

Paper

Monitoring the bulk milk antibody response to BVD: the effects of vaccination and herd infection status

R. E. Booth, M. P. Cranwell, J. Brownlie

Bovine Viral Diarrhoea Virus (BVDV) is a pestivirus in the flaviviridae family which affects cattle worldwide. Bulk milk (BM) antibody testing is frequently used as a relatively quick method of assessing herd BVDV exposure; however, an understanding of the effects of vaccination and historic infection is essential for test interpretation. This study investigated the trends exhibited by monthly BM antibody analysis in 14 herds split into three categories. Category 1 herds (vaccinating/no persistently infected (PI) animals) began the study with mid-positive BM antibody titres and experienced an estimated increase of 0.007 optical density (OD) units per month (equating to a rise of 0.35 OD units in 50 months). Category 2 herds (not vaccinating/no PI animals) began the study with mid-positive BM antibody titres and experienced an estimated decrease of 0.005 OD units per month with antibody levels in one category 2 herd taking 1290 days to decrease from mid-positive to negative. Category 3 herds (vaccinating/PI animals present) began the study with high BM antibody titres which plateaued within this range throughout the 50-month observation period. Vaccination was observed to cause transient increases in BM antibody in a number of herds in categories 1 and 3.

Introduction

Bovine Viral Diarrhoea Virus (BVDV) is a pestivirus in the flaviviridae family which affects cattle worldwide. Economic losses in infected herds are well recognised and primarily occur due to infertility and immunosuppression associated with high levels of secondary disease (Edwards and others 1986, Wray and Roeder 1987, Ellis and others 1988, Virakul and others 1988, McGowan and others 1993, Moerman and others 1993, Potgieter 1995, Houe 1999). In 2003, losses to the UK cattle industry were estimated at £40 million per year (Bennett and Ijpelaar 2003), whilst at the herd level Gunn and others (2004) indicated that, without control, BVDV infection of a beef suckler herd could cost £37 per cow per year equating to a loss of £37,000 for a 100-cow herd over a 10-year period.

The success of BVD control in Scandinavia (Sandvik 2004, Houe and others 2006) ensures that the disease is regularly the subject of interest in veterinary and farming publications. Most recently, Scotland has announced a control scheme for BVD which will be compulsory by 2013. For these reasons, practitioners are increasingly

likely to be approached by their clients to investigate and control BVD in their herds and, therefore, a good knowledge of disease pathogenesis, test selection and interpretation are vital. Three common principles underlie successful BVDV eradication and these are: (1) the need to distinguish BVDV infected from BVDV-free herds; (2) clearance of virus from infected herds through the identification and removal of persistently infected (PI) animals; (3) the adoption of effective surveillance mechanisms to monitor and maintain a BVDV-free state (Lindberg and Alenius 1999, Houe and others 2006). It is primarily in the first and third stages that bulk milk (BM) serology can play a useful role in BVDV control and surveillance. Accurate identification of BVDV-free herds at the outset of a control programme avoids unnecessary and costly searches for PI animals, thereby allowing resources to be concentrated on herds with proven infection. Furthermore, BM serology is a useful tool for ongoing surveillance of herds that have cleared the infection. However, it is unlikely to be appropriate for monitoring herds that have recently eradicated BVDV due to the persistence of BVDV antibodies in milk following natural infection (Houe 1999, Pritchard 2001, Valle and others 2001, Houe and others 2006). In these instances, other techniques, such as testing cohorts of young stock (YS) or first-lactation heifers for BVDV antibody are more appropriate (Niskanen 1993, Alenius and others 1996).

Throughout the initial stages of the Scandinavian eradication programmes, BM serology was extensively used to determine the status of the dairy herds involved. In Norway and Finland, seroprevalence of BVDV was determined to be 23 per cent and <2 per cent, respectively (Waage and others 1996, Kulkas 1997), whilst Sweden observed higher figures of 65 per cent (Alenius and others 1996). Seroprevalence figures for the UK showed that 87–95 per cent of herds are seropositive, thus demonstrating evidence of BVDV exposure (Paton and others 1998, Graham and others 2001, Humphry and others 2012), whilst the number of herds thought to be actively/recently infected ranges from 20.5 per cent to 65 per cent (Paton and others 1998, Graham and others 2001, Booth and Brownlie 2012,

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Humphry and others 2012). These seroprevalence figures vary with time and geographical region. The lower figure observed in Scotland by Humphry and others (2012) may be due to an increasing awareness of BVDV in more recent years. Conversely, Booth and Brownlie (2012) noted a figure of 59 per cent infected herds in a recent study in southwest England which, although analysing a smaller number of herds in detail, supports the figure reported by Paton and others (1998). Confidence can be placed in the Scandinavian BM seroprevalence figures since they elected to eradicate without the use of vaccine. Humphry and others (2012) and Paton and others (1998) present results that do not include vaccinated herds, whilst Graham and others (2001) acknowledge that their figures may be confounded by vaccine use. Whilst this emphasises the fact that vaccination may interfere with the interpretation of BM antibody, testing it also highlights the need for clarification of the issue at the individual farm level. Comprehensive figures for BVD vaccine uptake in the UK do not exist, however in many areas it is known to be high with 28/34 (82 per cent) of the herds investigated in southwest England vaccinating against BVDV (Booth and Brownlie 2012) whilst, in Scotland, Humphry and others (2012) report a figure of 41 per cent (150/370) of herds surveyed utilising BVDV vaccine. The effects of the combination of natural and vaccinal antibody, and the additional effect of annual revaccination on BM antibody results is often assumed, but not well documented. Several publications have investigated the use of specific ELISA tests in herds that utilised killed BVDV vaccines (Graham and others 2003, Makoschey and others 2007, Kuijk and others 2008, Alvarez and others 2012), but further work is required to elucidate how to use and interpret BM serology in vaccinating herds that have also experienced historic infection and thus have a natural seroprevalence. With this in mind, this paper investigates the use of BM BVDV antibody testing on working farms with differing vaccination policies, herd seroprevalence levels and BVDV infection status. It is hoped that the information in this communication illustrates how the BVD BM antibody responses presented in three different herd scenarios may aid the veterinary practitioner in managing BVDV infection in their clients' herds.

Materials and methods

The dairy herds contributing data to this paper are members of a pilot BVDV eradication scheme that was established in Somerset. Full details of the farms, their BVDV status, and how it was determined are described elsewhere (Booth and Brownlie 2012). Briefly, 41 farms were recruited in April 2006 onto a pilot BVDV eradication programme, based primarily in Somerset. Of these, 34 remained active in the study until the end of 2009 with 20/34 herds (59 per cent) beginning as infected. All herds underwent regular surveillance; a minimum of yearly YS serology and quarterly BM serology was performed. Where evidence of active BVDV infection existed (detected most commonly by seroconversion in YS cohorts), whole herd tests were conducted in order to identify and cull PI animals. Of the 34 active farms, 31 were dairy enterprises which were used to collect the data presented here. The farm numbers used throughout this manuscript are consistent with those used by Booth and Brownlie (2012) enabling cross-referencing between articles.

Bulk milk sampling

BM samples were taken into pots containing Bronopol preservative and submitted to Animal Health and Veterinary Laboratories Agency (AHVLA) Starcross for BVDV antibody ELISA testing (AHVLA test code TC0123). The BVDV BM ELISA is as described by Pritchard (2001); the test is an 'in-house' indirect (IgG) ELISA developed with antigen derived from the whole cell lysate of a culture infected with Oregon C24V. All results were reported as optical density (OD) ratios. AHVLA interpretation of the BM OD test results was that <0.1=negative, 0.1–0.35=low positive, 0.35–0.7=mid-positive and >0.7=high positive. The BM OD values are thought to equate to approximately <5 per cent, 5–25 per cent, 25–65 per cent and >65 per cent herd seropositivity within each sample result range, respectively. This result may be influenced by a number of factors, for example: calving pattern, number of cows contributing to the BM and their relative milk yields. There may

also be some test-to-test variation caused by the condition of the sample, such as the proportions of fat and colostrum present. It is for this reason that OD and seroprevalence classes are only estimates but do give a reasonable indication of antibody status. Since beginning data collection, the system of reporting BM antibody ELISA results through OD ratio has now been superseded, and AHVLA now use the commercially available Svanovir, BVDV 'in-direct' antibody ELISA kit (Svanova Biotech AB, Uppsala, Sweden) and report BM results as 'percentage positivities'. However, the current test is broadly comparable with the one described in this paper with results similarly split into negative, low, mid- and high positive groups with estimations of herd seroprevalence of 0–13 per cent, 5–30 per cent, 30–60 per cent and >60 per cent respectively. Further information regarding test details can be obtained from the AHVLA website (http://vla.defra.gov.uk/services/ser_test_bulkmilk.htm).

Longitudinal analysis of BM OD ratio

All dairy members of the Somerset scheme (Booth and Brownlie 2012) were asked to submit monthly BM samples; however, only 14 herds submitted samples with sufficient regularity for longitudinal analysis of their results. Fourteen herds were grouped as follows into one of three categories:

Category 1. Vaccinating and have no PI animals present (farms 3, 4, 8, 9, 11 and 25)

Category 2. Not vaccinating and no PI animals present (farms 13 and 24)

Category 3. Vaccinating and have PI animals present (farms 1, 5, 15, 26, 27 and 38).

Over the observed periods, all vaccinating farms used the vaccine Pregsure BVD (Pfizer, Sandwich, Kent) apart from farm 38 which used the vaccine Bovilis BVD (MSD Animal Health, Milton Keynes, Buckinghamshire). All vaccines were administered according to the datasheet recommendations from the manufacturer.

In order to smooth out the monthly variations in antibody titre, LOESS curves were fitted to illustrate the changes of BM antibody results over time for each farm using the 'xyplot< lattice>' routine in the statistical package R (The R Foundation for Statistical Computing). Individual line graphs were also produced for each farm involved to demonstrate the 'raw' data. For those farms that vaccinated their cattle, the graphs were standardised such that annual vaccination occurred on months 1, 13, 25, 37 and 49.

A mixed model was developed in SAS (SAS Institute, Cary, North Carolina, USA) to investigate the effect of time (month) within each category of farm, and also the differences between farm categories 1, 2 and 3. Farm was controlled for as a random effect. The model also provides an estimation of the starting BM antibody OD titre in month 1 for each category (the x-y intercept). SE was calculated for each variable examined.

Analysis of the correlation of BM OD ratio with herd seroprevalence

Where any of the 31 active dairy herds from the pilot eradication scheme underwent whole herd blood testing (WHT) to confirm their BVDV status, a BM sample was taken and all animals contributing to the BM tank were recorded as was the vaccination status of the herd. Whole herd testing included all bovines present on the day of the herd test and is described by Booth and Brownlie (2012); briefly, for cattle >six months of age a combination of antibody and antigen ELISAs were used, whilst those animals <six months were screened by pooled blood PCR. The proportions of seropositive and seronegative contributors to the BM sample were calculated and recorded alongside the herd BM antibody result. These data were available for 16 herds: farms 1, 3, 5, 7, 8, 9, 15, 18, 19, 20, 25, 26, 27, 37, 38 and 40 all of which were vaccinating with the exception of farm 19. Ten of these farms were included in the longitudinal analysis above. Farms 4, 11, 13 and 24 were included in the longitudinal analysis of BM OD ratio but are not included here since they consistently appeared BVDV-free and did not undergo WHTs. Farms 7, 18, 19, 20, 37 and 40 all began the pilot study as infected herds, and as such, underwent WHTs producing data enabling assessment of the correlation between BM antibody

TABLE 1: Farm status, category (infection/vaccination) and sample submission rate

Farm ID	Vaccinate?	Infected (PI)	Category	Samples submitted	Months observed	Average samples submitted per year	Percentage of months sample submitted (%)
3	Yes	No	1	27	43	8	63
4	Yes	No	1	34	41	10	83
8	Yes	No	1	19	37	6	51
9	Yes	No	1	15	37	5	41
11	Yes	No	1	24	41	7	59
25	Yes	No	1	17	42	5	40
				Average		7	56
24	No	No	2	31	43	9	72
13	No	No	2	20	33	7	61
				Average		8	66
1	Yes	Yes	3	6	43	2	14
5	Yes	Yes	3	12	41	4	29
15	Yes	Yes	3	14	42	4	33
26	Yes	Yes	3	7	42	2	17
27	Yes	Yes	3	7	42	2	17
38	Yes	Yes	3	12	34	4	35
				Average		3	24

PI, persistently infected.

titre and herd seroprevalence. Of these, farms 19 and 37 submitted BM samples with insufficient frequency for longitudinal analysis. The remaining four herds (farms 7, 18, 20 and 40) were not included in the longitudinal analysis since they only began vaccinating after joining the pilot study; thus the trends observed with BM antibody titre could not consistently be associated with both vaccination and PI presence.

Results

Longitudinal analysis of BM antibody OD ratio

It was intended that all 30 dairy enterprises active for the duration of the Somerset pilot scheme would be included in the longitudinal analysis of BM antibody OD ratio, but only 14 farms submitted samples regularly for analysis. Frequency of submission for each farm analysed in the longitudinal analysis is presented in Table 1 and illustrated in Fig 2a–c. Herds in category 3 (vaccinating/PI animals present) tended to submit fewer, less regular samples than those in categories 1 and 2 (vaccinating/no PI animals and not vaccinating/no PI animals, respectively). On average, category 3 farms submitted only 3 samples per year, whilst category 1 and 2 farms submitted an average of 7 and 8 samples per year, respectively. The serosurveillance conducted in YS cohorts (at least once a year) and through repeated BM antibody monitoring confirmed that no PI animals of any age were present throughout the period observed for herds in categories 1 and 2 and, additionally, provided confidence that there was no evidence of acute infection in these herds.

Fig 1a–c show the LOESS regression lines for each category of farm analysed during this study. The regression procedure allows general trends within each category to be examined, but this technique may ignore temporary increases in BM antibody levels. For this reason, the raw data and LOESS regression line for each individual farm is included in Fig 2. In some cases, variation between monthly sample results can be considerable and is described using specific examples below.

Analysis of the longitudinal data in a mixed model in SAS, revealed that there was a significant difference between categories 1, 2 and 3 ($P < 0.001$) indicating that PI presence and vaccination protocols significantly affect the results of BM testing. Within each farm category, there was also a significant change in BM OD ratio over time ($P=0.0013$) which is quantified below.

Herds in category 1 (vaccinating/no PIs), began the study in their first month of membership with antibody titres ranging between mid- and high positive (Figs 1a and 2a). The estimated starting BM antibody titre for farms in category 1 was an OD ratio of 0.6 (se 0.05) with a trend over time for this to increase by 0.007 OD units per month (se 0.002). The individual graphs also illustrate the upward trend of BM OD ratio for farms in this category (Fig 2a). However, it is also evident from Fig 2a that, in some herds, there appears to be a tran-

sient peak in BM antibody levels in the months following vaccination. This is evident in the raw data, but not where LOESS regression is applied. The postvaccination peaks subsequently fall until the next annual vaccination where the pattern is repeated—farm 3 consistently demonstrates this in months 13 and 25. The fact that these peaks are associated with vaccination supports the evidence that acute BVDV infection was not circulating on these units. Farm 11 illustrates that not all farms follow this trend.

Both herds in category 2 (not vaccinated/no PI animals) began the study with mid-positive BM antibody titres (Figs 1b and 2b). The estimated starting BM antibody titre for farms in this category was 0.4 OD units (se 0.09) with a trend for a gradual decrease over time of 0.005 OD units per month (se 0.003). Farm 24 is worthy of mention since it was the first farm in this study to become negative on BM antibody analysis. Starting at an initial titre of 0.359 OD units in month 1, it took 44 months to decrease to 0.01 OD units in month 44 of the study—this was 1290 days in total. It should be noted in Fig 2b that there is considerable variation between monthly samples despite the overall trend of a decreasing BM antibody titre. Farm 13 experienced a spike in BM antibody in month 24 although this was not sustained and continued to decline after the next sample was submitted in month 29. It should be noted that there were no indications of infection on this farm which consistently returned negative antibody results when cohorts of YS were sampled (data not shown), and subsequently continued to demonstrate low BM antibody titres.

For category 3 farms (vaccinated/PI animals present), all except farm 1 began the study with high positive BM antibody titres and rarely fell below this range throughout the duration of the study. Estimated start titres in month 1 for this category are 0.8 OD units (se 0.06), and the increase over the observed period was calculated to be 0.001 OD units per month (se 0.003). Of the six farms contributing to this group, farms 1, 15 and 38 had PIs in the milking herd for months 1–13, 12–13 and 10–17 respectively. All other PIs detected were in the YS and heifer groups. Farm 1 began with a mid-positive titre which increased to high positive in month 2. Further investigation on farm 1 identified two PIs in the YS and a three-year-old, first-lactation PI that had been in the milking herd for 136 days at the time of the initial mid-positive test. The PI had entered the milking herd in January 2006, and the farm was first sampled in June 2006. However, the month 2 BM antibody titre for farm 1 was 0.9, and this did not fall below 0.7 (AHVLA high-positive cut-off) in any of the subsequent samples. A notable feature in the category 3 farms was that, like category 1, some appeared to exhibit a transient increase in BM antibody titre following vaccination. This was not present in all cases, but appeared most pronounced for those herds that submitted regular milk samples around the point of vaccination. The regression curves appeared to closely follow the raw

data in Fig 2c, and this was most likely related to the lower submission of samples by farms in this category. Farm 15 should be highlighted because of two observed drops in BM antibody titre (months 12 and 35). During the study, farm 15 underwent several management changes resulting in large groups of the milking herd being dried off or sold.

Correlation of BM antibody OD ratio and herd seroprevalence

Where dairy herds underwent whole WHT, BM samples were collected on the day of testing (where available) in order to correlate herd seropositivity with the BM antibody OD ratio. The results are illustrated in Fig 3. It is evident from both Fig 3 and Fig 1a–c that negative BM antibody results were rare for the farms in this study with all farms starting with moderate to high antibody titres. The only consistent low positive and negative results were returned by category 2 farms in the longitudinal study. In Fig 3, mid- and high positive results occurred most frequently with 6/16 (38 per cent) and 9/16 (56 per cent) farms falling into these categories, respectively. The seropositivity ranges for each category in Fig 3 were; low positive (1 farm)=35 per cent herd seropositivity, mid-positive (6 farms)=41–97 per cent herd seropositivity, high positive (9 farms)=79–100 per cent

herd seropositivity. At the point of whole herd testing, 2/6 farms (Farms 5 and 19) returning mid-positive BM antibody results contained PI animals (Fig 3)—it should be noted that these PIs were in the YS. For those farms returning high positive BM antibody results at the point of whole herd testing, all contained PI animals although only farms 1, 15 and 38 had PIs of milking age.

Discussion

This study examined the effect of differing BVD infection states (active/recent/historic) and vaccination strategies on BM antibody test results. As mentioned previously, AHVLA now report BM antibody ELISA results as percent positivites, however, the trends noted in this publication are still of value and comparable with those that should be expected with the current test.

The longevity of the BM antibody response

Category 2 farms (not vaccinated/no PI animals) in the current study demonstrated a gradual decrease in BM antibody titre with farm 24 taking 1290 days to decrease from a mid-positive to negative BM antibody titre. Houe (1999) reported similar findings in an unvaccinated herd that did not experience infection over an observed period of 1000 days. The gradual decline in BM antibody titre noted is

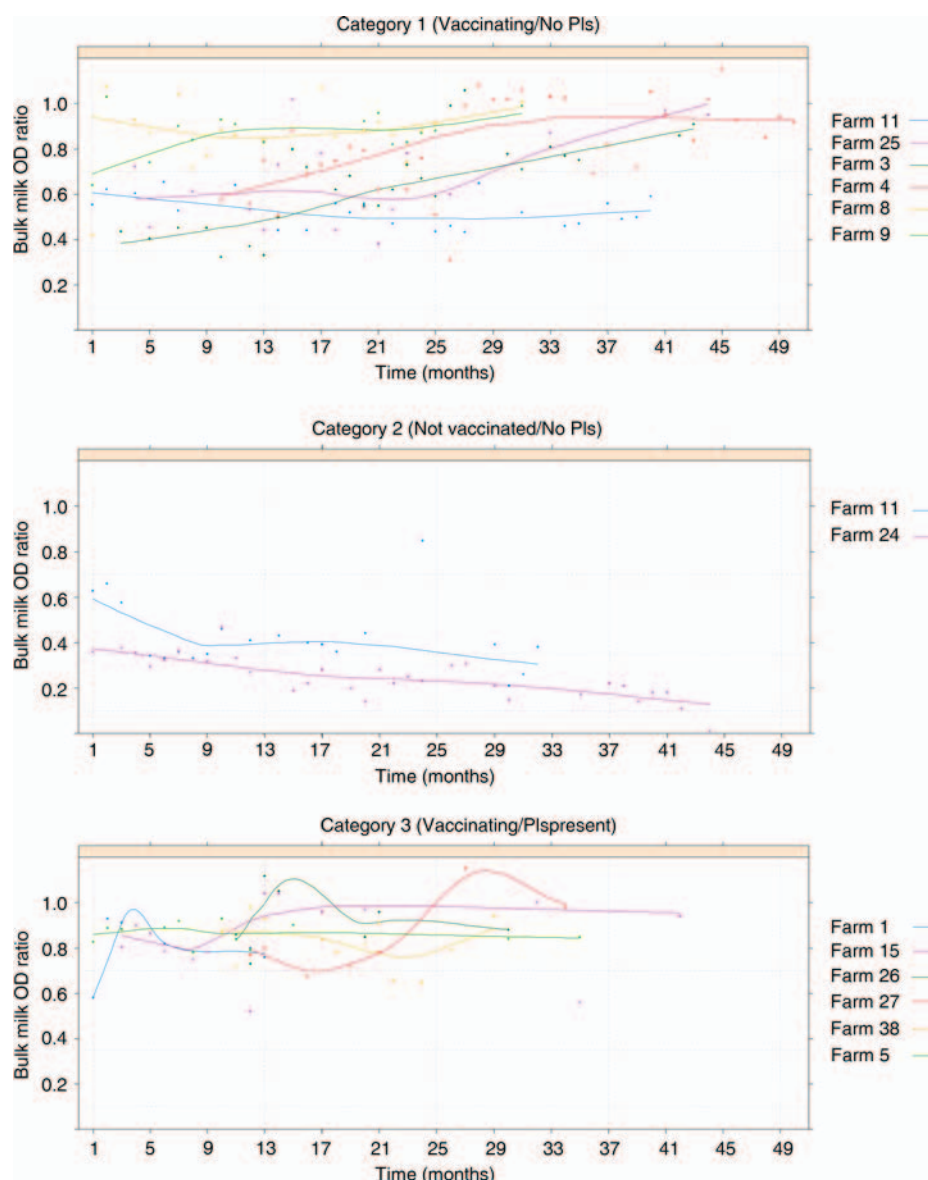


FIG 1: (a–c) LOESS regression curves illustrating change in bulk milk (BM) antibody titre over time in the three categories of herd investigated (span=0.7, degree=1). (a) Category 1 farms (vaccinating/no persistently infecteds (PIs)). (b) Category 2 farms (not vaccinating/no PIs). (c) Category 3 farms (vaccinating/PIs present). Vaccination (where applicable) occurs on months 1, 13, 25, 37 and 49—represented by the vertical gridlines. Low, mid- and high positive BM ranges are represented by the horizontal gridlines at 0.1, 0.35, and 0.7 OD ratio units for the range boundaries, respectively.

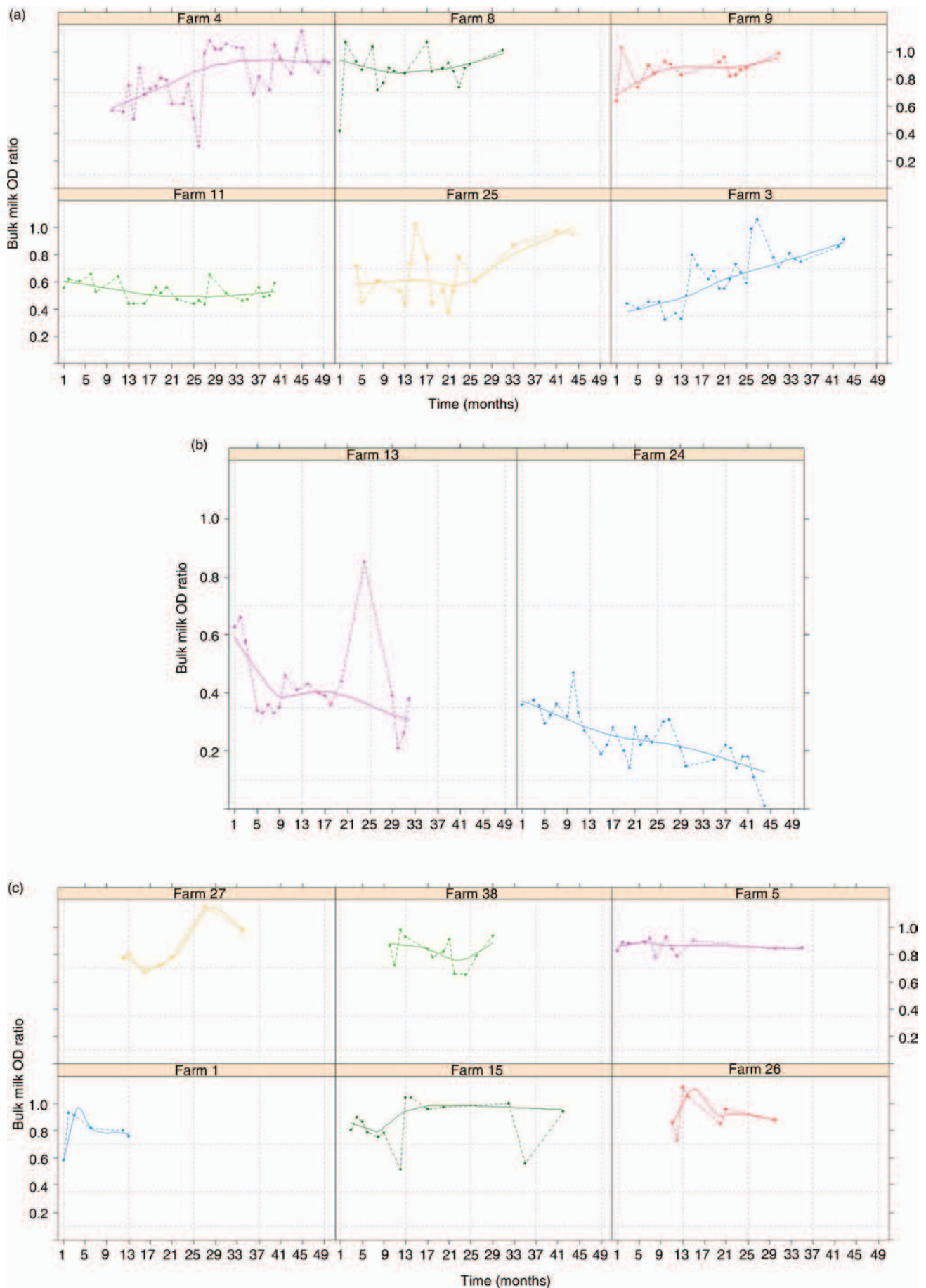


FIG 2: (a–c) Individual herd antibody graphs for each category of herd investigated. (a) Category 1 farms (vaccinating/no persistently infecteds (PIs)). (b) Category 2 farms (not vaccinating/no PIs). (c) Category 3 farms (vaccinating/PIs present). Dotted lines follow the raw data, whilst solid lines show the LOESS regression line for the individual farm (span=0.7, degree=1). Vaccination (where applicable) occurs on months 1, 13, 25, 37 and 49—represented by the vertical gridlines. Low, mid- and high positive bulk milk ranges are represented by the horizontal gridlines at 0.1, 0.35, and 0.7 OD ratio units for the range boundaries, respectively.

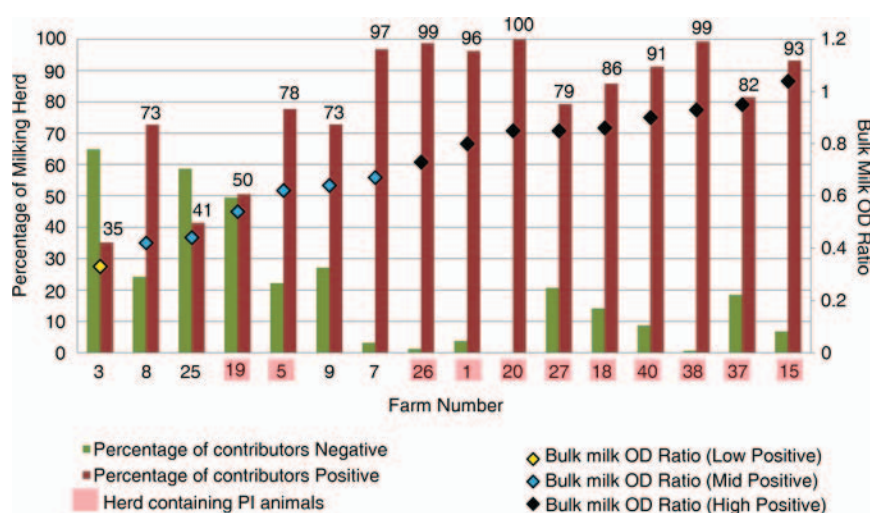


FIG 3: Herd seropositivity compared with bulk milk antibody OD ratio and category. Percentages of seropositive animals are stated above the red bar. Farms containing persistently infected animals are highlighted on the x axis in red.

likely due to the slow decline in antibody titre in individual animals (Moerman and others 1993) whilst also closely linked to culling rates, providing replacement animals are sought from BVDV free stock. The monthly variation noted between samples may be a result of the dynamic of individual cow milk volume and stage of lactation combined with the circulation of dry cows within the milking herd. In herds such as those presented in category 2, BM antibody testing is an effective test for BVDV surveillance since specificity is high in herds with low/negative seroprevalence. The spike in BM antibody titre noted for farm 13 in month 24 was highlighted to the farmer, and further milk samples were submitted in conjunction with YS antibody testing. Given that subsequent BM samples continued the pattern for antibody decline, and YS antibody testing did not indicate exposure to BVDV (data not shown), it was considered most likely that the month 24 result was test error.

For herds which have recently removed PI animals and do not vaccinate, high herd seroprevalence combined with the slow decline in natural antibody can result in low BM antibody test specificity for some years (Houe 1999, Lindberg and Alenius 1999, Valle and others 2001). As a result, testing YS cohorts and first-lactation heifer groups for antibody (to assess recent exposure) may be a more useful surveillance technique when BM antibody is high as a result of historic/recent infection (Lindberg and Alenius 1999). When conducting YS testing for serosurveillance, the selected animals should be unvaccinated and old enough such that colostral antibody has waned (>nine months is safest in the experience of the authors). It is also essential that selected cohorts are representative of the herd being assessed—testing only isolated groups which have had little exposure to other animals may not accurately represent herd status. In herds that do not vaccinate, the use of first-lactation milk samples can provide an accurate assessment of BVDV exposure. The application and interpretation of BM antibody testing in BVDV-vaccinated heifers is discussed below.

The effect of BVDV vaccination on the BM antibody response

The effect of BVDV vaccination on BM antibody is not widely reported in the literature. Pritchard (2001) indicated that BVDV vaccination of naive animals with a killed vaccine may not elicit a noticeable ELISA result upon antibody testing in the individual. However, the effect of vaccination of stock which are already seropositive is unclear. Evidence from this manuscript and other publications suggest that in the UK, >90 per cent of dairy herds have been exposed to BVDV (Paton and others 1998, Pritchard 2001) and, thus, seronegative herds could be considered rare. Furthermore, the use of different BVD vaccines in a herd over a series of years cannot be considered uncommon. With this in mind, herd categories 1 and 3 are of particular interest, since they provide data to aid interpretation of BM antibody results in the face of BVDV vaccination and historic/recent infection. Pregsure was the predominant vaccine used by the farms in this study and, whilst it is currently no

longer available, the remaining available killed vaccines are, in principle, similarly antigenic and likely to promote a BVDV antibody response.

The effect of BVDV vaccination on the BM antibody response in the absence of infection

The results from category 1 farms (vaccinated/no PI animals), indicate that in herds which are vaccinated annually and do not become infected, an increase in BM antibody over time can be expected. At the estimated monthly increase of 0.007 OD units per month, this would equate to a rise of 0.35 OD units over the 50-month observation period; an increase sufficient to move a herd from the bottom of the mid-positive range to a high positive result. The observed increase appears to be driven by yearly vaccination since no infection was detected in these herds during the observation period. It is evident that whilst the regression procedure highlights the upward trend, it does smooth out some transient peaks in the monthly results (Fig 2a). These transient increases in BM OD ratio appear to occur postvaccination and, perhaps, represent an anamnestic response to vaccination that is detected at the herd level. The frequency, magnitude and duration of these responses were not always consistent, hence, difficult to quantify but may depend to some degree upon which animals contribute to each sample in the natural cycle of dry cows, culls and replacement stock within a herd. Farm 3 demonstrates the postvaccine response most consistently appearing to experience transient BM OD ratio increases after vaccination in months 13 and 25, each of which subsequently decrease over a 3–4 month period to plateau until the next vaccination event. It is interesting to note that each plateau following vaccination was higher than that for the previous year, but this is not unexpected since the trend for these farms was for an increase in BM OD ratio with repeated vaccinations.

Farm 11 in category 1 was notable because it did not display either postvaccinal increases or the gradual rise in antibody titre with yearly vaccination that was observed for others in this group. Instead, farm 11 OD ratios appeared to plateau over the entire observation period. Replacement rate has not been assessed, but it is recognised that this will have an effect on the rate at which herd seroprevalence declines on a BVDV-free farm provided that replacements are home bred or acquired from a BVDV-free source (Houe 1999). Farm 11 did experience a high rate of laminitis cases due to nutritional problems, thus leading to a high cull rate (data not shown) with replacements sourced from BVDV-free farms which may account for the antibody plateau observed in Figs 1a and 2a.

The effect of BVDV vaccination on the BM antibody response in the presence of infection

The estimated monthly increase for category 3 farms was 0.001 OD units per month which equates to 0.05 OD units over a 50-month period. This is considerably less than the observation for category 1 farms. This essentially means that for category 3 farms, BM antibody levels appear to plateau at a high level for a prolonged period. The

plateau observed is most likely due to the recent presence of PI animals resulting in a high herd seroprevalence which, after PI removal, is maintained by annual herd vaccination combined with the length of time taken for antibody decline in individual animals.

Category 3 farms also appear to experience a transient increase in BM antibody following vaccination, although this appeared to occur less frequently than for category 1 farms. The lower sample submission by category 3 farms may explain the low frequency of the observed response to vaccine in some cases meaning that not all increases were detected. Where the increase does occur within category 3, it is apparent in both the regression curves and the line graphs of the raw data. The fact that the regression procedure also highlights the postvaccinal increases for category 3 farms (Fig 1c) may be due to a more 'herd wide' anamnestic response to BVDV vaccine, as vaccine and active infection will play a role in the immune response in these herds. The regression lines in Fig 2c for category 3 farms follow the raw data much more closely than those for category 1 farms. This is probably due to the lower degree of variation in the raw data as a result of lower sample submission by farms in category 3.

Farmer compliance with sample submission

Farms in category 3 submitted the least regular samples out of all groups tested which is curious since it is these farms that actually had active BVDV infection. This may reflect a whole issue of farmer attitude to compliance and commitment with herd health issues. Low sample submission rates seen in infected farms could reflect a less serious attitude towards BVDV control that partly explains the presence of infection on farms in this category. Alternatively, farmer perception might be that vaccination replaces the need for regular surveillance although one might also expect to see lower submission rates in category 1 farms if this were the case.

Alternative test protocols for surveillance in BVDV vaccinating herds

The results for category 1 and 3 farms in the longitudinal study presented here indicate that historic infection and vaccination can limit the value of BM antibody surveillance at the herd level for some time. The use of tests with 'Differentiating Infected from Vaccinated Animal' (DIVA) function have been investigated in relation to vaccine use and herd surveillance, although they may only be appropriate in specific circumstances. In theory, testing samples exclusively for antibody to the non-structural p80 (NS3) proteins can differentiate between infected and vaccinated animals since high p80 titres are not normally induced by killed vaccines. Several publications have addressed the specific use of p80 antibody ELISA testing in combination with the sole use of Bovilis BVD in herds with no pre-existing BVDV antibody (Makoschey and others 2007, Alvarez and others 2012) with both concluding that the test has good DIVA function under the conditions described. However, specific prerequisites to DIVA function at the herd level in the studies conducted by Makoschey and others (2007) and Alvarez and others (2012) were that the entire herd must be seronegative prior to investigation, and have only ever been vaccinated with Bovilis BVD; situations which are both rare in UK herds. Kuijk and others (2008) found that in a BVDV vaccinated herd that had recently cleared PI animals, BM antibody results were still positive despite testing with the p80 antibody ELISA test; the prolonged BM antibody response after natural infection explains this. Whilst p80 antibody ELISA testing may not be useful where historic infection and a level of seroprevalence in older cattle is expected, it may prove useful if utilised in a more targeted manner. Graham and others (2003) investigated the use of the p80 antibody ELISA in seronegative heifers vaccinated with inactivated BVDV vaccine. The results suggest that once seronegative replacement heifers reach milking age in a vaccinated herd that has older seropositive cattle, the p80 ELISA has the potential to be used in this group specifically enabling them to act as a sentinel group for the entire milking herd.

Interpretation of BM antibody results and classification of farm status

Whilst Fig 3 may lead readers to believe that high positive BM antibody results are always consistent with the presence of PI animals,

caution should be used before drawing this conclusion. The gradual increase in BM antibody titre experienced by category 1 farms (vaccinated/no PIs) over the course of the observed period has resulted in them achieving titres that are currently of similar magnitude to the starting titres for category 3 farms (vaccinated/PIs animals present). With many of the BVDV-free herds now returning high positive BM antibody results in the longitudinal survey, a high positive cannot be assumed to mean a herd is infected, without additional confirmation through other test procedures, such as examining seroprevalence levels in YS cohorts (>nine months of age) and first-lactation heifers. BM PCR testing may also be used at the point of herd screening, but it is important to note that the result only reflects the status of those animals contributing to the BM sample.

The data in Fig 3 also provides information relating BM antibody titre to herd seroprevalence. It is evident that the ranges of seropositivity observed in this study of 41–97 per cent and 79–100 per cent, in the mid- and high positive categories respectively, show some variation from those quoted in the AHVLA literature of 25–65 per cent and >65 per cent. It is curious that farm 7 had 97 per cent seropositive contributing animals and yet returned a mid-positive result. The result was, in fact, 0.67 OD ratio units which is only marginally below the high positive cut-off of 0.7 OD units. If the result for farm 7 is excluded from Fig 3, the range for farms returning mid-positive BM antibody results become 41–78 per cent antibody positive animals. The findings for herds in the high positive range are in line with those published by Niskanen (1993) where high ELISA absorbance values correlated with 87–100 per cent seropositivity, although the numbers of milking animals in the herds sampled by Niskanen (1993) tended to be fewer than those tested in the Somerset series.

An anomalous result may also explain the month 1 mid-positive result for farm 1 which occurred despite the prolonged presence of a PI in the milking herd at a time period where the cattle were housed and in close contact. However, Moerman and others (1993) reported similar findings in that complete seroconversion of a cohort of susceptible cattle exposed to a PI calf took three months, so perhaps the time taken here for the farm 1 milking herd seroconversion is not unreasonable.

Conclusion

Whilst BM sampling provides a rapid and cheap method of initial assessment, the dynamic nature of the BM antibody response to BVDV over time under the different conditions presented highlights the difficulty of diagnosing herd status from BM sampling alone. Vaccination status and the infection history of the herd need to be considered when interpreting the results of BM testing. The degree of variation between monthly samples and the potentially anomalous results in the data presented further highlight the difficulty of single BM sample assessment and underline the need to obtain at least two BM samples, so that any trends may be detected. Essentially, there is no BM antibody level above which presence of a PI animal is guaranteed. It is also difficult to define a lower limit below which a PI is not present. Ideally, a series of BM samples are required in combination with YS antibody tests and potentially BM PCR testing prior to making decisions regarding the course of action for BVDV control. The three categories of farm presented here are likely to be those which practitioners will encounter when investigating a herd BVDV problem, and thus it is hoped that the data provided will aid with interpretation of BM antibody results from the field.

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Monitoring the bulk milk antibody response to BVD: the effects of vaccination and herd infection status

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