = 29, c = 11 and d = 727 (Table IB). A total of 232 (23.5%) sera tested positive for T1 whereas 250 (25.3%) were found positive for T2. Both tests gave the same results on 948 sera (96.0%).

The general tendency of the separation of BVDV exposed and unexposed animals detected by each competitive ELISA was determined by the frequency histograms (Figs 1A and 1B) and the corresponding kernel densities (Figs 1C and 1D). Therefore, the two subpopulations of BVDV exposed and unexposed animals were well separated by both competitive ELISAs, considering the cut-off of 50% of inhibition. However, the subpopulations were more clearly separated by the E0-blocking ELISA (Figs 1B and 1D).

Please insert Figure 1 here

Indices of agreement between tests

The cross-classified test results (a = 221, b = 29, c = 11 and d = 727) were used to calculate the indices of agreement between the assays. The NS3- and E0-competitive ELISAs gave the same results on 91.7% of the positive results (P_{pos}), whereas the agreement on negative results (P_{neg}) was estimated to be 97.3%. The 95% confidence intervals ranged from 88.9% to 94.0% and 96.4% to 98.1%, respectively, for P_{pos} and P_{neg}.

Estimated true prevalence, test sensitivity and test specificity

Trace plots suggested that the chains were poorly mixing and the autocorrelation plots indicated that significant autocorrelations were present up to lag 30. Upon thinning with a thinning coefficient of 30, the model appeared to converge because the chains were properly mixing and the autocorrelations dropped to zero. The BGR plots corroborated these findings. The estimated Bayesian *P*-value of our model, 0.478, indicated no particular problems with model fit. The p_D estimated from the multinomial probabilities in R was 2.43 and the DIC was 21.62. The estimated p_D and DIC based on our model (2.66 and 21.85, respectively) were quite close to the optimal values. The estimated values of the sensitivity and specificity for both NS3- and E0-competitive ELISAs are summarised in Table II.

Please insert Table II here

As the outcome, the model also estimated an overall true seroprevalence of BVDV of 23.3% (95% credibility interval [Cr I]: 20.6–26.2). The estimated correlation between the two tests within the non-infected population was 0.25 (95% Cr I: –0.0026–0.59) and for the positive results it was 0.30 (95% Cr I: –0.01–0.77). The significant correlations of 0.25 and 0.30 provide evidence that the outcomes of the two tests are correlated for both non-infected and infected animals.

Sensitivity analysis

A sensitivity analysis was performed using several priors. For each model, validity criteria were assessed (the Bayesian *P*-value, the p_D value, the DIC value and the BGR statistic). The use of non-informative priors for all parameters led to a non-identifiable model due to the absence of convergence. The two extreme selected sets of priors and their corresponding posterior estimates are summarised in Table II. Beside the two extreme sets of priors shown in Table II, other prior distributions were applied and resulted in the absence of an interesting model (data not shown). These models included the following combinations of priors:

– non-informative prior for the seroprevalence and informative priors for the sensitivity (Se) and specificity (Sp) of NS3- and E0-competitive ELISAs

– non-informative priors for the Se and Sp of the NS3-competitive ELISA and informative priors for the seroprevalence and the Se and Sp of the E0-competitive ELISA

– non-informative priors for the Se and Sp of the E0-competitive ELISA and informative priors for the seroprevalence and the Se and Sp of the NS3-competitive ELISA.

Discussion

The primary objective of this study was to evaluate the diagnostic characteristics of two commercial competitive ELISAs based on the NS3 non-structural protein and the recombinant E0 protein of BVDV using a Bayesian approach. In addition, the results allowed estimation of the seroprevalence of BVDV in young bovines in southern Belgium.

In summary, the Se and Sp were estimated, respectively, as 97.2% (95% Cr I: 95.1–99.8) and 98.7% (95% Cr I: 96.6–99.9) for the first test (based on the NS3 non-structural protein) and 95.8% (95% Cr I: 91.1–99.7) and 96.1% (95% Cr I: 95.1–97.7) for the second test (based on the E0 protein). The results obtained with the two tests were not significantly different. In addition, using both ELISAs, the current BVDV exposure among young bovines in southern Belgium was estimated at 23.3% (95% Cr I: 20.6–26.2).

By definition, estimation of sensitivity and specificity of a diagnostic test requires knowledge of the true disease status of the animals on which the test is applied. This status is given by a ‘gold standard’ test. In the absence of a ‘gold standard’ test, a Bayesian approach is helpful to estimate test sensitivity, specificity and prevalence, as has been done for several diseases (19, 20, 27, 28, 29, 30). This is also the case for estimation of the BVDV prevalence (29). Moreover, the World

Assembly of Delegates of the OIE recently added the Bayesian approach to the OIE *Terrestrial Manual* as a tool to estimate the sensitivity and specificity of diagnostic tests (12).

Prior available information on Belgian BVDV prevalence (16) and on the sensitivity and specificity of the NS3-competitive ELISA (2, 21) were included in our estimation process. For the sensitivity and specificity of the E0-competitive ELISA, priors derived from a surrogate dataset were used because information was available only for an indirect ELISA (9). Prior knowledge may reduce the number of parameters to be assessed by the model. However, the posterior estimates resulting from the Bayesian analysis will be a combination of the data resulting from both ELISAs and the prior knowledge (30). It has been found that the prior information may influence posterior estimates in the course of the analysis (20). Indeed, in this study, priors were represented by ranges of values (test characteristics) obtained by merging estimates produced in the course of several epidemiological studies (using different conditions and methodologies), and they may not necessarily be relevant to the current situation. Nevertheless, the results obtained from the sensitivity analysis influenced the posterior estimates more than the prior information. Ultimately, the choice of priors allowed robust results to be obtained. This robustness is important for trade purposes, especially because BVD is a transboundary disease. In situations where prior information on sensitivity and specificity is lacking, it is recommended to use a sufficiently representative sample to accurately estimate the true prevalence and test characteristics. In addition, a sensitivity analysis should be systematically performed.

In addition to the main scope of this paper, the model was used to estimate the true seroprevalence of BVDV in southern Belgium. A relatively low true prevalence (23.3%) was found when compared with the previously reported true prevalence of 32.9% estimated by Sarrazin *et al.* (16) in Belgium. However, comparisons of these results have some limitations related to differences in sampling designs and strategies, and in the ELISAs used. Indeed, several differences exist between the present and the past (16) studies: the study period (2014

in this study *versus* 2009–2010 in the past study), the area (southern Belgium for this study and Belgium as a whole for the past study), the ELISA (different manufacturers) and the method used for the estimation of prevalence [Bayesian approach based on two ELISAs in the present study *versus* estimation of true prevalence based on the apparent prevalence and the Se and Sp claimed by the producer and using the Rogan and Gladen formulae (31), for the past study]. In addition, in both studies, the true seroprevalence was estimated without differentiating between vaccinated (modified-live vaccines) and naturally infected animals.

The posterior estimates given by the Bayesian model are based on serological test results of the NS3- and E0-competitive ELISAs. Considering the estimates of test characteristics (Table II), the best combination of sensitivity and specificity was obtained for the NS3-competitive ELISA, with a Youden index (Se + Sp – 1) of 0.96, followed by the E0-competitive ELISA with a Youden index of 0.92 (32). The index for both NS3- and E0-competitive ELISAs is similar to the index (0.94) of another commercially available BVDV ELISA (2).

A good level of agreement between the two diagnostic tests was obtained when considering positive results obtained by both ELISAs (97.3%). Similarly, a significant correlation between the two tests was found for negative results (91.7%). This could be related to the high specificity of both tests (estimated during the Bayesian analysis for both tests) (Table II) and the good separation between the two subpopulations (BVDV exposed and unexposed animals) obtained using both tests (especially the E0-competitive ELISA) (Fig. 1). This separation seems to be more pronounced than for another commercially available ELISA (2). A good level of agreement has been also reported for tests with high specificity in other diseases (33).

The major immune proteins of BVDV are the envelope glycoproteins E0 and E2 and the non-structural protein NS3 (9). The agreement between ELISAs using these immunogenic proteins and the VNT is considered excellent in the literature (6), in particular for the E2

protein (34). The E0 and NS3 proteins are more conserved among BVDV strains (10, 11), allowing in some cases the detection of antibodies that cannot be measured by the VNT with BVDV type 1 but can be detected easily with western blot (9). A possible explanation for this observation is that the VNT only detects antibodies with neutralising activity, but these antibodies do not represent the total population of anti-BVDV antibodies (9). These results highlight that the VNT should not be considered as a gold standard (i.e. a perfect test with Se and Sp equal to 100%) but as a reference test (with high Se and Sp but not equal to 100%), and ELISAs based on E0 or NS3 could represent a promising surveillance BVDV diagnostic tool to minimise false negative serological reactions (9). Indeed, the use of ELISAs (NS3 and E0) that have literature evidence of excellent agreement with the VNT as reference tests certainly contributed to the good estimation of the true prevalence of BVDV in this study. In addition, the NS3 protein is essential for BVDV replication (35) and it is consequently expressed by wild-type as well as live attenuated vaccine virus. Recently, among three commercially available inactivated BVDV vaccines, one did not interfere with the detection of anti-NS3 antibodies in bulk tank milk of vaccinated herds, showing that a putative differentiating infected from vaccinated animals (DIVA) strategy could be implemented (36). In this context, the use of both E0- and NS3-competitive ELISAs could allow discrimination of BVDV free, naturally infected and vaccinated herds.

Importantly, virological testing of all newborn calves is a valid method for rapidly detecting PI animals, but not for monitoring the BVDV-free status of a herd (37). BVDV surveillance should rely on the combination of virological tests performed systematically on newborn calves and serological tests performed at the herd level (38).

In summary, the results of this study support the use of an ELISA based on the E0 or NS3 protein as an efficient diagnostic tool to be applied in the surveillance of BVDV. Using the two ELISAs in parallel allowed estimation of a true prevalence of BVDV of 23.3% in young bovines in southern Belgium. However, because the use of a

live attenuated vaccine cannot be ruled out, the estimated true seroprevalence combines naturally infected as well as vaccinated animals.

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Table I
Contingency table showing results for NS3-blocking ELISA (T1) and E0-blocking ELISA (T2)

A) Codification

		T1		
		Pos	Neg	Total
T2	Pos[a]	*a*	*b*	*a+b*
	Neg[b]	*c*	*d*	*c+d*
	Total	*a+c*	*b+d*	*n*[c]

[a] Positive result
[b] Negative result
[c] Total number of samples tested by the two diagnostic tests (*a+b+c+d*)

B) Observation

		T1		
		Pos	Neg	Total
T2	Pos	221	29	250
	Neg	11	727	738
	Total	232	756	988

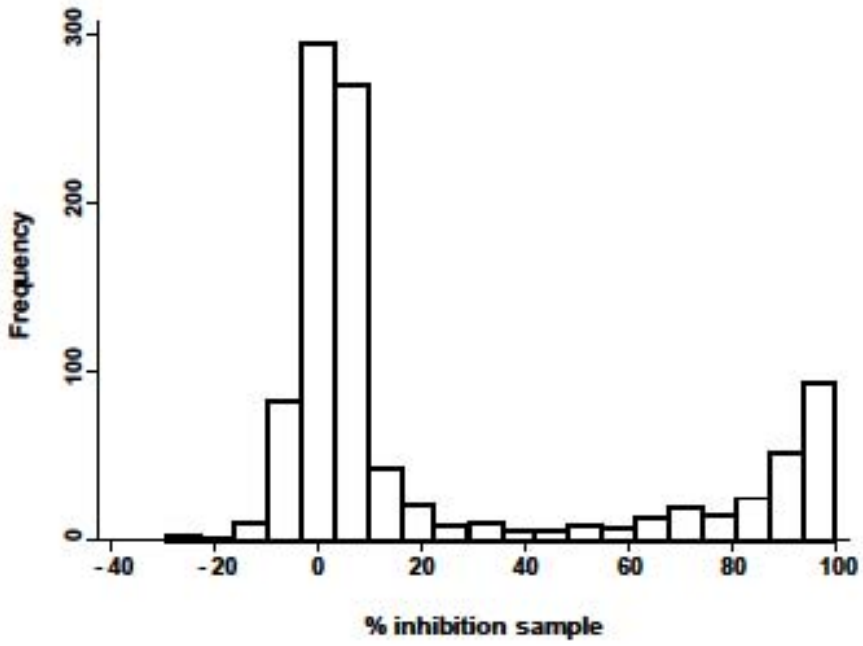
Table II
Sensitivity and specificity estimates for NS3-blocking ELISA and recombinant E0-blocking ELISA using a Bayesian approach

Scenario	ELISA	Parameter	Uniform prior	Posterior estimates (Cr. I[a])
Informative priors	NS3-blocking	Prevalence	[0.20,0.40]	0.233 (0.206–-0.262)
		Se[b]	[0.95,1]	0.972 (0.951–0.998)
		Sp[c]	[0.95,1]	0.987 (0.966–0.999)
	E0-blocking	Se	[0.60,1]	0.958 (0.911–0.997)
		Sp	[0.95,1]	0.961 (0.951–-0.977)
Non informative priors	NS3-blocking	Prevalence	[0,1]	0.243 (0.003–0.988)
		Se	[0,1]	0.557 (0.058–0.975)
		Sp	[0,1]	0.813 (0.381–0.993)
	E0-blocking	Se	[0,1]	0.625 (0.106–0.988)
		Sp	[0,1]	0.790 (0.266–0.987)

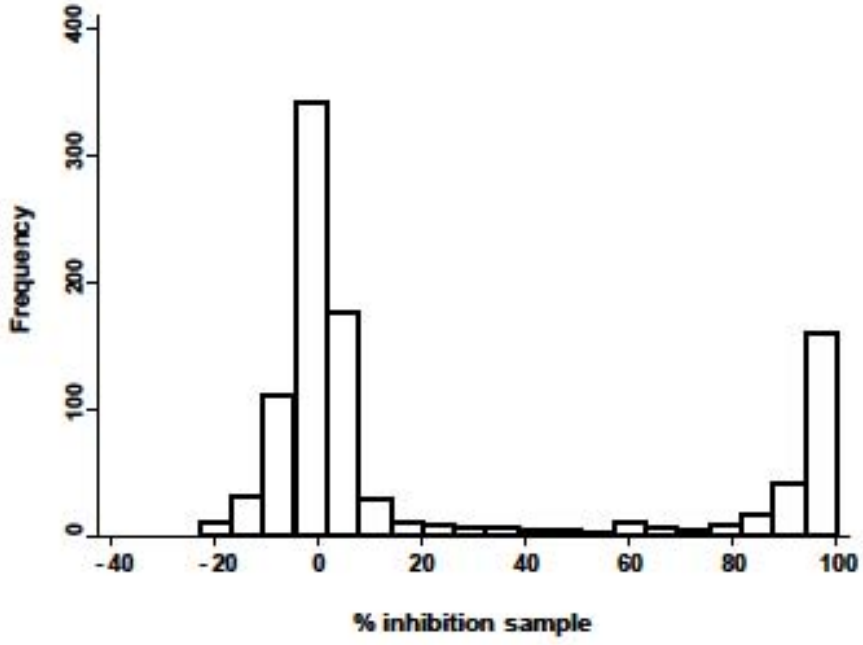
[a] Credibility interval
[b] Sensitivity
[c] Specificity
ELISA: enzyme-linked immunosorbent assay
Priors were estimated according to available literature information. Previous study estimated the prevalence of bovine viral diarrhoea virus (BVDV) exposure to be 33% (8). For sensitivity and specificity of the NS3-blocking ELISA, priors were available (2, 21). For sensitivity and specificity of the E0-blocking ELISA, priors were obtained from a surrogate dataset because only information based on an E0-indirect ELISA was available (9).

Figure 1.

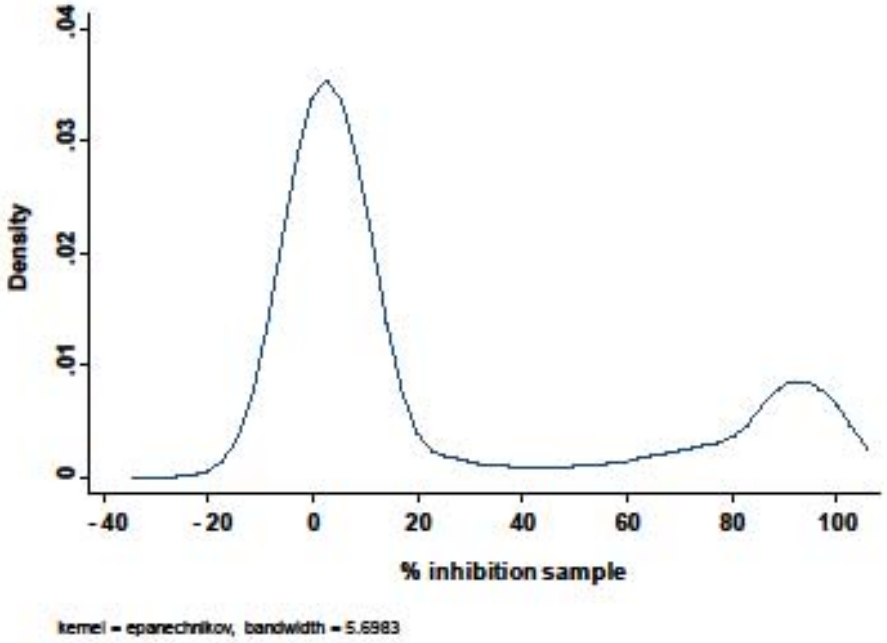
[A]


Frequency
0
100
200
300
-40
-20
0
20
40
60
80
100
% inhibition sample

[B]


Frequency
0
100
200
300
400
-40
-20
0
20
40
60
80
100
% inhibition sample

[C]


Density
0
.01
.02
.03
.04
-40
-20
0
20
40
60
80
100
% inhibition sample
kernel = epanechnikov, bandwidth = 5.6983

[D]

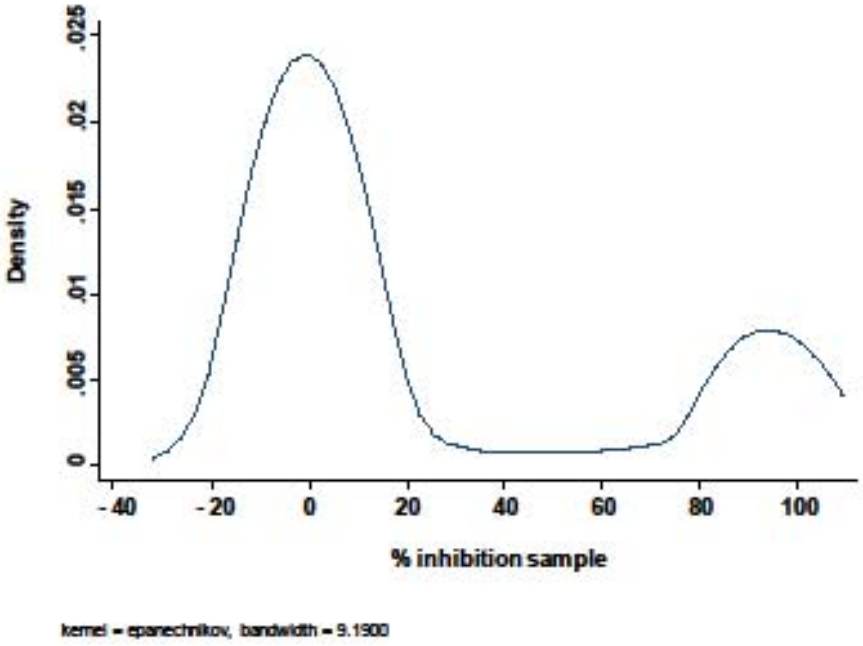

Density
0
.005
.01
.015
.02
.025
-40
-20
0
20
40
60
80
100
% inhibition sample
kernel = epanechnikov, bandwidth = 9.1900

Fig. 1

Frequency histogram of percentage of inhibition for each blocking ELISA test ([A] and [B]) and corresponding kernel density estimation ([C] and [D])

ELISA: enzyme-linked immunosorbent assay
[A] and [B]: frequency histograms of percentage of inhibition for NS3-blocking ELISA and recombinant E0-blocking ELISA, respectively
[C] and [D]: kernel density estimation for NS3-blocking ELISA and recombinant E0-blocking ELISA, respectively
Percentage of inhibition on the *x*-axis line should be read as –40 to 100%

Appendix 1

WinBugs code for estimating prevalence and test characteristics of NS3-blocking ELISA and E0-blocking ELISA

ELISA: enzyme-linked immunosorbent assay

```
model
{
r[1:4] ~ dmulti(p[1:4], n)
p[1] <- pi*(SeElisaNS3*SeElisaE0+covDp) + (1-pi)*((1-SpElisaNS3)*(1-
SpElisaE0)+covDn)
p[2] <- pi*(SeElisaNS3*(1-SeElisaE0)-covDp) + (1-pi)*((1-SpElisaNS3)*SpElisaE0-
covDn)
p[3] <- pi*((1-SeElisaNS3)*SeElisaE0-covDp) + (1-pi)*(SpElisaNS3*(1-SpElisaE0)-
covDn)
p[4] <- pi*((1-SeElisaNS3)*(1-SeElisaE0)+covDp) + (1-
pi)*(SpElisaNS3*SpElisaE0+covDn)
ls <- (SeElisaNS3-1)*(1-SeElisaE0)
us <- min(SeElisaNS3,SeElisaE0) – SeElisaNS3*SeElisaE0
lc <- (SpElisaNS3-1)*(1-SpElisaE0)
uc <- min(SpElisaNS3,SpElisaE0) – SpElisaNS3*SpElisaE0
pi ~ dunif(0.2,0.4)
SeElisaNS3 ~ dunif(0.95,1)
SpElisaNS3 ~ dunif(0.95,1)
SeElisaE0 ~ dunif(0.60,1)
SpElisaE0 ~dunif(0.95,1)
covDn ~ dunif(lc, uc)
covDp ~ dunif(ls, us)
rhoD <- covDp / sqrt(SeElisaNS3*(1-SeElisaNS3)*SeElisaE0*(1-SeElisaE0))
rhoDc <- covDn / sqrt(SpElisaNS3*(1-SpElisaNS3)*SpElisaE0*(1-SpElisaE0))
r2[1:4] ~ dmulti(p[1:4],n)
for (i in 1:4)
{
d[i] <- r[i]*log(max(r[i],1)/(p[i]*n))
d2[i] <- r2[i]*log(max(r2[i],1)/(p[i]*n))
}
bayesp <- step(sum(d[]) – sum(d2[]))
}
list(r=c(221,11,29,727), n=988)
```