DIAGNOSTIC TESTS TO DETECT AND ERADICATE BVDV IN HERDS

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in the second part of their article, consider diagnostic techniques for bovine viral diarrhoea virus at the herd level and advise on four steps to control it

ABSTRACT

Bovine viral diarrhoea (BVD) virus is an economically important pestivirus affecting cattle worldwide. With the increasing number of European BVD eradication programmes, there is growing pressure for farmers in England and Wales to follow suit. Excellent diagnostic tests exist in combination with a thorough knowledge of the disease pathogenesis and epidemiology, making BVD eradication a real possibility. This article focuses on control at farm level, describing the use and interpretation of laboratory tests and issues surrounding effective control.

Keywords: BVD, persistently infected (PI) animals, biosecurity, BVD vaccination, BVD eradication.

OUR earlier article (*VT*44.20) reviewed the pathogenesis of bovine viral diarrhoea virus (BVDV) and highlighted the numerous bovine viral diarrhoea (BVD) eradication schemes that are either completed or under way throughout Europe.

We also mentioned the excellent regional schemes that have been implemented across England and Wales and the need to maintain the momentum these programmes have gathered. As yet, we have no national scheme for England or Wales and the development of one is the subject of much discussion. As such, this article will focus on control of BVD at farm level, using commonly available and reliable diagnostic techniques. Much of this discussion reiterates the excellent advice offered by the Cattle Health Certification Standards (CHeCS) and further details can be found in the CHeCS technical document found at checs.co.uk

BVD control at farm level

The authors often divide BVD control at farm level into four steps and these are highlighted in **Figure 1**.

In our opinion, proof of herd exposure to BVDV through seroprevalence testing (step two) is essential before going down the route of costly hunts for persistently infected (PI) animals (step three) and this can be determined accurately and at relatively low cost, provided the cohorts of animals to be screened are carefully identified.

For many farmers, this step builds a body of evidence alongside disease history (step one) that will help persuade them to go further down the route of BVD eradication. For those farmers where BVD exposure is shown to be unlikely by step two, efforts can be concentrated into protecting the herd (step four). This type of framework may be adapted to apply to many of the infectious diseases that we deal with on farm.

To take each step in turn:

Step one – farm disease history, biosecurity and vaccination

Disease history can provide vital clues as to the likelihood of BVD being present on a farm – previous confirmation of mucosal disease, previous bulk milk test results or diagnosis of BVD as a cause of abortion can all provide evidence the disease is likely to be present.

Considering BVD as a possible cause of poor calf health and/or suboptimal fertility is also important, as these may not immediately be associated with BVD infection since the disease often underlies these more overt problems in a herd.

Assessing biosecurity at this early stage can highlight weaknesses in the current farm policies and allow them to be addressed. Excellent biosecurity is essential for preventing incursion of disease on to a farm and it is often overlooked, potentially seen as an inconvenience, even though it has the potential to impact on multiple diseases in one go. Bought in animals and over the fence contacts are among the most important issues that need to be considered with respect to biosecurity and BVD, and these issues will be covered in the detail they deserve in a future article.

For immediate reference, the CHeCS technical document and the web-based herd management tool myhealthyherd.com (myhealthyherd.co.uk) can be referred to for further information on assessment of the biosecurity risks associated with BVD.

Reviewing farm vaccination protocols, if used, permits discussion to ensure vaccines are being used correctly. This can extend beyond BVD and can be used as an opportunity to review vaccination policies for multiple diseases. Some of the common issues experienced with BVD vaccine use are discussed by Meadows (2010) and include incorrect doses (volume and/or route of injection), incorrect timing of the primary course with respect to first service and incorrect intervals between the two injections that form the primary course.

Failure to correct these issues could result in a vaccine programme that is unable to provide sufficient protection in the face of challenge and provides a false sense of security as well as wasting resources.

Vaccines can be an important facet of a rounded infectious disease control programme, but it is important to note that BVD vaccination without measures to control PI animals is unlikely to result in eradication of the disease from an infected herd (Booth and Brownlie, 2012). Further information on vaccination to control infectious diseases and the correct use of BVD vaccines can be found in a review by Brownlie (2014).

Step two – assessing herd exposure to BVD

Testing a proportion of the epidemiological groups on a farm should be performed to ascertain whether exposure to BVD has occurred in any of these groups. In our opinion, this is one of the most important steps because, for a small amount of money, herd exposure can be assessed and this determines whether there is any need to go on a PI hunt (step three) or whether time and money should be spent on regular surveillance and enhanced biosecurity which, in many cases, will include ensuring a robust vaccination plan is in place.

Most testing at this stage should hinge around the use of targeted antibody testing to assess exposure of the selected cohorts. This can include bulk milk, pooled first lactation heifer milk and blood samples from five to 10 youngstock from each management group between nine and 18 months of age.

Even though it is a test for virus, many schemes may aim to include bulk milk PCR testing at this stage and this is perfectly acceptable provided the farmer is aware a negative result does not mean a negative or unexposed herd, but simply that a PI animal did not contribute to the milk sample tested at this point. Bulk milk PCR is also highly relevant to step three when screening for PI animals.

Step three – whole herd testing to identify PI animals

If step two indicates exposure has occurred, then step three involves the identification of PI animals with a view to culling them and thus removing these sources of infection from the herd (**Figure 2**).

At its quickest, this step may be performed by testing all live animals in one day for virus using a combination of the tests detailed in **Table 1**. Some of the options and technical issues for whole herd testing are described further in the discussion of tests for BVD virus.

Whole herd testing and initial PI removal is followed by a period of testing all newborns for 12 months to identify any PI animals that were in utero at the point of the initial herd test. A period of 12 months without the identification of a PI calf is required and if one is identified then 12 months of testing must begin again. For some however, whole herd testing can present a logistical and financial problem so it needs to be considered whether protocols should be put in place to allow a more gradual search for PI animals. We have, in the past, likened this type of search to a loan – it is easier to pay off in small chunks over a longer time period, but you pay more money overall in interest. Likewise, removing the disease in one go is more expensive in the first instance, but you don't suffer prolonged losses by retaining a PI animal for longer than you need to.

Step four – regular surveillance and maintaining/enhancing biosecurity

Step four essentially forms a regular repeat of step two with the emphasis on regularly assessing herd exposure, combined with biosecurity improvements and robust vaccination programmes. One-off testing is only relevant to

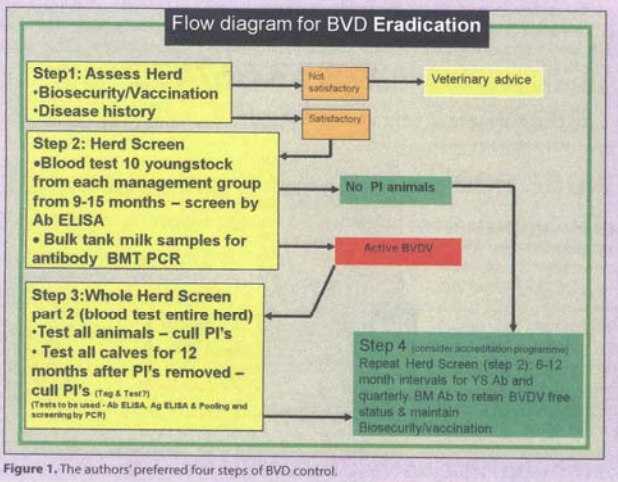

Figure 1. The authors' preferred four steps of BVD control.

Figure 2. Can you spot the PI animal in this photo? Lab tests were needed to identify it because they don't always stand out from the crowd.

when the tests were taken, whereas regular surveillance can help detect disease incursions early and, with careful thought, can help pinpoint the source of infection, avoiding the need for costly whole herd searches should exposure become evident.

At this point, herds may wish to consider joining an accreditation programme as this can provide a framework for surveillance and may also give some herds a sales advantage. Indeed, many breed societies have made it a condition their members must be part of an official CHeCS accreditation programme before selling animals through official sales.

To implement these steps, knowledge of the tests and their interpretation is vital. The following discussion focuses on the common tests mentioned in **Tables 1** and **2** and their use in practice.

Antibody tests

Antibody tests really indicate exposure in the case of BVD. They can be performed on blood or milk samples and are suitable for testing cohorts of animals from the separate epidemiological groups present on a farm. The tests available are detailed in **Table 2**, which has been adapted and updated from Brownlie et al (2000).

It should be remembered animals that have come into contact with field virus seroconvert and their antibody titre will rise following infection and remain high for four or more years.

From approximately day 14 of infection, the viraemia decreases along with the degree of nasal shedding and the animal recovers. With the exception of prolonged testicular infection in the bull (*VT*44.20), these animals are considered to be no longer infective. This is in contrast to Johne's or infectious bovine rhinotracheitis antibody-positive animals where these animals are infected and potentially infective for life.

Bulk milk antibody testing

Performing a bulk milk antibody test measures the antibody titres in all animals in the milking herd – youngest to oldest – and a positive result may mean one of several things.

- It may be measuring a herd-wide response to recent infection – all animals exposed.
- It may be measuring the antibody response to historic infection, thus detecting the response elicited by the older animals present in the milking group that had experienced BVD exposure some years ago. Refining the groups tested further could provide an up to date status for the herd – consider testing a pooled milk sample from the first lactation heifers present and/or expand the herd screen to include youngstock.
- There is some evidence to suggest regular vaccination, especially in animals that have been previously exposed to field virus, can increase bulk milk antibody titre (Booth et al, 2013).

A negative result is likely to mean the herd is largely naïve and unexposed, but regular tests are required to ensure this status is maintained, and thought should be given to protecting these herds. Mid-positive results are slightly more difficult to interpret without further testing – are they increasing (recent infection) or decreasing (historic exposure)? If the former, a titre increase can be expected on a future test approximately four weeks after the first. If the latter, the titre will decrease gradually and may not differ significantly from one month to the next. Natural variation between monthly or quarterly samples does occur as dry cows move into and out of the milking group, so thought should be given to this dynamic when interpreting a series of bulk milk antibody tests.

Blood testing for antibody

While bulk milk testing is a relatively quick and cheap method of gaining some insight into a herd's BVD status, it only concentrates on one epidemiological group; the milking herd, which is also the older animals present. Of course, it is not suitable for the beef herd either.

Considering the youngstock on a farm is vital to obtaining an analysis of herd exposure as a whole. In beef herds, these youngstock are often run with adults for a significant time and so may be representative of both the youngstock and adult groups.

Table 1. Diagnostic tests available for detection of BVDV or BVDV antigen (*adapted and updated from Brownlie et al, 2000*).

Test	Single animal or group	Requires	Measures	Interpretation
Immunoperoxidase staining	Single animal	Blood* or tissue sample	Viral antigen	Test provides a positive or negative result
Antigen ELISA	Single animal	Blood* or tissue sample	Viral antigen	Test provides a positive or negative result
Virus isolation	Single animal	Blood*	Live virus	Test provides a positive or negative result
Bulk milk PCR	Pooled milk sample from up to 300 animals	25ml of bulk tank milk with specific preservative in sample bottle†	Viral RNA	Positive result indicates presence of at least one PI animal in milking group at the time of sampling. This test does not screen animals that did not contribute to the bulk tank at the point of sampling
Pooled blood PCR	Individual samples from up to ten animals are pooled at the laboratory	Up to 10 individual blood* samples per pool	Viral RNA	Positive result indicates presence of at least one PI animal in the pool tested. Follow up with antigen testing of all contributors
Individual blood PCR	Single animal	Blood*	Viral RNA	Positive result indicates an infected animal. Antigen testing 28 days later is recommended to determine whether PI or acute infection

**Confirm the blood sample required with the laboratory. †Discuss with laboratory regarding submission of sample.*
PCR polymerase chain reaction, PI persistently infected

Discussion with the client is required to define the different epidemiological youngstock groups present between nine and 18 months of age, and issues such as groups kept in separate barns and certainly those kept on separate premises need to be considered.

Once determined, testing a small number of five to 10 animals from each of these groups will provide good information on BVD exposure within each group (Houe et al, 2006). These tests are often referred to as youngstock "check" or "spot" tests and they form a major component of ⇨ page 10

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POSTOPERATIVE ANALGESIA IN AGGRESSIVE FELINE PATIENTS

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Case notes

MR Bunting's three-year-old, male, neutered, Bengal cat named Swipe is presented to your surgery for a pelvic fracture repair.

Swipe is a difficult and temperamental patient at the best of times, but the pain and fear associated with his injury have turned him into a veterinary nightmare of claws and teeth.

Question

While you think you may just about manage to anaesthetise Swipe for his operation, you are worried how you and your nurses – or even his owner – will manage to provide adequate analgesia postoperatively.

Answer

Pelvic fracture repair is a painful and invasive procedure. Once the fracture has been stabilised, the patient will often be in less pain, but nonetheless, strong analgesics are still likely to be required to provide adequate pain relief.

As with most analgesic regimens, an NSAID should be provided (unless contraindicated), but this class of drugs alone may not prove to be sufficient in the immediate postoperative period.

If the patient can be hospitalised, and if intravenous access can be placed and maintained, then intravenous opioids can be given to effect. However, some scared or aggressive cats may settle better, and may be less stressed, at home. In this case, analgesia will have to be provided orally or by other means.

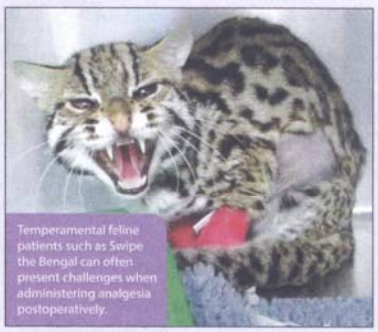
Most feline patients will refuse to ingest drugs that are not extremely palatable and because of this, and because of the size of these patients, the repertoire of drugs that can be given orally is very limited.

Tramadol (2mg/kg to 4mg/kg twice a day) is a drug for which the clinical effects in cats (mostly via the oral route) are poorly studied, probably due to the poor palatability of the pill. This drug is not licensed for use in cats in the UK.

Buprenorphine (0.02mg/kg three times a day) and, more recently, methadone (0.6mg/kg four times a day) given via the oral transmucosal route (off licence) have been shown to be effective (although less so than when given via the intravenous route) and these can often be used for postoperative analgesia in the owner's hands. However, if the patient is also aggressive towards the owner then oral transmucosal medication is not an option.

For such cats, the most effective "hands-off" way of providing strong analgesia is probably the off licence application of a transdermal fentanyl patch (12.5 micrograms to 25 micrograms fentanyl/hour).

Drawbacks of these patches are that the onset of analgesia is delayed, and that the plasma fentanyl concentrations are variable, but, if combined with an NSAID, fentanyl patches will generally provide an adequate level of analgesia for approximately five days.



Temperamental feline patients such as Swipe the Bengal can often present challenges when administering analgesia postoperatively.

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Take home message

To summarise, although aggressive felines can be very difficult to both handle and manage, there is the possibility of being able to provide adequate hands-off postoperative analgesia in these patients.

⇨ continued from page 9

BVD monitoring through CHeCS.

It is essential to appreciate individual antibody testing in animals less than six months of age will often pick up maternally derived antibodies (MDA) and thus animals under six months are unlikely to return interpretable results following antibody testing. For this reason they must not be included in such group testing.

Occasionally, MDA will not wane until nine months of age and, for this reason, the authors recommend to go no younger than eight or nine months of age when considering youngstock testing.

In reality, this may not be possible on all farms and so if animals as young as six months need to be included in the youngstock test, consideration should be given as to whether positive results are due to MDA or BVD exposure.

In these instances retesting the group four weeks later can clarify matters by revealing a titre that is either decreasing or increasing. If the former, the original result would likely be due to MDA; if the latter, there is a high possibility of exposure. Retesting positive individuals in this way can also be useful when a "check" test of 10 animals returns one out of 10 antibody-positive – is this early exposure of the group or one younger animal with MDA? Retesting and applying the same principles to an increase or decrease will help determine the answer.

Vaccination and antibody test interpretation

There is some question about the effectiveness of BVD vaccination on the results of BVD antibody tests. There are claims NS3, previously known as p80, antibody ELISA tests will only return a positive result if an animal has been infected and not if vaccinated; however, the literature does not always support this, especially if testing is undertaken within 12 weeks of vaccination (Booth et al, 2013). Furthermore, if a vaccinated animal subsequently becomes infected, it will seroconvert and recover in 28 days, but then be p80/NS3 antibody-positive for some years.

Table 2. Diagnostic tests available for detection of BVDV antibody *(adapted and updated from Brownlie et al, 2000)*.

Test	Single animal or group	Requires	Measures	Interpretation
Individual blood antibody ELISA	Single animal blood sample	Blood*	IgG antibody	Test provides a positive or negative result with indication of OD ratio or PP depending on the laboratory. Considered alongside the laboratory defined cut-offs some interpretation can be given as to the level of antibody detected with higher results associated with recent infection although, once raised, antibody titres may persist for some time. Seroconversion is confirmed by an increase in titre between paired samples
Bulk milk ELISA	Group test (although individual or pooled milk samples can be tested using the ELISA)	25ml of bulk tank milk with specific preservative in sample bottle	IgG antibody	Test provides a positive or negative result with indication of OD ratio or PP depending on the laboratory. Considered alongside the laboratory defined cut-offs some interpretation can be given as to the level of antibody detected, with higher results associated with recent infection although, once raised, antibody titres may persist for some time
Serum neutralising antibody test	Single animal	Blood*	Serum neutralising antibodies	Test is not routinely employed, but is available. Measures neutralising antibodies as distinct from antibodies, which may not play a role in immune protection

**Blood sample required by most laboratories is one clotted blood tube (red).*
IgG immunoglobin G, OD optical density, PP percent positivity.

Virus tests

The available lab tests for detection of BVD virus or BVD antigen are noted in **Table 1**. While still offered by some laboratories, virus isolation and immunohistochemistry are rarely used for on farm BVD diagnostics, with the preference being to use easier, cheaper and quicker techniques, such as ELISA and PCR. The most common virus tests used in practice are those testing for viral antigen using ELISA and those that look for viral RNA via PCR.

The distinction between antigen and RNA tests may seem pedantic as both are always positive in the PI animal (with the exception of antigen ELISA tests performed in the presence of MDA). However, in an acutely infected animal, antigen is only normally detectable by ELISA from around day three to 10 postinfection, whereas a PCR test may remain positive for 50 days, and in some cases up to 100 days, following acute infection (Collins et al, 2009).

The aim of these virus tests is to identify PI animals so, when confirmed, they can be culled. PI animals will typically form between one to three per cent of an infected herd (Booth and Brownlie, 2012; Houe, 1999). It is not the purpose of this article to document all the different options for herd testing, but the details in **Table 1** will allow the appropriate targeted use of each of the available virus tests.

Whether a decision is made to test the whole herd for virus in one go or to spread the effort and cost over time, the aim is to test all animals to confirm their PI status.

The major diagnostic gap to be aware of when using the available virus tests is that for those animals under 30 days of age, antigen ELISA on blood is not considered suitable, as a small amount of MDA may mask any circulating antigen from the ELISA. PCR or ear notch testing are considered suitable for animals less than 30 days of age. In addition to testing all individuals, there are also options for indirect testing of adults by testing calves. When using techniques such as tag and test antigen ELISA, where a newly tagged animal is identified as non-PI, this confirms the dam is also not PI while a PI calf places suspicion on the mother. The potential for mismothering should be considered when conducting this type of testing.

Conclusion

We hope this article provides a framework for structured BVD control at herd level. An excellent understanding of the disease pathogenesis and epidemiology, combined with reliable BVD lab tests, makes eradication a real possibility.

In the individual and committed herd, eradication is achievable in as little as 18 to 24 months and can only offer health and production advantages to the farmer.

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