Bovine herpesvirus 1: within-herd seroprevalence and antibody levels in bulk-tank milk

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Summary

The aim of the present study was to establish a relationship between the results obtained with the enzyme-linked immunosorbent assay (ELISA) technique for antibodies (against bovine herpesvirus 1) in serum and those in milk at the herd level. For this purpose, 275 samples of bulk-tank milk were analysed with glycoprotein E (gE)

antibody ELISA and 207 more were analysed with glycoprotein B (gB) antibody ELISA (482 in total). All of these samples came from dairy herds whose seroprevalence was also evaluated. The results of this study were then used to analyse the sensitivity of the bulk-tank-milk test in detecting herds with a high risk of active infection (>60% seroprevalence) and its specificity in detecting those with few (<20%) or no seropositive animals. In regard to the reference test (results in blood serum), the sensitivity of the bulk-tank-milk test in detecting herds with >60% seropositive animals was 100% for both gE and gB ELISAs. The specificity figures, for gE and gB ELISAs, respectively, were 88.4% and 99.1% for infection-free herds and 72.6% and 96% for herds with <20% seroprevalence. In a quantitative approach, Pearson's correlation coefficients, reported as a measure of linear association between herd seroprevalences and transformed optical density values recorded in bulk-tank milk, were –0.63 for gE ELISA and 0.67 for gB ELISA.

Keywords

Bovine herpesvirus 1 – Bulk-tank milk – Enzyme-linked immunosorbent assay – Glycoprotein B – Glycoprotein E – Serology – Within-herd prevalence.

Introduction

Infectious bovine rhinotracheitis (IBR) is a highly contagious disease that affects cattle worldwide. The disease is caused by *Bovine herpesvirus 1* (BoHV-1), in the subfamily *Alphaherpesvirinae* of the *Herpesviridae* family.

Through eradication programmes, some European Union (EU) countries and regions have managed to reach the status of 'BoHV-1 free', and others are presently adopting control programmes. A third group of countries, including Spain, have no specific official BoHV-1 programme (1). Common control measures against infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) include screening, surveillance, precautions at borders and farm biosecurity measures. In several EU countries, screening and surveillance for IBR

are based on periodic testing of samples of bulk-tank milk (BTM) taken from all dairy herds (2, 3, 4). The adaptation of the enzyme-linked immunosorbent assay (ELISA) technique for detecting antibodies in BTM samples constitutes a cheap and reliable test since it provides information about the status of a large group of animals from a single sample.

In Galicia (north-west Spain), a voluntary BoHV-1 programme was initiated in 2004. As regards laboratory analysis, the programme was based on periodic testing of BTM samples by antibody ELISA. When a positive BTM result was obtained, heifers from nine to 18 months of age, which had been born on the farm, were tested individually, using the same technique. The presence of one or more seropositive results in this group was indicative of recent exposure to the virus, which might still remain on the farm. All cattle purchased from other farms were also tested.

This study was designed to examine the relationship between within-herd seroprevalence and BTM antibody levels against BoHV-1, as measured by antibody glycoprotein B (gB) and glycoprotein E (gE) ELISAs.

Materials and methods

The study was performed in 2012 in Galicia (north-west Spain), the Spanish region which produces the most milk (35% of total Spanish milk production).

To evaluate the relationship between the level of antibodies in a sample of BTM and the within-herd seroprevalence, 482 samples of BTM were taken from 482 dairy herds and serum was collected less than one week later from every animal that was lactating. Of 482 herds, 275 (196 of which had never been vaccinated and 79 of which had been vaccinated against BoHV-1 with marker vaccines) were analysed (both individual serum and BTM samples) with an ELISA that detects antibodies against gE of the virus (Idexx IBR gE antibody, Idexx Laboratories, the Netherlands), and a further 207 of the 482 herds (none vaccinated against BoHV-1) were analysed with an

ELISA that detects gB-specific antibodies against BoHV-1 (Idexx IBR gB antibody).

All analyses were performed by following the recommendations of the manufacturer, including the procedures for the determination of cut-off points. In the gE ELISA, values were expressed as a sample-to-negative (S/N) ratio (samples were considered positive at an S/N ≤ 0.8), and in the gB ELISA as % blocking (positive at a % blocking ≥ 45).

Initially, for analysis, herds were categorised as (5):
– negative (0% within-herd seroprevalence)
– low positive, including infection-free herds (≥0–20%)
– mid positive (>20–60%)
– high positive (>60%).

On those farms analysed with gE ELISA, the within-herd seroprevalence distribution of vaccinated and non-vaccinated herds was compared by means of the Chi-square (χ^2) test.

The sensitivity (Se) of the BTM test (with respect to the reference test: serum samples) was evaluated in terms of the proportion of high-positive herds which the BTM test classified as positive. Specificity (Sp) was evaluated in terms of the proportion of infection-free or low-positive herds which the BTM test classified as negative.

Scatter plots were created by evaluating the correlation between within-herd seroprevalence and antibody levels in BTM using a quantitative approach along with the corresponding Pearson correlation coefficients (ρ).

Results

Of the 275 farms analysed with gE ELISA, 95 had 0% seroprevalence, 124 had ≥0–20% seroprevalence, and 21 had a seroprevalence of more than 60%. The remaining 35 were in the interval 20–60% (Table I). There were no differences between the within-herd seroprevalence distribution of unvaccinated and vaccinated herds ($p = 0.896$). Of the 207 herds analysed with gB ELISA, 107 were free from infection (0%

seroprevalence). In all, 199 had ≥0–20% seroprevalance and two had a seroprevalence of more than 60%. The remaining six were in the group 20–60%.

Both the gE and gB BTM ELISAs classified herds with a high seroprevalence (>60%) as positive (100% Se). The Sp when testing infection-free herds was 88.4% for the gE ELISA and 99.1% for the gB ELISA. When considering low-positive herds (<20% seroprevalence), the Sp values decreased to 72.6% for gE ELISA and 96% for gB ELISA (Table I). Among those herds whose BTM samples were classified as negative, the highest within-herd seroprevalence observed was 24.5% for farms analysed with the gE ELISA and 28% for those analysed by gB ELISA.

In addition, 30 out of 35 (85.7%) herds from the group with a seroprevalence of between 20% and 60% tested positive in the BTM test with gE ELISA, whereas only two out of six herds (33.3%) from the same group tested positive with gB ELISA.

The scatter plots showed moderate to good correlation between within-herd seroprevalences and transformed optical density values from BTM samples for the two ELISAs in a quantitative approach, which was also reflected in the *p* values: -0.635 for gE ELISA (-0.622 in unvaccinated herds and -0.690 in vaccinated herds) and 0.670 for gB ELISA ($p < 0.001$ for all coefficients) (Fig. 1).

Discussion

A previous study using a gB ELISA indicated that the correlation between the BTM blocking percentage and the within-herd seroprevalence was 0.86 (3). As in the present paper, this earlier study considered that this test could not be accurate enough to distinguish infection-free and low-positive herds. Herds containing only a few reactors often tested negative using the BTM test (3). Immunoglobulin G (IgG) concentration methods would increase the ability to differentiate between infection-free and low-positive herds (6). The determination of IgG in pooled milk samples before and after concentration indicated concentration factors from 11 to 30 (6, 7).

However, this method can be difficult to implement as a routine procedure in a diagnostic laboratory.

For the particular case of gE ELISAs, seroconversion against this antigen in vaccinated animals was shown to be delayed (for more than 28 days) or even undetectable (8, 9).

The authors included separate estimations for herds with a low seroprevalence (≥0–20%) and those with no positive animals (0%). Low-positive herds could be considered basically naïve, and in those herds active infection is unlikely (5). Although herds with a low seroprevalence might contain some animals in which the infection is latent, they are still relevant to the present study and to areas where the prevalence is high. These herds are the main candidates to acquire the status of being disease-free in the short term, providing that reactivation does not occur.

However, in vaccinated herds, given that IBR vaccination protects such herds, seroconversion of 10–15% of the milking cows is rare, and reinfections may remain undetected for a long time (7).

There appeared to be no difference in terms of prevalence between herds that were not vaccinated and those that had been vaccinated. However, the explanation could be that farmers only start vaccination programmes after their farms are affected by an infection, which means that the seroprevalence is already high. Another possible explanation is the use of 'conventional vaccine' before joining the programme, in which conventional vaccines are not allowed.

On the other hand, the high percentage of false-positive reactions with gE ELISA (infection-free herds testing positive in BTM, representing 15.6% of the studied population) is striking, since the main limitation described for this test was Se (8, 9). A previous study, using a blocking ELISA, indicated that this could be due to binding of non-specific protein in the wells of the test plate (10). In any case, in the present paper, this situation was scarcely observed when using gB ELISA (in herds from the same population) since only one of the 107 infection-free herds tested positive in BTM.

It is necessary to take into account the fact that discrepancies between individual serum and BTM results could exist due to varying contributions of individual cows to BTM (11).

The study showed an acceptable correlation between the level of antibodies in BTM samples and within-herd prevalence, especially when analysed by gB ELISA. An initial herd classification could be determined and then monitored with additional samples to help control BoHV-1 infection over time. The main limitation seems to be related to the fact that, when gE ELISA was used in BTM, a significant number of infection-free herds (0%) tested positive.

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Table I
Cross-classification of the results of an antibody enzyme-linked immunosorbent assay from samples of bulk-tank milk and within-herd prevalences from serum samples

Bulk-tank-milk result	Within-herd prevalence							
	gE ELISA				gB ELISA			
	0%	≥0–20%	>20–60%	>60%	0%	≥0–20%	>20–60%	>60%
Positive	11	60	30	21 (100%)	1	8	2	2 (100%)
Negative	84 (88.4%)	159 (72.6%)	5	0	106 (99.1%)	191 (96%)	4	0
Total	95	219 (95 + 124)	35	21	107	199 (107 + 92)	6	2

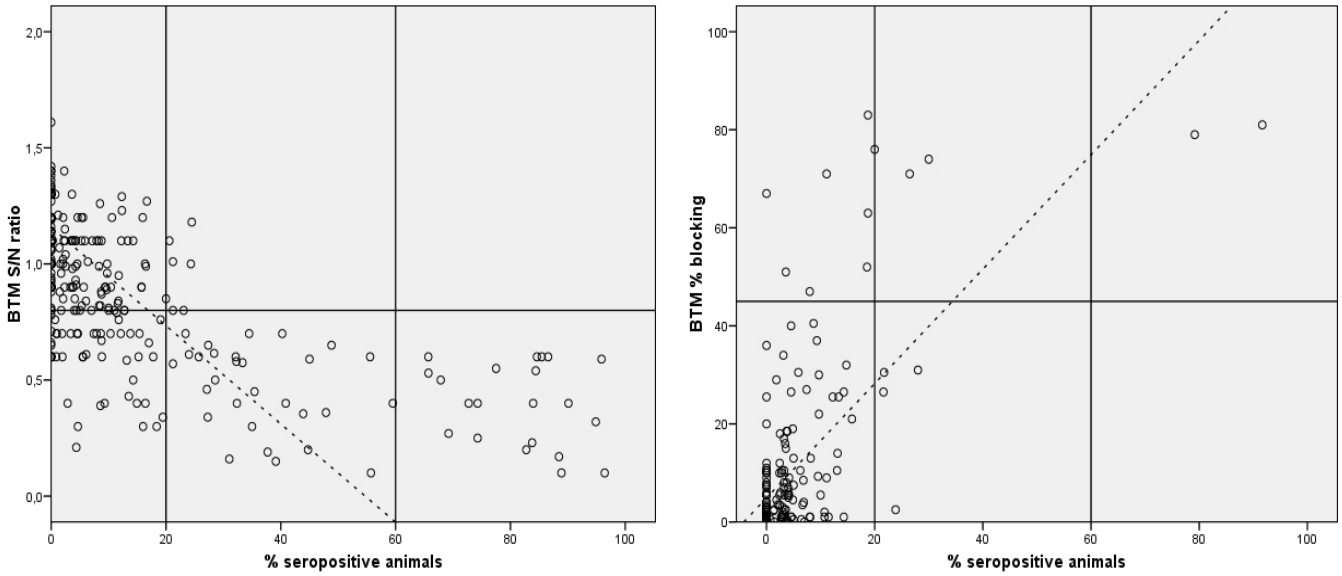
ELISA: enzyme-linked immunosorbent assay

gB: glycoprotein B

gE: glycoprotein E

Fig. 1

Scatter plots of within-herd prevalence (*x*-axis) and the level of antibodies in bulk-tank milk (*y*-axis), expressed as S/N ratio (gE ELISA, *n* = 275) and percentage blocking (gB ELISA, *n* = 207)



BTM: bulk-tank milk

gB: glycoprotein B

gE: glycoprotein E

S/N: sample to negative