



Impact of three inactivated bovine viral diarrhoea virus vaccines on bulk milk p80 (NS3) ELISA test results in dairy herds

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ABSTRACT

Bovine viral diarrhoea virus (BVDV) is endemic in many countries and vaccines are used as a component of control and eradication strategies. Surveillance programmes to detect exposure to BVDV often incorporate the use of bulk milk (BM) testing for antibodies against BVDV p80 (NS3), but vaccination can interfere with these results. The aim of this study was to evaluate whether BVDV vaccines would confound BM testing for specific antibodies in a nationally representative group of commercial dairy farms in the Republic of Ireland. A total of 256 commercial dairy herds were included in the statistical analysis. Quarterly BM or serum samples from selected weanling heifers (unvaccinated homeborn youngstock) were assessed by ELISA for antibodies against the BVDV p80 subunit and whole virus. Wilcoxon rank-sum and receiver operating characteristic (ROC) analyses were used to examine differences among groups vaccinated with one of three commercially available inactivated BVDV vaccines. Two of the three vaccines showed evidence of interference with ELISA testing of BM samples. ROC analysis highlighted that one vaccine did not reduce the discriminatory power of the BVDV p80 ELISA for identification of herds with evidence of recent BVDV circulation, when compared with unvaccinated herds; thus, administration of this vaccine would allow uncomplicated interpretation of BM ELISA test results in vaccinated seropositive herds. Seasonal differences in BM antibody results were identified, suggesting that the latter half of lactation is the most suitable time for sampling dairy herds containing predominantly spring calving cows. The results of the present study are likely to prove useful in countries allowing vaccination during or following BVDV eradication, where BM testing is required as part of the surveillance strategy.

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Introduction

Bovine viral diarrhoea virus (BVDV) is endemic in many countries (Lindberg and Houe, 2005). Control of BVDV involves removing persistently infected (PI) animals from the herd, ensuring biosecurity and vaccination of susceptible animals (Moennig et al., 2005). Several countries, including Ireland¹, have initiated eradication programmes (Ridpath, 2012; Ståhl and Alenius, 2012) and many of these countries are close to or have achieved BVDV-free status (Bitsch et al., 2000; Valle et al., 2005; Presi et al., 2011).

Detection of BVDV-specific antibodies in bulk milk (BM) samples is routinely used to determine herd health status, prior to introduction of BVDV control measures and for surveillance purposes after implementation of a control programme (Houe et al., 2006). BM

analysis is often combined with selective (spot) testing of young stock for serum antibodies against BVDV, to determine whether there is evidence of recent virus circulation (RVC) in a herd, since BM results often reflect historical rather than current antibody responses (Mars and Van Maanen, 2005; Laureyns et al., 2010).

Use of a BVDV vaccine is a confounding factor when interpreting test results for immunoassays designed to detect antibodies against BVDV, since it can be difficult to differentiate vaccinated from infected animals (DIVA). This issue has led some Scandinavian countries to prohibit the use of BVDV vaccines during, and subsequent to, BVDV eradication (Greiser-Wilke et al., 2003; Barrett et al., 2011). However, vaccination is often a feature of BVDV control and eradication strategies in high seroprevalence countries, such as Ireland (Makoschey et al., 2007; Barrett et al., 2011), thereby restricting the usefulness of BM testing as a surveillance tool.

Previous studies have examined the impact of vaccination against BVDV on antibody testing under controlled conditions (Graham et al., 2003; Makoschey et al., 2007; Raue et al., 2011). Small scale field studies have been conducted on a single Dutch farm (Kuijk et al.,

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¹ See: www.animalhealthireland.ie (accessed 23 October 2014).

2008), on two (Álvarez et al., 2012) and three (González et al., 2014) Spanish dairy farms, and on 34 UK farms, the majority of which used a vaccine that is no longer commercially available (Booth et al., 2013). These studies investigated whether the use of a p80 (NS3) subunit ELISA has the potential to allow differentiation between infected animals and those receiving inactivated BVDV vaccines.

Such differentiation might be possible, since administration of an inactivated form of the virus should lead to limited exposure of the immune system to the p80 non-structural protein, which is produced predominantly during viral replication (Graham et al., 2003; González et al., 2014). Although some of the inactivated BVDV vaccines evaluated seemed to generate an antibody response that was not detected with the p80 ELISA, more recent studies have suggested that DIVA is not straightforward (Raue et al., 2011; González et al., 2014), but might be possible at the herd level, with the use of BM samples rather than individual sera (Álvarez et al., 2012).

The aim of the present study was to undertake a large scale, farm-based study of the impact of BVDV vaccination on BM testing in a high seroprevalence region. A commercially available BVDV p80 ELISA was evaluated for use in BM testing in a nationally representative group of dairy farms in the Republic of Ireland, where three different commercial BVDV vaccines were being administered.

Materials and methods

Study population and sample collection

A detailed description of the sample population used in the present study has been provided by O'Doherty et al. (2013). Stratified proportional sampling, based on herd size and geographical location, was used to randomly select and invite farmers on 500 Herdplus farms from the Irish Cattle Breeding Federation (ICBF) database² to participate in the study without incentives. A total of 312 commercial dairy farms were subsequently recruited.

BM samples were submitted from each herd on four occasions (March, June, August and November) in 2009. Farms were visited from October 2009 to January 2010 to sample weanling heifers (unvaccinated homeborn youngstock). Blood samples were obtained by coccygeal venepuncture from 20% of the weanling group, with a minimum of five heifers sampled per farm. Serum samples were obtained following centrifugation of clotted blood samples for testing by ELISA. Where possible, only weanlings >270 days of age were sampled, although this was not achieved on all farms. Accurate weanling age, based on calf registration data, was accessed from the ICBF database.

The vaccination status of each herd was determined by questionnaire. Herds for which there was not a complete set of four BM samples ($n = 3$), which did not return the vaccination questionnaire ($n = 4$), which did not supply a vaccine brand name ($n = 19$), which recorded positive weanlings <270 days of age ($n = 22$) or for which weanling ages were unavailable ($n = 8$) were excluded from the study; of the 312 farms initially recruited, a total of 256 herds were eligible for statistical analyses.

Sample analysis

BM samples at each time point were analysed at an accredited laboratory (National Milk Laboratories) using a BVDV p80 blocking ELISA (Institut Pourquier), demonstrating a sensitivity (Se) of 95.0% and specificity (Sp) of 97.7% for BM (Beaudeau et al., 2001). Heifer serum samples were also tested using this assay (Se 97.6%, Sp 97.3%). On the basis of optical density at 450 nm (OD_{450}), results are reported as % inhibition, i.e. $1 - (OD_{450} \text{ of the analysed sample} / \text{mean } OD_{450} \text{ of the negative control}) \times 100$, with cut-off values for positivity of $\geq 55\%$ and $>60\%$ inhibition applied for BM and serum, respectively.

BM samples submitted in March and August were also tested in an approved laboratory (Enfer Diagnostics) using an indirect BVDV ELISA against whole virus antigen (IDEXX Laboratories; Se 96.3%, Sp 99.5%) (Hashemi Tabar et al., 2011). Results are reported as S/P values, i.e. $(OD_{450} \text{ of sample} - OD_{450} \text{ of negative control}) / (\text{mean } OD_{450} \text{ of positive control} - OD_{450} \text{ of negative control})$, with the positive cut-off value for BM samples set at 0.2.

Herd classification

Herds were classified on the basis of geographical density (high density dairy; low density dairy), enterprise type (dairy livestock; mixed livestock) and herd size (31–65 cows; 66–99 cows; >99 cows). Calving data from the ICBF database were used

to determine the calving season of each herd (spring calving; not spring calving, i.e. spring–autumn, SA; year-round, YR), as described by O'Doherty et al. (2013). Vaccination status (vaccinated; unvaccinated) was determined by questionnaire, requesting date of vaccination and product used (Bovilis BVD, MSD Animal Health; Bovidec, Novartis Animal Health; PregSure BVD, Zoetis Animal Health).

Herds were also classified in terms of the presence of positive test results in unvaccinated weanlings. Herds having at least one weanling >270 days of age with detectable antibodies against BVDV were classified as RVC (Houe, 1994; Handel et al., 2011; Løken and Nyberg, 2013); herds not recording any seropositive weanlings were classified as no recent virus circulation (NRVC). Herds containing seropositive young stock <270 days of age were excluded from this part of the analysis, due to potential interference from maternally derived antibodies (MDA).

Statistical analysis

Microsoft Excel (MS Office 2010) was used for descriptive statistics and graphical representations. Statistical analyses including Wilcoxon rank sum analysis, generalised estimating equations and receiver operating characteristic (ROC) analysis were performed using Stata (Version 12). Power calculations were performed using G*Power (Version 3.1.9).

Eight datasets were constructed, consisting of varying combinations of unvaccinated and vaccinated RVC and NRVC herds. The normality of each dataset was assessed using the Shapiro–Wilk normality test and visually using ladder of powers histograms constructed in Stata (Version 12). Differences between vaccinated and unvaccinated herds of varying BVDV circulation status (RVC and NRVC) were examined using non-parametric Wilcoxon rank sum analysis. ROC analysis was completed to examine the discriminating power (area under the curve, AUC) of average BM results (classification variable) in identifying RVC herds (reference variable) in the unvaccinated herd datasets and vaccine brand datasets. An ROC analysis will yield AUC values between 0.5 and 1.0; the closer the value to 1.0, the greater the discriminating power of BM results in distinguishing RVC from NRVC herds (Greiner et al., 2000). Vaccinated and unvaccinated herds were assumed to have equal levels of seroprevalence, based on existing data in Ireland (Cowley et al., 2012). Three ROC analyses were completed to determine the Se and Sp of potential BVDV p80 ELISA positive cut-off values in groups incorporating unvaccinated herds and herds vaccinated with Bovilis BVD ($n = 144$), Bovidec ($n = 175$) or PregSure BVD ($n = 151$).

Generalised estimating equations (GEEs) were used to investigate seasonal patterns in BM test results across vaccination status and vaccine brand. Herd was included as a repeated measure, a Gaussian distribution was assumed and an exchangeable correlation structure was applied. Associations between vaccine brand and explanatory variables, i.e. region, herd size, calving-season, mean of quarterly BM test results (positive vs. negative) and evidence of RVC (at least one positive weanling vs. no positive weanlings) were examined by multinomial logistic regression. As a first step, explanatory variables recording P values ≤ 0.15 in a univariate analysis were included in the multivariate model. A manual backwards elimination with a forward step was completed for all models, with variables recording P values ≤ 0.05 being maintained in the final model. All second level interactions were also examined.

Results

The distribution of study herds across region, herd size, calving season and type of enterprise is shown in Appendix: Supplementary Table S1. Herd BM, RVC and vaccination status are shown in Appendix: Supplementary Table S2. In the Pourquier p80 ELISA, $\geq 55\%$ inhibition results were recorded in BM from 94/118 (79.7%) unvaccinated herds, indicating a high prevalence of seroconversion against the BVDV p80 antigen from natural viral infection. Of the vaccinated herds suitable for statistical analysis ($n = 149$), 68/149 (45.6%) used Bovidec, 44/149 (29.5%) used PregSure BVD and 37/149 (24.8%) used Bovilis BVD. The most common time of year to vaccinate against BVDV was January to April, when 153/190 (80.5%) of herds were vaccinated. A total of 2171 serum samples from weanlings (mean age 291 days, range 109–549 days) were analysed, with 543/2171 (25.0%) testing seropositive for BVDV; at least one seropositive weanling >270 days of age was identified in 87/256 (33.9%) study herds.

Quarterly median BVDV p80 ELISA BM results for vaccinated and unvaccinated herds across vaccine brand over time are shown in Fig. 1. Corresponding Wilcoxon rank-sum z and P values are shown in Table 1. Using the Pourquier p80 ELISA, there were significant increases in median percentage BM ELISA inhibition values over the entire lactation in NRVC herds vaccinated with Bovidec (10.8–24.1%; $P < 0.01$) or PregSure BVD (14.0–26.4%; $P < 0.01$), but not in NRVC

² See: http://www.icbf.com/?page_id=56 (accessed 23 October 2014).

Table 1
Range of Wilcoxon rank sum *z* and *P* values for vaccinated and unvaccinated recent virus circulation (RVC) and no recent virus circulation (NRVC) herds across month of sampling (March to November).

Vaccine	Pourquier BVDV p80 subunit		IDEXX whole BVDV antigen ^a	
	<i>z</i> value range ^b	<i>P</i> value range ^b	<i>z</i> value range ^b	<i>P</i> value range ^b
	Vaccinated NRVC vs. unvaccinated NRVC		Vaccinated NRVC vs. unvaccinated NRVC	
Bovidec	-4.96 to -3.58	<0.01	-5.12 to -5.07	<0.01
Bovilis BVD	-1.49 to -0.59	0.1–0.6	-3.62 to -2.96	<0.01
PregSure BVD	-6.51 to -4.46	<0.01	-6.16 to -5.16	<0.01
	Vaccinated NRVC vs. unvaccinated RVC		Vaccinated NRVC vs. unvaccinated RVC	
Bovidec	-1.44 to 0.23	0.15–0.8	-0.92 to 0.48	0.4–0.6
Bovilis BVD	2.38–3.15	<0.05	0.16–1.52	0.1–0.9
PregSure BVD	-3.47 to -1.39	<0.01–0.2	-3.96 to -3.78	<0.01
	Vaccinated RVC vs. unvaccinated RVC		Vaccinated RVC vs. unvaccinated RVC	
Bovidec	-0.96 to -0.01	0.3–1.0	-1.37 to -0.51	0.2–0.6
Bovilis BVD	-1.34 to 0.14	0.2–0.9	-1.37 to -1.79	0.07–0.2
PregSure BVD	-2.53 to -0.65	0.01–0.5	-3.94 to -2.99	<0.01

^a Data represent March and August samples and were analysed only as a means of verifying vaccine administration. These data were not used for seasonal analyses.

^b All *z* and *P* values for each month of sampling are within the ranges listed. Where a single figure is listed, i.e. *P* < 0.001, all data points yielded the same value.

herds vaccinated with Bovilis BVD (0.46–10.9%; *P* values 0.14–0.55 at different sampling dates), compared to unvaccinated NRVC herds (Fig. 1A; Table 1).

NRVC herds vaccinated with Bovilis BVD had significantly lower median BM ELISA percentage inhibition values at each sampling time point than unvaccinated RVC herds (March: *P* = 0.02; June: *P* = 0.009; August: *P* = 0.003; November: *P* = 0.002), while NRVC herds vaccinated with Bovidec recorded no significant difference (March: *P* = 0.7; June: *P* = 0.15; August: *P* = 0.8; November: *P* = 0.8). PregSure BVD vaccinated NRVC herds recorded elevated % inhibition values compared to unvaccinated RVC herds in March (*P* = 0.035), June (*P* = 0.001) and August (*P* = 0.008), with no significant difference (*P* = 0.16) recorded in November (Table 1). There was no significant difference in ELISA values in herds with evidence of RVC, regardless of vaccination status or vaccine brand administered (Fig. 1B; Table 1).

There was a significant increase (*P* < 0.01) in whole virus antigen ELISA values in BM from NRVC herds vaccinated with any of the three BVDV vaccine brands in comparison with NRVC unvaccinated herds; animals vaccinated with the PregSure BVD vaccine had the highest values (Fig. 2; Table 1). Power calculations relating to the most pertinent between-group comparisons are outlined in Appendix: Supplementary Tables S3 and S4. With the exception of the March comparison between Bovilis BVD NRVC and unvaccinated RVC herds, sample sizes were of sufficient power (≥0.8) to detect reported between-group differences.

ROC analysis of BM and spot test results on serum samples from weanling heifers in unvaccinated herds yielded an AUC of 0.78. Bovilis BVD vaccinated herds recorded similar powers of discrimination between RVC and NRVC herds, yielding an AUC of 0.77 (*P* = 0.91; Fig 3A; Table 2). Herds vaccinated with Bovidec or PregSure BVD yielded AUC values of 0.50 and 0.55, respectively, indicating that RVC and NRVC herds could not be distinguished using these vaccines (Figs. 3B, C; Table 2). A p80 ELISA percentage inhibition positive cut-off value for BM samples between 83.3% (Se 71.4%, Sp 72.6%) and 86.5% (Se 65.3%, Sp 81.0%) correctly classified >70% of unvaccinated and Bovilis BVD vaccinated herds (*n* = 144).

Examination of the effect of seasonality on ELISA test results in unvaccinated herds showed significantly increased ELISA % inhibition

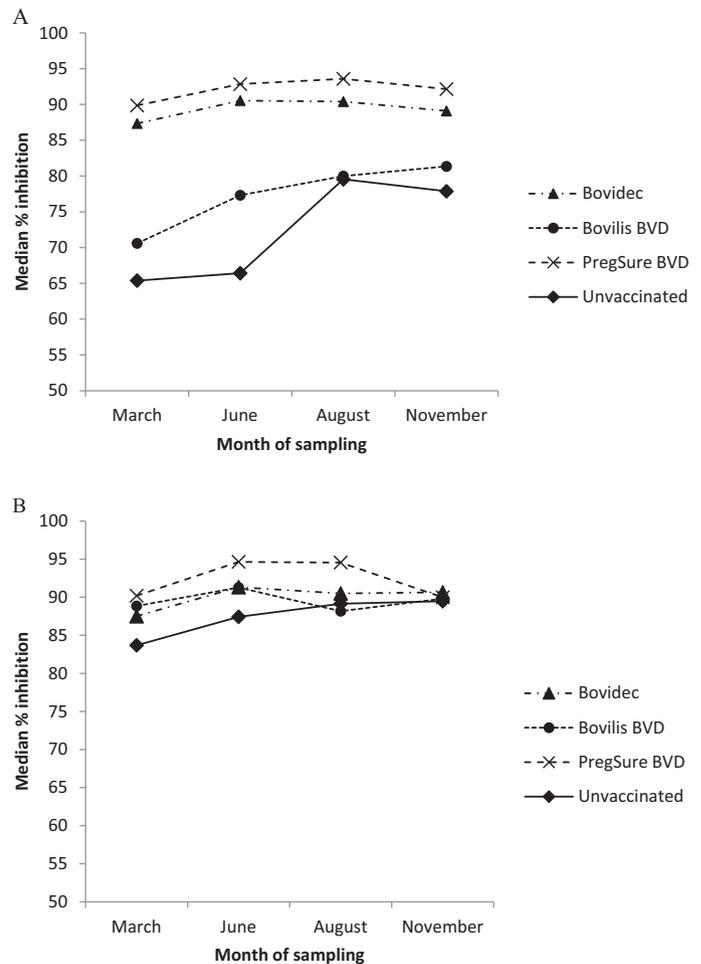


Fig. 1. Quarterly median bovine viral diarrhoea virus (BVDV) p80 ELISA test results (% inhibition) across (A) no recent virus circulation (NRVC) vaccinated (Bovidec, Bovilis BVD or PregSure BVD) and NRVC unvaccinated herds, and (B) recent virus circulation (RVC) vaccinated and RVC unvaccinated herds.

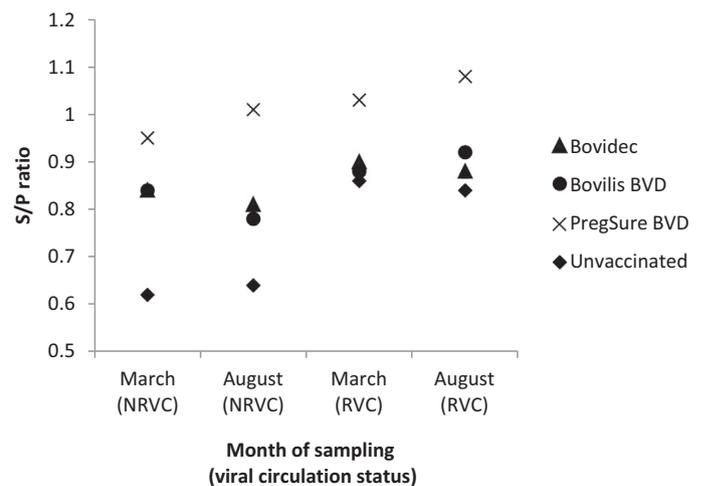


Fig. 2. Median bovine viral diarrhoea virus (BVDV) antibody S/P values measured in bulk milk (BM) using the IDEXX whole virus antigen ELISA in unvaccinated and vaccinated no recent virus circulation (NRVC) herds in March and August 2009.

Table 2

Bulk milk p80 ELISA receiver operating characteristic (ROC) analyses of unvaccinated (UV) and vaccinated (Bovilis BVD, Bovidec, PregSure BVD) herds for differentiation of herds with recent virus circulation (RVC).

Vaccine status	n	AUC ^a	Standard error	95% Confidence interval	P value
UV	107	0.78	0.05	0.69–0.86	
Bovilis BVD	37	0.77	0.09	0.59–0.94	
UV vs. Bovilis BVD					0.91
Bovidec	68	0.50	0.07	0.36–0.64	
UV vs. Bovidec					<0.01
PregSure BVD	44	0.55	0.11	0.33–0.75	
UV vs. PregSure BVD					0.05

^a ROC area under the curve.

Table 3

Multinomial logistic regression analysis of vaccine brand use across region, herd size, calving season and type of livestock enterprise.

Explanatory variables in comparison of vaccine brands	Relative ratio	95% Confidence interval	P value
Bovidec vs. Bovilis BVD			
Mixed vs. dairy only	0.49	0.21–1.20	0.1
Bulk milk positive vs. negative ^a	1.49	0.39–5.63	0.6
High density vs. low density*NRVC vs. RVC	9.77	1.23–77.45	0.03
PregSure BVD vs. Bovilis BVD			
Mixed vs. dairy only ^b	0.26	0.09–0.67	<0.01
Bulk milk positive vs. negative ^a	8.49	0.88–8.81	0.06
High density vs. low density*NRVC vs. RVC	4.57	0.44–47.10	0.2
PregSure BVD vs. Bovidec			
Mixed vs. dairy only	0.52	0.23–1.16	0.1
Bulk milk positive vs. negative ^a	5.67	0.66–48.60	0.1
High density vs. low density*NRVC vs. RVC	0.47	0.08–2.83	0.4

Explanatory variables included in the model were Region, Recent infection, Region × Recent infection, Enterprise and Bulk milk status (Model $P < 0.01$). * Interaction between explanatory variables 'Region' and 'Recent infection'.

^a Bulk milk positive and negative is based on the mean of quarterly bulk milk ELISA results.

^b Relative to herds vaccinating with Bovilis BVD, herds vaccinating with PregSure BVD were less likely (relative ratio = 0.26) to operate a mixed livestock enterprise compared to a dairy only herd.

values in the latter half of the year, regardless of infection status. Seasonality of test results in vaccinated herds was more variable (see [Appendix: Supplementary Table S5](#)).

A number of associations were highlighted between explanatory variables and the use of each vaccine brand ([Table 3](#)). An interaction between explanatory variables, region and RVC highlighted that, compared to herds vaccinated with Bovilis BVD, herds vaccinated with Bovidec were more likely to record positive weanlings in a high dairy density region than a low density region ($P = 0.03$). In addition, relative to herds vaccinated with Bovilis BVD, mixed enterprise herds were less likely to use PregSure BVD than herds managing dairy stock only (relative ratio, RR = 0.26; $P < 0.01$).

Discussion

Vaccination has a role to play in the control of BVDV infection, once PI animals have been identified and removed from the herd ([Makoschey et al., 2007](#); [Ståhl and Alenius, 2012](#)). Although the immunological protection offered by BVDV vaccines is limited when naïve cattle are exposed to PI animals shedding large amounts of virus ([Ståhl and Alenius, 2012](#)), vaccination can be a valuable tool in protection against clinical disease. However, the use of vaccines can compromise surveillance strategies based on the use of serological assays to determine if there has been exposure to BVDV in herds. In the present study, an inactivated vaccine was identified that could be used alongside a BVDV subunit ELISA to DIVA.

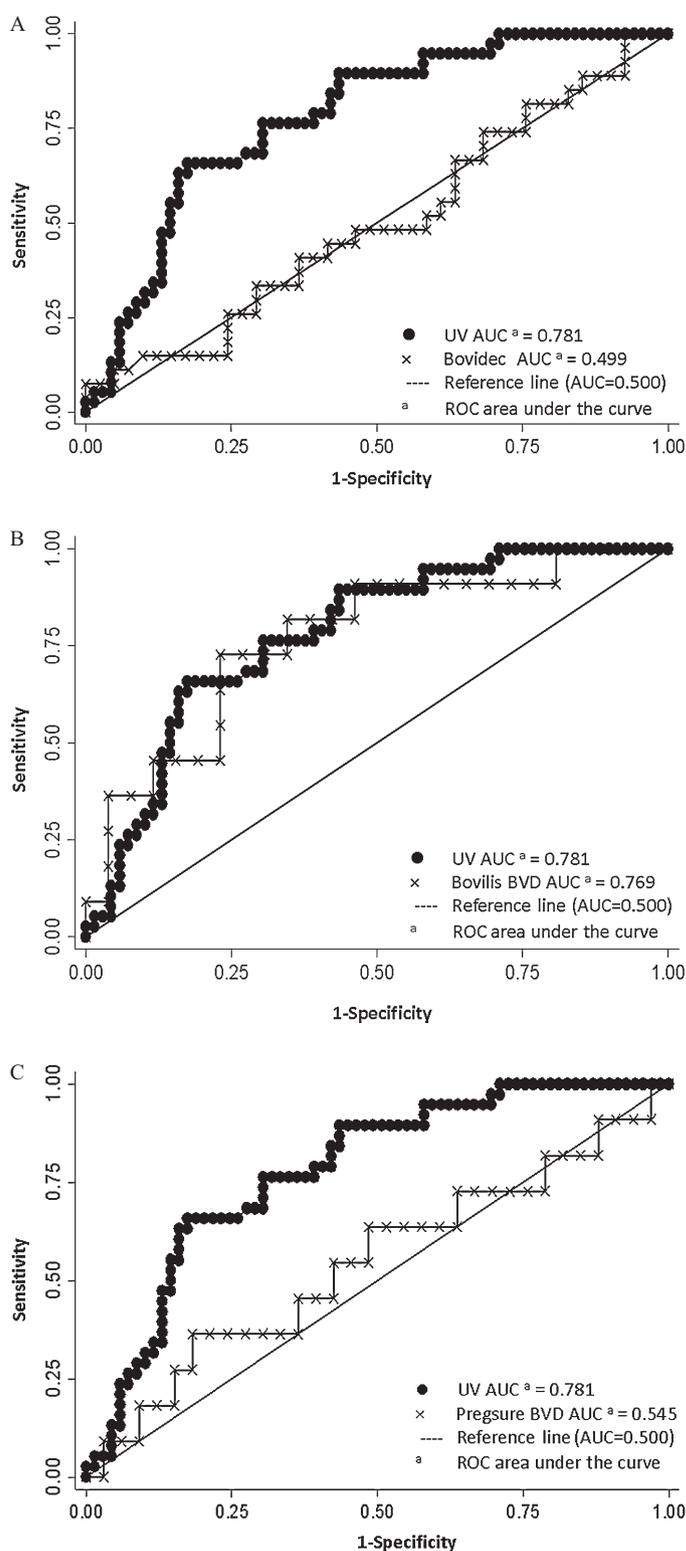


Fig. 3. Receiver operating characteristic (ROC) curves comparing discriminatory power of BM testing to identify recent virus circulation (RVC) herds in unvaccinated (UV) and herds vaccinated with (A) Bovidec, (B) Bovilis BVD and (C) PregSure BVD.

In the present study, a relatively large proportion of spring calving herds were recruited. Such herds have a relatively stable composition over a lactation period and animals are vaccinated at a similar time of the year. These factors potentially reduce the fluctuations

in BM test results reported by Booth et al. (2013), thereby allowing more definitive conclusions to be drawn. The high BVDV seroprevalences reported in Irish dairy herds in the current study (80%) and by Cowley et al. (2012) (98%) should minimise differences in BVDV status between vaccinated and unvaccinated groups, thereby allowing meaningful comparisons across study groups. A potential weakness of the present study is that only vaccines administered in 2009 were recorded and the impact of prior vaccine use on results cannot be assessed.

In an ideal situation, vaccines should be an integral component of a BVDV eradication and surveillance programme, provided vaccination has minimal interference with BM antibody testing. Certain conditions should be met, before a particular vaccine would be considered to be suitable for use in BVDV control programmes. The median percentage inhibition value of unvaccinated NRVC herds should not differ from that of vaccinated NRVC herds using the p80 ELISA, i.e. administration of the vaccine should not result in significantly increased levels of p80 antibodies. The median percentage inhibition value of unvaccinated RVC herds should be significantly greater than that of vaccinated NRVC herds using the p80 ELISA, i.e. it should be possible to distinguish vaccinated herds from those with evidence of recent viral transmission and infection. The median S/P value (whole virus ELISA) of a vaccinated group should not be significantly lower than that of other vaccine groups examined, i.e. the vaccine should be administered correctly and should induce a BVDV antibody response. Finally, ROC analysis of BM and spot testing of vaccinated herds should not be significantly different from unvaccinated herds, i.e. AUC values in vaccinated and unvaccinated herds should indicate comparable power ($AUC > 0.5$; $P > 0.05$) in distinguishing RVC from NRVC herds.

Under field conditions in a high seroprevalence region, Bovilis BVD was the only one of three vaccines that satisfied the stated criteria, when used in conjunction with the Pourquier p80 ELISA. Bovilis BVD vaccinated herds did not differ significantly from unvaccinated herds in the parameters included in the statistical analysis. Additionally, ROC analysis of unvaccinated and Bovilis BVD vaccinated herds yielded a similar BM positive cut-off value for detecting RVC herds to that suggested by Thobokwe et al. (2004) (80% inhibition) in a study in New Zealand dairy herds, in a production system similar to that in Ireland.

In contrast, Bovidec and PregSure BVD appear to elicit BVDV p80-specific antibody responses, limiting their usefulness in surveillance programmes, where a large proportion of seropositive herds are present. It should be noted that the antibody response to Bovilis BVD may be transient (Álvarez et al., 2012); furthermore, all farmers in the current study administered a single annual booster dose to their herds, contrasting with the three dose regime outlined by Álvarez et al. (2012). Further research is required to better characterise the serological response to inactivated BVD vaccines immediately post-vaccination and how this might affect BM testing.

Inappropriate vaccine use or vaccine failure could have influenced the study outcome. However, it is considered to be unlikely that this would be biased towards any particular vaccine brand. The BM antibody test results against whole BVDV were not significantly different between Bovidec and Bovilis BVD vaccines, suggesting that the differences observed in the Pourquier p80 ELISA were specific to the vaccine brand. The elevated antibody reactivity against whole BVDV observed with the PregSure BVD vaccine is possibly due to increased immunogenicity associated with the specific adjuvant used in this vaccine (Demasius et al., 2013). The PregSure BVD vaccine has recently been withdrawn, due to links with neonatal pancytopenia in calves (Deutsken et al., 2011; Euler et al., 2013).

Seasonal variation in BM test results was evaluated, since it has been reported that BVDV antibody levels in milk are inversely related to the amount of milk produced (Niskanen et al., 1989). The majority of herds in the present study calved in the spring, with peak

lactation occurring 9 weeks post-calving (Quinn et al., 2005); a significant increase in antibody values was recorded in unvaccinated herds in the latter half of the year, as anticipated. This indicates that autumn might be a more suitable period for sampling unvaccinated herds, to avoid false negative results. Although significant differences between sampling times were also recorded in vaccinated herds, none were of a magnitude to warrant suggesting an optimal time of year for BM sampling in vaccinated herds. The variation in individual animal responses to vaccination, as outlined in previous studies (Raue et al., 2011), highlights the usefulness of herd level BM testing, which avoids issues with sampling at different times post-vaccination.

Further research is required into farm level risk factors, which might account for the increased likelihood of seropositive weanlings on farms using Bovidec compared to Bovilis BVD. It is possible that Bovilis BVD performs better in reducing the levels of new/recent infection in dairy systems or, alternatively, that farmers experiencing issues with active BVDV infection are more likely to select Bovidec. Similarly, herds vaccinated with PregSure BVD tended to be more likely to record a positive mean annual BM p80 test result, which again may indicate increased use of this vaccine in herds experiencing problems with BVDV, rather than inferior vaccine performance. The significantly elevated values recorded in PregSure BVD vaccinated herds for both BVDV p80 and whole virus antigen ELISAs may be the main contributing factor. These findings should be investigated further to allow evidence-based recommendations with regard to BVDV vaccine use.

Conclusions

Administration of Bovilis BVD results in minimal interference with BM testing for antibodies against BVDV using the Pourquier p80 ELISA. Therefore, use of this vaccine is compatible with BVDV eradication and surveillance programmes.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at [doi:10.1016/j.tvjl.2015.03.025](https://doi.org/10.1016/j.tvjl.2015.03.025).

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