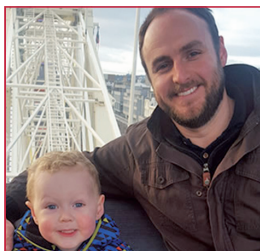


# An approach to investigating a herd level BVD breakdown via the Scottish BVD eradication scheme

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## Introduction

Bovine viral diarrhoea virus (BVDV) is a common pathogen that causes a range of syndromes in cattle including respiratory disease, reproductive failure, immunosuppression and mucosal disease resulting in economic losses (*reviewed in Lanyon et al., 2014*). BVD, a pestivirus infection of cattle, is one of the most economically damaging viral diseases affecting cattle in the UK. Farmers, vets, scientists and government have been working together on creating a national bovine viral diarrhoea (BVD) eradication plan for Scotland since 2009 (*Voas, 2012*). The basis of such schemes is the identification and removal of persistently infected (PI) cattle from herds, combined with husbandry changes to prevent infection being reintroduced (*Lindberg and Alenius, 1999*). Central to an efficient BVD control strategy is the prevention of foetal infection before it becomes immunocompetent. This is because infection of a naïve dam before 120 days of pregnancy may lead to the birth of an immuno-tolerant PI animal. This 'carrier' can shed virus throughout their life and become the main means of BVD spread in the herd (*Laureyns et al., 2010*). This report describes an investigative approach chosen with the aim of eradicating BVD from a dairy herd.

## Farm background

The herd of 280 Holstein dairy cows are housed year-round and milked three times daily with an average 305-day yield of 10,100 litres milk produced. Solids average at 3.9% butterfat

and 3.1% protein. All breeding animals are vaccinated annually against BVD, Leptospirosis and Infectious Bovine Rhinotracheitis. Annual replacement rate runs at around 28%. Only artificial insemination was performed using Holstein sires. Vet performs weekly routine scanning and current calving interval is 396 days. All calves are BVD antigen ear tag tested within twenty-four hours of birth. Bull calves are sold to a calf rearer once a negative BVD antigen result has been returned. Heifers are now reared in the local area but historically had been transported 100 miles away to a rearing unit. Previously heifers left the herd at around six months of age and returned one month before calving was expected.

## Case description and clinical reasoning

Annual compulsory BVD check test screening had been performed on the farm since 2010 in accordance with the Scottish Government requirements (*Scottish Government, 2011*). This consisted of blood testing five homebred, unvaccinated, animals aged between 9 and 18 months of age from each management group. These samples were checked for BVD antibodies by blocking enzyme linked immunosorbent assay (ELISA). See figure 1 for further information on the ELISA technique. The annual screen in 2010, 2011 and 2012 each returned ten negative results for BVD antibodies. The herd was then given a 'negative' status by ScotEID, the administrative database.

When the check test was performed in 2013, ten out of the ten heifers blood sampled had positive BVD antibody titres. Optical densities between 4 and 8% were returned. Interpretation of results is explained in figure 1. A two-pronged investigation was then initiated, consisting of adult milking herd screening and individual animal ear tag testing.

A polymerase chain reaction (PCR) test was performed for BVD antigen on a bulk milk sample to screen the contributing animals. A second bulk milk sample was taken sixty days after the first. Both samples gave a negative result for BVD antigen.

Ear tag testing of calves, within two days of birth, was commenced. All calves born since the 2012 check test, that remained in the herd, were tested for antigen by ear tag. Only around 40% of these animals were available. Over the next twelve months seventeen antigen positive calves were found by ear tag and a blood sample was taken three weeks later to identify BVDV-PI animals. Of these seventeen, thirteen tested BVD antigen positive, three tested negative and one animal died before testing could occur. All confirmed PIs were culled as soon as possible.

The dams of all confirmed PI calves were antigen tested by blood sample, none of which returned a positive result. These dams were all found to be heifers. These animals had been vaccinated pre-insemination with an inactivated virus vaccine (Bovidec, Elanco Animal Health) whilst away from the main premises. After questioning the head stockperson, it was found that unbeknownst to the herd owner the rearing unit had started buying stores. The heifers were housed in the same accommodation as these animals.

Heifers are no longer reared away from home after a neighbouring farm ceased business and these sheds are now rented. The herd now uses a modified live virus vaccine (Bovela, Boehringer Ingelheim Limited). The animal that introduced BVD to the rearing unit was not identified. However, several staff members have contact with livestock from other premises. It is possible that this was the source of introducing BVDV. A local contractor is used for slurry spreading. Insufficient disinfection of shared machinery between premises could also have been the source of BVDV.

Over the two years since the removal of the last PI from the home premises no further PI animals have been identified.

## Discussion

The approach described above is based upon guidance given by the Scottish Government BVD scheme and the Belgian eradication model as described by *Laureyns et al.*, (2010). Due to monetary constraints, a compromise in testing had to be made. Another method of investigation would have been to perform a bulk milk PCR antigen test, then blood test all animals, down to 3 months of age not contributing to the bulk milk sample, for antigen by ELISA. All animals less than 3 months and all calves born for the next 12 months would be BVD ear tag tested. The most cost-efficient method is to pool blood samples in groups of 20 and test for antigen by PCR (*Munoz-Zanzi et al.*, 2000). The same author found as prevalence increases beyond 3% the benefit of pooled testing drops. Since no adult PI animals were identified in this case the approach would have been unnecessary.

Antibody testing is appropriate for BVD surveillance because the antibody response to a natural BVD infection is detectable in both serum and milk using the blocking ELISA (*Graham et al.*, 2003). For serum samples, a negative/positive inhibition percentage cut-off value of 50% gave a sensitivity and specificity of 96.9% and 97.8% (*Beaudeau et al.*, 2001).

After a positive antibody result is returned further investigation is required. According to *Brownlie*, (2014), there are two routes for controlling BVD infection: ensure that all PI animals are recognised and removed from other livestock or ensure all dams are protected in early pregnancy from BVD infection, by good biosecurity and/or vaccination. In this case both approaches were used concurrently.

To screen the milking herd, testing of bulk milk samples by PCR for antigen was chosen as it provides a rapid and sensitive method of screening herds for the presence of BVDV infections (*Radwan et al.*, 1995). The RT-PCR technique showed 100% specificity and sensitivity in detecting PI lactating animals in a bulk milk sample (*Drew et al.*, 1999). The bulk milk PCR is effective in samples with 300 contributing animals as discussed via telephone with Dr. Paul Burr, working at Biobest Laboratories,



in 2012. There was a possibility that not all animals would have been tested using this regime due to milk being excluded on that day (examples being dry cow therapy and mastitis treatments). However, as any PI dam will always give rise to a PI calf it would be detected in the follow up to an antigen positive ear tag result.

BVD tag and test ear tags perform a skin biopsy with the sample retained being sent for antigen testing. Antigen ELISAs performed on ear skin tissue samples have been shown to be reliable and independent of the presence of colostral antibodies (Kuhne *et al.*, 2005, Hill *et al.*, 2007). A publication by Zimmer *et al.*, (2004) advocates a combination of reverse-transcriptase PCR and ELISA testing if using blood samples, because the antigen ELISA can give false negatives if maternal antibodies are too high. Blood sampling of calves less than 3 months old for BVD antigen was avoided on this basis.

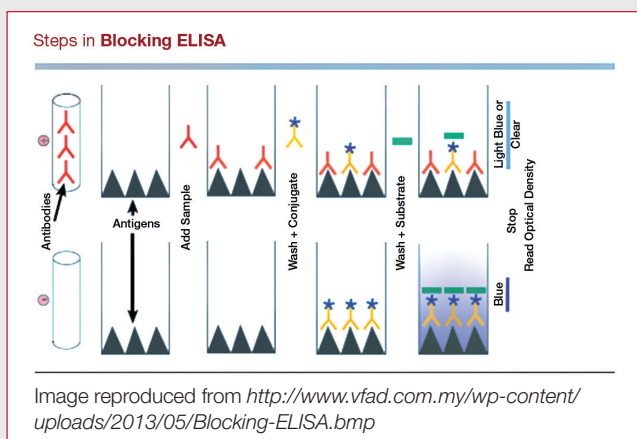
To identify a PI animal a period of three weeks between ear tag and blood sampling should be allowed as recommended by Munoz-Zanzi *et al.*, (2000). This allows any transiently viraemic animal to clear the infection as was the case in the three animals that re-tested negative.

Studies examining BVD vaccination on farms found that less than 30% of BVD vaccines are given correctly (Brownlie, 2014). The product datasheet for Bovidec (Elanco Animal Health) states 'the primary vaccination course comprises 2 doses of vaccine separated by a three-week interval and should be completed at least 7 days before service' (NOAH, 2017). The stockperson revealed that it was often up to six weeks before heifers received a second vaccination. To improve compliance a change of product to Bovela (Boehringer Ingelheim Limited) was made as a single vaccination three week pre-service confers sufficient protection (NOAH, 2017). This vaccine has been shown to protect pregnant animals and their foetuses from infection (Meyers *et al.*, 2007, Platt *et al.*, 2017).

Eradication of BVD from herds is worthwhile due to its economic significance although there have been few farm level estimates of the losses associated with BVD infection of the UK dairy herd (Gunn *et al.*, 2003). The intricacies of the costs and benefits involved in the eradication of BVD are beyond the scope of this report. Highlighted during this investigation is the need for good management compliance at farm level with regards to biosecurity and use of vaccines.

## Appendix 1: Blocking ELISA technique

In a blocking ELISA, presence of specific antibodies prevents the trapping of test antigen between a layer of immobilized capture antibodies and a reporting layer of



enzyme-linked antibodies, which are also virus specific. A positive sample causes a reduction of the OD which is expressed as a percentage inhibition, relative to the OD of a negative reference serum (Sandvik, 2005).

### Interpretation of BVD antibody ELISA results

- Optical density 0-30% - Positive titre for non-vaccinal antibodies
- Optical density 31-49% - Inconclusive titre for antibodies
- Optical density 51-100% - Negative titre for non-vaccinal antibodies

Inconclusive titres may arise from vaccination with inactivated vaccines, or be due to altering levels after recent non-vaccinal exposure. Repeat testing of such samples after a period of four weeks can be useful to monitor changes.





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