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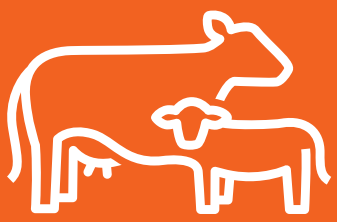
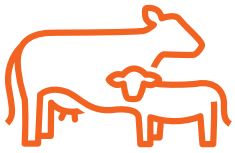


BVDV

Guidelines for BVDV
(Bovine Viral Diarrhoea
Virus or Bovine Pestivirus)
Testing for Veterinarians

2010

Guidelines for BVDV Testing for Veterinarians



These guidelines have been adapted from guidelines developed by the BVDV Technical Advisory Group – a group of veterinary researchers and practising veterinarians who provide services to dairy and beef herds, and feedlots. The primary objective of these guidelines is to improve decision making on investigation and control of BVDV infection in cattle.

Could BVDV infection be contributing to the problems in this herd or management group?

Step 1: Consider the evidence

- Look at the clinical history of the herd or management group.
- Undertake clinical examinations and diagnostic testing for BVDV. Include investigation of the likely contribution of other potential infectious and non-infectious causes of reduced performance.
- To help interpret the results, consider other evidence that may approximate when BVDV infection occurred in the herd or group. If a PI animal has been detected, determine its age – its dam is likely to have become infected 5-8 months prior to the birth of the PI animal.

Outcome

- If you conclude that BVDV is likely to be contributing to the problems detected in this herd or management group, go to Step 2.
- If you conclude that BVDV is unlikely to be contributing to the problems detected in this herd or management group consider how to decrease the risk of BVDV infection.

Step 2: Determine the proportion of heifers or cows in this herd that are likely to be susceptible (i.e. not immune) to BVDV infection during the next mating period

- Do a serological profile of this herd or management group.
- Decide whether this herd or individual management groups within the herd will have a high or low proportion of animals susceptible during the next mating period.

Outcome

- High proportion of susceptible animals anticipated during the next mating period.
- Low proportion of susceptible animals anticipated during the next mating period.

Step 3: Assess the risk of exposure to BVDV infection during the next mating period

- Look at all the possible ways that BVDV could be introduced to and/or transmitted in this herd or management group.
- Decide whether this herd or management group will have a low or high risk of exposure to BVDV during the next mating period.

Outcome

Assess the anticipated status of this herd/management group during the next mating period.

The herd or management group will fall into one of four categories:

1. High % of heifers or cows susceptible/high risk of exposure.
2. High % of heifers or cows susceptible/low risk of exposure.
3. Low % of heifers or cows susceptible/high risk of exposure.
4. Low % of heifers or cows susceptible/low risk of exposure.

Step 4: Options for controlling BVDV in this herd or management group

- Look at all the possible ways that BVDV could be introduced to and/or transmitted in this herd or management group.
- Decide whether this herd or management group will have a low or high risk of exposure to BVDV during the next mating period.

Outcome

In discussion with the herd manager, decide which of the following control options or combination of control options best suit this herd or management group:

1. Vaccination.
2. Partial vaccination.
3. Autovaccination.
4. Removal of PI animals.
5. Biosecurity measures.
6. No specific management to control BVDV.

Assessing the contribution of BVDV to reduced reproductive performance

Sampling and test requirements vary with the time at which loss is identified

Examples include:

1. Reduced reproductive performance

- lower than expected conception rate and/or delayed time to conception, reduced pregnancy rate, abortions, spread-out calving pattern usually identified by a reduced pregnancy rate at pregnancy diagnosis. If BVDV infection has contributed, infection will have occurred one to several months before the event is observed. Collect serum samples from representative affected animals (approximately 10 animals) and test for antibodies using the BVDV AGID test.

Determine the proportion of positive animals and whether there is evidence of recent infection based on the strength of AGID reactions.

2. Abortions and reduced calving rate

- may be evidence of abortion or just failure to calve. If there is no foetal material available, sample as (1) above. If a foetus is available collect:

- Pericardial fluid to test for IgG level and BVDV antibody
- Fresh lung and spleen to test for BVDV antigen using antigen-capture ELISA or BVDV virus using virus isolation or PCR
- Fixed tissues for histopathology.

3. Stillbirths and reduced viability of calves

- unexpectedly high incidence of stillbirths, congenital defects, mortality in young calves, weak, stunted and 'poor doing' calves.

If BVDV is contributing to these problems, calves will have been infected at some stage during gestation. Collect samples as in (2) above and test for both BVDV antigen or virus and BVDV antibodies. (Remember that the ability to detect antigen may be compromised in calves <5 months old due to the possibility of residual colostral antibodies. Testing of skin biopsy samples (ear notch testing) overcomes this problem).

4. Weaner and yearling disease

- some or all of these will be persistently infected animals if BVDV is contributing to the problems. These animals were most likely to have been infected with BVDV between about 30 and 90 days of gestation. Test for BVDV antigen in the blood or skin biopsy if the animal is alive, or from the spleen/lung if the animal is dead. Also, fix tissues for histopathology.

5. Mucosal disease

- clinical diagnosis of mucosal disease. Presenting signs include persistent fever; ulceration of gum, mouth and gut; lameness and ulcers between the claws; bloody diarrhoea and death.

6. Post-mortem results

- evidence of changes consistent with BVDV infection (in foetuses and calves: cerebellar hyporplasia; in weaners, yearlings and adults: mucosal disease).

7. Increased incidence of infectious respiratory or enteric diseases

- unexpectedly high incidence of diseases such as bovine respiratory disease (BRD) or calf scours.

How is an AGID serological profile obtained?

When obtaining a serological profile, the structure of the herd must be taken into account. Review the herd structure considering the number of management groups and how these groups are segregated by husbandry management and age.

If animals in a management group are the same age

- collect serum from an appropriate number of randomly selected animals.

If there are multiple ages in the management group

- sample an appropriate number of animals from each age category (e.g. heifers, first calvers, mature cows). The optimal group profile will include samples from heifers to be joined, joined heifers, first calvers and mature cows. Recording the age of cows is valuable and assists with interpretation of results.

If there are multiple management groups

- each group should be sampled if they have been managed discreetly during the current (or previous) breeding season.

The precision of the serological profile will depend on how many animals are tested.

What does an AGID test serological profile tell you?

The AGID serological profile indicates:

- The seroprevalence of BVDV antibodies in the herd or management group; that is, what proportion of animals have been exposed to BVDV and are now immune, and what proportion of animals are susceptible to infection
- An estimate of how recently the exposure may have occurred within the herd or management group.

Estimating the percentage of susceptible cattle in a herd or management group

The percentage of tested animals that are seronegative enables you to estimate the percentage of animals in the herd/group that are susceptible. It is important to consider the confidence interval for the estimate when interpreting these results. If you sample a larger number of representative animals, the confidence interval will be narrower and so your estimate of the percentage of animals in the group/herd that are susceptible will be more precise.

Table 1 shows the 90% confidence intervals for various numbers of animals tested and for the various percentages that test negative. For example, from the table, if you test 20 animals and 6 (30%) are seronegative, you can be reasonably sure that the percentage of seronegative animals in the herd/group is between 14% and 51%. And if you test 30 animals and they all test negative, you can be reasonably sure that the percentage of seronegative animals in the herd/group is at least 90%.

Table 1. Determining the precision of the serological profile with different numbers of animals tested

Number of animals tested			
% of animals tested that were seronegative	10	20	30
(Likely % of animals in herd/group that are seronegative)			
0 (i.e. all tested positive)	0-26	0-14	0-10
30	9-61	14-51	17-47
50	22-78	30-70	34-66
70	39-91	49-86	53-83
100 (i.e. all tested negative)	74-100	86-100	90-100

Table 2.

Interpreting the strength of the AGID serological results		
0	Negative	Susceptible animal
1 or 2	Weakly positive/positive	Exposure to BVDV less likely to have happened within the last 12 months
3	Strongly or very strongly positive	Recent infection, usually in the last 3-9 months

What diagnostic tests are available to detect BVDV antibodies and what do they tell you?

- Vaccination history must be known to properly interpret results.
- Although the scoring systems for these tests have been standardised nationally, reading of the results is subjective; results may therefore vary slightly from laboratory to laboratory but the herd profile should not change significantly.

Test	Interpretation	Advantages/Disadvantages
AGID	Used to measure seroprevalence and to detect recent infection (especially within last 9 months) Test will detect antibody (Ab) from at least several years before Ab reaction measured and quantified: negative = susceptible animal 1 or 2 (weekly positive/positive) = exposure to BVDV less likely to have happened within the last 12 months ≥3 (strongly or very strongly positive) = recent infection, usually in the last 3-9 months **Samples from pregnant females that yield this result indicate the female may be carrying a PI foetus**	• Moderately high sensitivity (in the period of about 2 years postinfection); high specificity • Quick test, sample quality less important than VNT – good for samples • Can detect Ab 2 weeks post-infection with maximum reaction usually about 5-12 weeks post-infection • Will not usually detect Ab post-vaccination • Vaccinated cattle will still develop AGID titres if naturally exposed to BVDV. This can be used to monitor if BVDV is still circulating in a herd that is vaccinated
Virus neutralisation test (VNT)	Used to measure seroprevalence; difficult to predict time of infection • Most very high titres (>1280) indicate infection in last 9-12 months • Ab titre measured as a reciprocal of serum dilution: ≤10 = Negative 20, 40, 80, 160, 320 etc. to >5120 = positive	• Detects Ab 2 weeks post-infection but titres rise for up to 9 months – difficult to interpret 'rising' titres on positive paired samples • Detects vaccine Ab and good for comparison of titres in sequential samples from vaccinates • Good test for detection of low levels of Ab; need very good quality samples • Sensitivity and Specificity of test varies depending on antigenic similarity between reference strain used in test and herd strain

Test	Interpretation	Advantages/Disadvantages
Antibody ELISA	Used to measure seroprevalence by detecting Ab. May be done on blood or milk samples	As for Virus Neutralisation Test ELISA tests typically detect antibodies sooner after infection/vaccination than VNTs TEGO blood collection devices allow blood collection by producers at their convenience. Samples do not require refrigeration. PI testing can be done on the same samples if necessary
BVDV Antibody ELISA for Milk	May be used to measure the BVDV antibody level in milk from individual animals, or more commonly in a sample of milk taken from the vat at the completion of milking. There is a correlation between this latter measurement and the proportion of seropositive animals in the milking herd, and the concentration of antibodies in seropositive animals The results of this test are expressed as a S/P ratio (Sample/Positive: Control ratio) • Very low values (approximately 0.25 or less) are indicative of very low seroprevalence in the milking group • Very high values (approximately >1.0) in an unvaccinated herd are indicative of high seroprevalence in the milking group, and may suggest there are many animals that have been infected relatively recently, or sometimes that there may be one or more PI animals in the herd • S/P values between 0.25 and 1.0 include herds with a wide range of seroprevalence which require further investigation (e.g blood sampling for an AGID serological profile) before recommendations can be made about BVDV management Very large changes in the S/P ratio indicate changes in the BVDV status of the herd, which require review of the BVDV management of the herd. For example, a significant drop in the SP ratio may indicate a herd is more susceptible. A significant increase in S/P ratio may indicate the recent introduction of a PI	• Recent vaccination can result in a significant increase in the S/P ratio • Care should be taken that vat samples are representative of the milking group • Easy to collect, inexpensive test. Facilitates regular monitoring of the BVDV status of the milking herd • Doesn't assess the other management groups in the herd i.e. dry cows, heifers, bulls

What are the tests for identifying Persistently Infected animals?

- PI animals will have an ongoing negative or weak antibody reaction and a positive test for antigen.
- Care should be taken in interpreting results from testing blood samples from animals less than six months of age as colostral antibodies may reduce the sensitivity of the antigen capture ELISA test in detecting PI animals. The preferred samples for testing calves under 6months of age to detect PI animals are ear notch (skin tissue) or hair.

Test	Sample	Cost/sensitivity/ turnaround time	Advantages/disadvantages
Antigen capture ELISA	Leukocytes Blood clot Serum Plasma Tissues Skin e.g. ear notch Hair, including the roots	Relatively low cost/ high sensitivity Turnaround: 1-2 days (serum, plasma, skin) 2-3 days (leukocytes, tissues)	• Can be used for whole herd/group testing and for detecting individual PI animals • Infrequently detects transiently infected animals if testing blood or serum but may occasionally detect transient infections with skin as the sample • Skin testing (Ear notch testing) The advantage of using a skin sample is that colostral Abs do not interfere with the test • Must re-test test positive animals 3 weeks later to confirm persistent infection • Cost of skin testing is less than blood testing because blood samples have to be spun down and serum separated off • Ensure cross contamination of samples does not occur, and samples are appropriately identified for individual animals

Test	Sample	Cost/sensitivity/ turnaround time	Advantages/disadvantages
Polymerase chain reaction (PCR)	Serum Plasma Tank Milk	Moderate cost/very high sensitivity 2-3 days turnaround	• Not most cost-effective method of determining individual PI animals • Good for screening of pooled samples from whole herd/group bleeds. If a pool tests positive, the individual samples are re-tested to identify infected individual(s) • Must re-test suspected PI animals 3 weeks later to confirm persistent infection (rather than transient infection) • There is potential for cross-contamination • In tank milk, a negative test provides confidence of no exposure on up to 400 milkers Positive pools suggest presence of a PI and require individual screening to find these animals.
Virus isolation (VI)	Serum Blood Tissues	High cost/high sensitivity 1-3 weeks turnaround	• Not preferred test for routine herd screening due to cost and turnaround time • Must re-test suspected PI animals 3 weeks later to confirm persistent infection (rather than transient)

Options for controlling BVDV in a herd or management group

1. Vaccination

Important consideration

Vaccination becomes more appropriate as the proportion of susceptible cattle in the herd/group increases and as the risk of exposure increases. If you are planning to conduct an artificial breeding program, strong consideration should be given to vaccinating breeding females prior to conducting the program.

What are the benefits of vaccination?

- Properly conducted, vaccination of a herd/management group will result in a high proportion of the females being immune to infection, which minimises the likelihood of losses if the herd/group is exposed to BVDV.

What are the disadvantages of vaccination?

- Vaccination is not 100% effective. There will still be a proportion of susceptible animals in the herd/group despite vaccination so adequate biosecurity and monitoring procedures are still required to prevent re-introduction and transmission of the virus and subsequent infection of susceptible animals. It may also be advisable to consider testing all the cattle in the herd/group to identify and cull PI animals. PI breeding females will produce PI calves even if vaccinated.
- Vaccination must be maintained to ensure long-term protection. If vaccination of a herd following BVDV infection is maintained for several years, the herd is likely to become free of infection; it then follows that in the absence of vaccination, this herd will experience an increase in the proportion of susceptible animals over time unless re-exposed to BVDV from an external source.

What is the vaccination protocol for a herd or management group?

- **Whole herd/group vaccination program**
  – in the first year each breeding female should be vaccinated twice with the second vaccination occurring at least 4 weeks before commencement of mating. Thereafter, females should be vaccinated once a year, preferably 4 weeks prior to the start of mating.
- **Replacement heifer vaccination program**
  – heifers should be vaccinated twice, with the second vaccination occurring 2-4 weeks prior to the commencement of mating.
- Previously unvaccinated cows and bulls will require a primary course of vaccination consisting of two doses of vaccine, with an interval of 4 weeks – 6 months between doses. The primary course of vaccination should preferably be completed 2-4 weeks prior to joining/insemination.
- It is important that Pestigard® vaccine is handled and administered correctly.
- The vaccine should be stored between 2°C and 8°C and protected from light.
- When ready to use the vaccine, it must be gently mixed (vigorous shaking may cause the product to foam) and kept thoroughly mixed during use.
- All the vaccine must be used within 30 days of opening.
- The dose of vaccine on all occasions is 2 mL. The vaccine should be injected subcutaneously.
- Protective immunity is expected to develop within 14 days of the second dose. Pestigard® is safe for use in pregnant cows.

	Heifers		Cows	1st Season or New Bulls		Bulls
Schedule	**Primary Course**		**Annual Booster**	**Primary Course**		**Annual Booster**
Timing	**1st Dose:** 6-8 weeks pre-joining	**2nd Dose:** 2-4 weeks pre-joining	2-4 weeks pre-joining	**1st Dose:** 6-8 weeks pre-joining	**2nd Dose:** 2-4 weeks pre-joining	2-4 weeks pre-joining
Farm Management Flexibility	1st Dose may be given up to 6-months pre-joining		May be given at pregnancy testing or branding. Pregnant cows can be vaccinated	1st Dose may be given up to 6-months pre-joining		

2. Partial vaccination

(Vaccination of part of the herd or management group that is more likely than the rest to become infected and experience losses due to BVDV infection).

Important considerations

This option is commonly implemented to reduce the likelihood of BVDV infection in replacement heifers. Often, due to the fact that replacement heifers are reared in isolation to the adult herd, there may be a high proportion of susceptible animals in this group. Analysis of the results of serological profiling of the heifer and cow herds/groups and consideration of the timing of introduction of the replacement heifers to the cow herd relative to the commencement of mating will enable assessment of the likely benefits of implementing this option.

What are the advantages of partial vaccination?

- The major advantage of this option is the reduced number of vaccine doses required for the herd.

What are the disadvantages of partial vaccination?

- Infection in the unvaccinated animals may result in losses and maintenance of infection in the herd/group.

What is the protocol for vaccinating heifers only in a herd or management group?

- Heifers should be vaccinated 4 weeks – 6 months apart, with the second vaccination occurring 4 weeks prior to commencement of mating. If the heifers are going to be maintained in isolation from the cow herd until after calving, it may be beneficial to vaccinate them again prior to entry into the cow herd.

3. Autovaccination

(Controlled, natural transmission of infection using PI animals)

Important considerations

If this approach to inducing immunity is to be used, it is critical that autovaccinator cattle (i.e. the animals you suspect are PI animals) are tested and confirmed to be PI animals.

Further, the PI animals must be clearly identified so that they are not inadvertently mixed with cattle in the early stages of pregnancy.

The PI autovaccinator cattle should be placed in a small yard with the replacement heifers each year at least 16 weeks prior to the start of mating and kept separate from breeding females at all other times.

As PI cattle have a significantly shorter life expectancy than normal cattle, if the autovaccinator cattle die and there are no other PI cattle in the herd, the herd will progressively become more susceptible to infection over time.

What are the advantages of autovaccination?

- Yarding susceptible cattle with a PI animal for a period of several days should result in a high proportion (>80%) becoming infected and subsequently developing long-lasting immunity. To increase the likelihood of exposure of the susceptible cattle to the PI animal(s), the cattle should be fed and watered at a single point in the yard, and regularly moved around in the yard.

What are the disadvantages of autovaccination?

- You cannot be guaranteed that all cattle in the group exposed to the PI animal will become infected and hence immune because of issues such as the number of animals in the group and the behaviour of the PI animal (it may be a 'shy' animal). Most studies that have induced infection using this method have exposed a relatively small numbers of heifers (<30) to a single PI. There is a lack of information on how long much larger mobs of heifers should be exposed to a single PI animal to ensure the majority become infected. A possible way to address this problem would be to draft large heifer mobs into several smaller groups in the cattle yards, and then rotate the PI between pens of heifers every couple of days for a period of about 2 weeks.
- Exposure to infection using this method can be variable and some susceptible cattle may still be present in herds/management groups. Accordingly, the seroprevalence of BVDV antibodies in exposed groups should be assessed.
- There is an increased risk of infectious disease among cattle, particularly young cattle exposed to a PI animal due to the immunosuppression associated with transient BVDV infection. Investigation of outbreaks of bovine respiratory disease in weaners has been linked to the presence of PI calves in the weaner calf group subsequent to outbreaks of foetal infection in breeding herds.

4. Removing PI animals from the herd or management group

Important considerations

If this option is implemented then farmers must recognise that, over a period of several years, the proportion of susceptible cattle in their herd/group will increase substantially. If the risk of exposure to infection continues to be high then options to enhance the immunity of the herd/group (e.g. vaccination of the herd/group) and/or reduce the risk of exposure to infection (e.g. implement biosecurity measures) should be strongly considered.

For maximum benefit, PI animals need to be removed prior to the subsequent joining to prevent exposure of seronegative pregnant females and their unborn calves. If a producer aims to eradicate BVDV from their herd/management group, several rounds of herd/group testing may need to be done, followed by ongoing monitoring, to verify freedom from infection. This approach on its own will only be successful if the majority of cows are immune to BVDV from previous exposure, which generally only occurs in situations of high stocking density and smaller herd sizes.

What are the advantages of removing PI animals from a herd/group?

- As PI animals are the major source of infection, their removal from a herd or group will significantly reduce transmission of BVDV.
- If all PI animals are removed prior to joining, the risk of in utero exposure of the subsequent calf crop to BVDV will be low. This minimises or eliminates the proportion of PI calves introduced into the herd/group.
- In situations of high-stocking densities, the removal of PI animals may improve the efficacy of a BVDV vaccination program, because the animals are not constantly being challenged.

What are the disadvantages of removing PI animals from a herd/group?

- In large herds or management groups, removing PI animals may not be an option on economic grounds because of the large cost involved in sampling and testing individual animals.
- Removing PI animals prior to the subsequent joining is difficult in herds with long joining periods and requires the handling of fairly young calves. Females that abort calves infected with BVDV or deliver stillborn calves may also act as a source of infection of early pregnant cows running with them.
- Removal of PI animals also means that, over a period of several years, the proportion of susceptible cattle will increase substantially if no further PI animals are introduced into the herd or management group. Removal of PI animals from the herd should be combined with control options to minimise the risk of re-introduction of BVDV to the herd.
- Several rounds of testing may be required to identify all PI animals and unless all older cows in the herd have developed natural immunity to BVDV, foetal infection with BVDV and birth of PI animals will continue to occur. Vaccination of the herd may be necessary to ensure herd immunity is maintained.

5. Biosecurity measures

Important consideration

In situations where there is a high percentage of susceptible cattle in the herd or management group, the consequences of infection are high if biosecurity fails.

How can you minimise the risk of BVDV introduction from outside animals?

- Establish a closed herd and only use semen and embryos from confirmed BVDV-free donors.
- Test all introduced cattle, including bulls, to confirm that they are not persistently infected.
  - until test results are available, introduced cattle should be quarantined away from the main herd.
  - as transiently infected cattle can sometimes transmit BVDV, introduced cattle should be quarantined for a minimum of 6 weeks.
  - in the case of pregnant females, no tests are available to determine whether they are carrying a PI foetus – introducing pregnant females should be avoided or these females should be quarantined on the farm and their calves tested to confirm that they are not persistently infected.
- Minimise over-the-fence contact between breeding females and neighbouring cattle. This can be achieved by avoiding grazing females in boundary paddocks, or in some cases, using double-fencing.
- Vaccinate cattle that are going to be transported off-farm and later returned (e.g. travelling to agricultural shows, agistment, markets) 8 weeks prior to movement.
- Avoid mixing different groups immediately prior to and during mating. This will minimise the risk of *in utero* transmission of BVDV and the risk of the birth of a PI calf.
- Clean cattle handling, transport and husbandry equipment, and protective clothing between different herds/groups of cattle, and particularly between properties.

To effectively control BVDV, frequently a combination of control measures such as vaccination and implementation of biosecurity measures is recommended.

For further information please review
www.bvdvaustralia.com.au

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