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Prevalence of exposure to bovine viral diarrhoea virus (BVDV) and bovine herpesvirus-1 (BoHV-1) in Irish dairy herds

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ABSTRACT

Bovine viral diarrhoea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) are contagious bovine viral agents. The objectives of this study were to use quarterly bulk milk and 'spot' testing of unvaccinated youngstock to establish the national prevalence of exposure to BVDV and/or BoHV-1 in Irish dairy herds. Seasonality of bulk milk ELISA results was also examined. From a geographically representative population of 305 dairy herds, 88% and 80% of herds yielded mean annual positive bulk milk readings for BVDV and BoHV-1, respectively. Of these, 61% were vaccinated against BVDV and 12% against BoHV-1. A total of 2171 serum samples from weanlings having a mean age of 291 days yielded 543 (25%) seropositive for BVDV, and 117 (5.4%) seropositive for BoHV-1. A significant seasonal trend in bulk milk antibody ELISA readings and herd status was recorded for BVDV, with more herds categorised as positive in the latter half of the year.

1. Introduction

Bovine viral diarrhoea (BVD), caused by BVD virus (BVDV), and infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus 1 (BoHV-1), are highly contagious viral diseases of cattle (Moennig et al., 2005; Muylkens et al., 2007; Nandi et al., 2009). Both exhibit a worldwide distribution (Lindberg et al., 2006; Thiry et al., 2006) and are listed as notifiable diseases by the Office International des Epizooitic[1] (OIE). Although OIE-listed diseases, compulsory national control programmes for BVDV and BoHV-1 do not exist in many countries (Ackermann and Engels, 2006; Heffernan et al., 2009).

Where regulation does exist, successful BVDV eradication has been achieved through the use of 'test and cull' protocols involving removal of persistently infected (PI) individuals (Heffernan et al., 2009; Lindberg et al., 2006; Moennig et al., 2005; Presi et al., 2011; Ridpath, 2012; Ståhl and Alenius, 2012; Valle et al., 2005). In the case of BoHV-1, vaccination with marker/DIVA (**D**ifferentiating **I**nfected from **VA**ccinated) vaccines (Mars et al., 2001; Nandi et al., 2009; Nardelli et al., 2008; van Oirschot, 1999) constitutes the primary method of control and eradication in high prevalence regions. In January 2013, a mandatory national eradication programme for BVD, coordinated by the Animal Health Ireland (AHI), was introduced in the Republic of Ireland (Graham et al., 2013). As yet, a co-ordinated approach to BoHV-1 control does not exist in Ireland.

In order to determine the necessity for, and measure ongoing success of an eradication programme, it is useful to conduct prevalence studies to obtain baseline data (Heffernan et al., 2009; Lindberg et al., 2006; Lindberg and Alenius, 1999; Paisley et al., 2001). National prevalence studies, however, are often prohibitively expensive (Thrushfield, 2005). The advent of bulk milk testing overcomes this issue and reliable antibody detection bulk milk test procedures have been developed for both BVDV and BoHV-1 (Beaudeau et al., 2001; Nylin et al., 2000). Bulk milk analysis for BVDV antibodies, however, does not readily distinguish between vaccinated and unvaccinated herds (Lindberg et al., 2006). This issue has been overcome in the case of BoHV-1 with the advent of BoHV-1 gE-deleted DIVA vaccines. Due to legislative requirements,[2] all BoHV-1 vaccines administered in the Republic of Ireland since December 31, 2004 are DIVA vaccines (Simon, 2004).

Additionally, bulk milk BVD antibody readings may reflect historical rather than current herd viral status (Brülisauer et al., 2010; Lindberg and Alenius, 1999). To overcome this issue, it is useful to test unvaccinated homeborn youngstock (weanlings) for antibodies against BVDV, i.e. a 'spot test' (Houe, 1992, 1994; Mars and Van Maanen, 2005). Positive antibody readings in this population, once maternal antibodies have dissipated, can be indicative of current

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[1] www.oie.int.
[2] Diseases of Animals Act 1966; Control on Animal and Poultry Vaccines Order 2002; S.I. 528 of 2002 www.irishstatutebook.ie.

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or recent viral circulation (Houe, 1992, 1994; Lindberg and Alenius, 1999), and as such provide a useful adjunct to bulk milk testing.

Although preliminary surveillance studies have indicated high levels of both BVDV and BoHV-1 in the Irish national cattle population (Cowley et al., 2011, 2012; O'Grady et al., 2008; O'Neill et al., 2009), national prevalence data for BVD and BoHV-1 exposure among a geographically representative sample of Irish dairy farms are not available. In addition, evaluation of longitudinal BVD and BoHV-1 bulk milk data over a single lactation in a predominantly spring-calving dairy system has not been reported previously. The primary objective of this study, therefore, was to use bulk milk analysis and spot testing of Irish dairy herds to generate national prevalence data for both BVD and BoHV-1, while investigating the usefulness of this diagnostic strategy in an Irish context.

2. Materials and methods

2.1. Sample population and survey

The study was licenced by the Irish Department of Health and Children in 2009, meeting all legislative requirements for research involving animals in the Republic of Ireland at the time of the study.

A detailed description of the sample population used in this study is outlined in O'Doherty et al. (2013). Briefly, stratified proportional sampling based on herd size and geographical location was used to randomly select and invite 500 herds from the Irish Cattle Breeding Federations (ICBF) database to partake in the study on a non-incentivised basis. Over the 2009 lactation, four bulk milk samples (23 March, 8 June, 31 August and 2 November) were submitted by post in a standardised kit from each participating farm. Each study farm was visited between October 2009 and January 2010 to collect blood samples by coccygeal venepuncture from 20% of the replacement heifer group (weanlings for spot test) on each farm, with a minimum of five weanling heifers sampled on each farm. All heifers were homeborn and not vaccinated against BVDV. Where possible, only weanlings over 270 days of age were sampled, although not achievable in all cases. Accurate weanling age based on calf registration data was downloaded from the ICBF database.

2.2. Sample analysis

Commercially available enzyme linked immunosorbent assay (ELISA) kits were used to test bulk milk samples for the presence antibodies against: (i) BVD p80 (NS3) protein, (Institut Pourquier, France); (ii) Ultrapurified IBR lysate (Institut Pourquier, France) in BoHV-1 unvaccinated herds; and (iii) IBRgE, (IDEXX laboratories, USA) in BoHV-1 vaccinated herds. Weanling serum samples were also tested for antibodies against BVD p80, ultrapurified IBR lysate, and IBRgE with serum adapted positive cut-off values applied as outlined by kit manufacturers (Table 1). All analyses were completed by commercial accredited laboratories; BVD p80 and IBR lysate by National Milk Laboratories Ltd. (UK), and IBR gE by Enfer Diagnostics Ltd. (Ireland).

2.3. Herd classification

Calving data from the ICBF were used to determine calving-season of each herd (spring-calving and non-spring-calving, i.e. spring-autumn [SA] and year-round [YR]) as described by O'Doherty et al. (2013). Vaccination status (vaccinated [V] and unvaccinated [UV]) was determined by questionnaire, with date of vaccination, product used, and class of animal vaccinated (cows, yearling-heifers, weanlings) recorded. In all cases, kit-manufacturer positive cut-off values were applied to ELISA outputs in order to classify herds as 'positive' or 'negative'. Herds were classified as positive or negative at each of the four sampling time points (longitudinal data). Additionally, a mean annual ELISA result for each herd (herd status data) was calculated to provide an overall bulk milk classification for each herd. Herds were also categorised on the basis of combined BVDV and BoHV-1 bulk milk antibody status, i.e. negative for both viral antibodies, positive for BoHV-1 and negative for BVD, negative for BoHV-1 and positive for BVD, and positive for both viral antibodies.

Finally, herds were classified with regard to the presence of seropositive unvaccinated weanlings. Two datasets were constructed with weanlings either categorised 'positive aged ≥180 days of age' or 'positive aged ≥270 days of age' to both assess and minimise potential interference from maternally derived antibodies (MDAs) (Fulton et al., 2004). Herds having at least one weanling serologically positive for either BVDV or BoHV-1 were classified as having 'evidence of recent viral circulation' (RVC) (Houe, 1992; Handel et al., 2011). Herds not recording a positive weanling or recording a positive weanling under either 180 or 270 days of age, depending on the dataset, were classified as 'not having evidence of recent viral circulation' (NRVC).

2.4. Data analysis

Descriptive analysis and graphical representations were completed in Excel (MS Office 2010). Normality of the data was assessed visually using ladder of powers histograms, with normality of residuals assessed using normal probability plots and kernel density estimate plots constructed in Stata (Version 12). True prevalence was calculated using the Rogan–Gladen estimator in the survey toolbox version 1.04 (www.ausvet.com.au (Cameron, 1999)). Pearson's chi-squared, Fisher's exact, univariable and multivariable logistic regression, generalised estimating equations (GEE), multinomial logistic regression, Wilcoxon rank sum, and Hosmer–Lemeshow test of goodness of fit analyses were carried out using Stata (Version 12).

Seasonal trends in true prevalence for both diseases were tabulated. In addition, box plots of %inhibition, %S/P, and S/N ratio for BVDp80, IBR lysate, and IBR gE, respectively, at each sampling time

Table 1
ELISA kit performance data and positive cut-off values for BVD and BoHV-1assays used in this study.

Test	BVD P80 Milk	IBR Lysate Milk	IBR gE Milk	BVD P80 Serum	IBR lysate Serum	IBR gE Serum
Sensitivity	95.0%	100%	72.0–88.4%	97.6%	98.7%	100%
Specificity	97.7%	99.6%	100%	97.3%	99.9%	>99%
Positive cut-off	≥55	≥25	≤0.8	>60	>55	≤0.60
(Kit)	%Inhibition[a]	%S/P[b]	S/N ratio[c]	%Inhibition[a]	% S/P[b]	S/N ratio[c]
Within-herd prevalence	≥30%[d]	Not available	10.0–15.0%[e]	n/a	n/a	n/a

[a] %Inhibition = [1 – (OD 450 of analysed sample / mean OD 450 of negative control)] × 100.
[b] %S/P = (OD 450 of sample – OD 450 of negative control) / (mean OD 450 of positive control – OD 450 of negative control) × 100.
[c] S/N ratio = (sample mean – absorbance 650 nm)/negative control mean.
[d] Beaudeau et al., 2001.
[e] Wellenberg et al, 1998; Kramps et al., 1994.