Veterinary Herd Investigation Protocol following positive test results for BVD virus

Includes explanatory notes on a series of tools which have been developed on the ICBF database to assist with herd investigations.

For further information on herd biosecurity see information from Animal Health Ireland at www.biosecurity.ie.
AIM
This document is designed to provide a structured framework for the conduct of a herd investigation following the birth of a PI or TI calf, with emphasis on...

...IDENTIFYING THE SOURCE OF THE VIRUS, REMOVING ALL PI ANIMALS AND REVIEWING HERD BIOSECURITY

It is assumed that the additional (i.e. confirmatory re-testing of the calf to exclude transient infection and testing of the dam of positive calves) and enhanced testing (TASAH sampling) required by the BVD Regulations (2017) (S.I. 30 of 2017) and the Notification and Control of Diseases Affecting Terrestrial Animals (No. 2) Regulations (2016) (S.I. 130 of 2016) have been carried out. From 2020, access to BVD data in ICBF is automatically granted to the veterinary practitioner designated to carry out the investigation by the herd owner.

IDENTIFY THE SOURCE OF THE VIRUS
A series of tools have been developed on the ICBF database to assist with herd investigations. These are available through the BVD Practice Dashboard (Figure 1. BVD Queries/BVD Dashboard). In addition, the Dashboard provides a live listing of the status of all herds to which you have been granted access.

Use the ‘Select Year’ function to choose to view data for either a single year or for all years.

![Figure 1. BVD Practice Dashboard](image-url)
The Herd Dashboard screen (Figure 2) presents:

- **Herd/farmer details.**
- A summary of the status of all animals in the herd (see Appendix for an explanation of each status).
- A series of options, including including ‘TASAH Sample List/TASAH TI Sample List’, ‘Contiguous Herds’ and others associated with herd investigations.
- Individual listing and BVD details of all animals currently in the herd.
- (Individual listing and details of all animals that have left the herd is available through ‘View status/generate results for animals that have left the farm’ under ‘Please choose one of these options’).
STEP 01 Determine time period when each calf was exposed \textit{in utero}

Assuming that the dam has been tested with negative results, each PI calf has been born as a result of exposure of their dam during the “window of susceptibility” in early pregnancy—typically between 30 and 120 days of gestation (Figure 3).

The effects of BVDV infection on reproduction

\textbf{Figure 3.} Time-dependent outcomes of \textit{in utero} infection with BVD virus
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Select ‘Investigate’ from ‘Please choose one of these options’. This will open a screen showing a range of information on each PI calf and its dam (Figure 4). Based on the recorded birth date and a 282 day gestation, the dates of opening (30d) and closing (120d) of the window of susceptibility for each PI calf are shown. Note that while these are the generally accepted limits, they should not be treated as absolute time boundaries.

Where more than one PI has been born (as in the example above), click on ‘Date of birth’ to sort by birth date. With multiple PIs, the source of infection could potentially have been continuously present from the date that the first dam entered the window of susceptibility (16-Sep-13 in Figure 4) to the date that the last dam left the window (26-Jan-14).

This would be the expected situation where the source of infection is internal to the herd e.g. the presence of an unidentified PI animal in the herd.

However it is also possible that infection was present for a shorter period of time while all dams were within the window of susceptibility (28-Oct-13 to 15-Dec-13). This would be the expected situation where infection originated from a ‘point source’ as a result of a one-off event e.g. an animal breaking in, boundary contact, indirect exposure.

Check the identity of the dam to see if she is homebred or not and the date of birth or entry to the herd (these will be the same for most homebred animals, but will differ if the animal has left the herd and returned e.g. following contract rearing).

Check the entry-to-calving interval to see if the animal entered the herd while pregnant with the PI calf and is therefore a possible Trojan dam.

Figure 4. Herd Investigation screen showing details of PI calves and their dams.
STEP 2  Determine the location of the exposure

Determine the location of the dam(s) during the window of susceptibility identified in Step 1. Bear in mind that this could have happened outside the herd if the dams were pregnant when introduced. In Figure 4 one dam calved within ~7 months of introduction (Entry to calving interval) and could therefore have been a Trojan (investigated further under STEP 3). Note that where the animal was less than 120 days in calf when introduced (as in this case; still in the window of susceptibility) it is possible that the foetus became infected after introduction. Where the animal was more than 120 days in calf, then it is highly likely that it was carrying a PI calf when introduced.

Consider the age of the dam(s) of the PI calves as this will indicate the particular management group or groups that were exposed. E.g. in Figure 4 all dams appear to be first-calving heifers. Were all of these animals managed as a single group? If yes, this suggests contact of only this group with a source of virus. Where dams have a range of birth dates, this suggests exposure of the adult herd and/or multiple management groups to a common source of virus.

During this period were the dams:

- On the home farm or an out-farm?
- Housed or at pasture?
- Grazing contiguous to farm boundaries?
- Outside the herd for part or all of this period?

STEP 3  Investigate potential sources of exposure

Within herd source  Source external to herd

These may be broadly divided into sources WITHIN-HERD and OUTSIDE THE HERD, and are summarized in Figure 5 and Figure 8.

![Figure 5. Summary of main within-herd sources of infection with BVDV leading to birth of PI calves](image-url)
CONSIDER:

1. **Presence of unidentified PI cattle in the herd. Are there any animals in the herd without a negative status?**

   Click on ‘All non-negative’ in the Status Summary (Figure 2) to generate a list of these animals (Figure 6). These should be identified and tested. In the example in Figure 6, this comprises 9 animals in total, including 6 animals with an UNKNOWN status, 2 animals with a DAMPI status (the two untested dams of positive calves from Figure 4) and the one calf that has had an initial positive result (INIPOS) and remains alive. Click on ‘Excel’ or ‘PDF’ to convert this to a saveable file in the chosen format or ‘Print’ to generate an immediate hard copy. In addition, from 2019 onward, there is a requirement to test additional animals that could reasonably have led to the birth of the PI(s) or TI(s) triggering the investigation through their having a false negative status. This includes animals that have a single negative BVD status (assigned directly or indirectly) either at the time of the WOS of the PI or the initial positive test of a TI. Note that animals with a single direct negative test which have also produced one or more calves that have also tested negative will be excluded.

   A listing of all animals to be sampled is generated by selecting the ‘TASAH Sample List’ or the ‘TASAH TI Sample List’ option on the BVD Dashboard (Figure 2). Note that this list also contains those the ‘All non-negative’ animals referred to above.

   In dairy herds in addition to the blood sampling a bulk tank milk sample will be taken to be tested by RT-PCR for the presence of BVD virus.

![Figure 6. Listing of animals currently in the herd with a non-negative status.](image)

2. **Contact with a known PI animal.** The listing in Figure 6 will show if there are older PI animals retained in the herd. Select the option ‘View status/generate declaration for animals that have left the herd’ to investigate if PI cattle were identified in the herd previously.

   a. Were any identified?
   b. When were these removed?
   c. Does their date of removal overlap with the window(s) of susceptibility of the dam(s)?
3. **Introduced animals.** Potential means of introduction (in decreasing order of likelihood) include:

   a. PI animals. Note that with time the proportion of animals traded that are of unknown status is decreasing. All cattle born after 1.1.13 must have a negative test result to move. The majority of older females will have produced at least one negative calf and therefore have an indirect negative status (INDINEG).

   b. Trojan dam. Was the PI calf born to a homebred or to a purchased animal? If purchased, did the date of conception precede the purchase date? If yes, it is possible that the calf became PI before purchase. If the dam was purchased when more than 4 months (120 days) in calf, the window of susceptibility would have closed before the dam joined the herd. Where a potential trojan dam is identified as a plausible cause of a PI birth, AHI will identify the source herd(s) where the dam was located during the WOS to determine if a recognised source of infection was present, triggering the required TASAH investigations in the source herd(s). Where infection was not identified, suggesting either a breach of biosecurity or the presence of an unidentified source of infection, a separate TASAH investigation may be assigned to the source herd(s).

   c. Transiently infected (TI) animals. These are considered a much lower risk than PI animals but cannot be excluded. Following TI, virus is typically shed at low levels and for a short period (up to 14 days). When investigating the potential for purchased animals to have introduced BVD virus through transient infection consider the following:

      i. What was the BVD status of the farm of origin (did it have any positive results; when was the last positive removed)?

      ii. Did the introduced animals move directly from the farm of origin or had they the opportunity to mix with cattle from other herds (particularly those of unknown status) e.g. at markets or during transport?

      iii. Does the herd have a quarantine policy for added animals and was it applied to the introduced animals? Did this include testing for virus? (a virus and antibody negative result for an added animal even at the time of the investigation will exclude the possibility of it having been TI at the time of introduction).

      iv. How long after introduction did the added animals first have contact with the dam(s) that went on to produce a PI calf or calves- the longer this period, the lower the risk.

Select the option ‘Purchase history’ (Figure 2) to view a full listing of all introduced animals (use the ‘Select year’ function to refine dates for which information is presented).

Information provided includes the herd of origin (and their birth herd, if different; Figure 7). If you have permission to view the data from these herds, you may link through to these herds to investigate further.

Where home-born animals have left the herd under investigation and subsequently returned (e.g. from a contract rearer or associated herd) the number of the herd under investigation will be shown as the birth herd.

The pregnancy status of the animal when introduced, based on age at purchase and subsequent calving date is also shown. Where an animal was pregnant, the status of the calf is also shown to help identify possible Trojan births.

In the example shown, one dam could have been a Trojan—though from Figure 4 it cannot be excluded that infection occurred after introduction.
4. **Other species.** BVD virus is predominantly associated with cattle but it can infect other ruminant species (sheep, llamas, alpacas) and pigs. Sheep may also be infected with Border disease virus (BDV), a pestivirus related to BVDV and which has occasionally been detected in cattle in other countries (but not in Ireland to date). BVDV has been detected in sheep in Ireland, but at a lower prevalence (flock and animal level) than that seen in cattle, suggesting that the main direction of transfer is from cattle to sheep rather than sheep to cattle. If sheep are on the same holding, particularly if co-grazing with cattle, consider screening for evidence of infection. Antibody testing of a proportion of the flock will indicate if they have had extended contact with a PI animal (ovine or bovine) but individual sampling is required to identify any PI sheep present (pooled RTPCR testing is the cheapest test method for screening large numbers of blood samples for evidence of virus); check with with the National Reference Laboratory before submitting samples.

![Figure 7. Listing of introduced animals, including purchase date, pregnancy status at purchase and their current test status](image-url)
Veterinary Herd Investigation Protocol following positive test results for BVD virus

**Figure 8. Summary of main external sources of infection with BVDV leading to birth of PI calves**

Transient infection of pregnant cattle during the window of susceptibility that led to the birth of PI calves may occur through either direct or indirect means.

1. **Direct exposure** occurs when cattle in the herd under investigation acquire the virus through contact with cattle in other herds. Knowing the location(s) where exposure may have occurred, consider:
   
a. Were the cattle grazing at a boundary during this period?
      
i. Was there cattle from neighbouring herds on the other side of the boundary at that time?
   
   ii. Was the quality of the boundary sufficient to prevent nose to nose contact (3m gap) or the break in (or out) of cattle (is the farmer aware of any instances where this occurred)?
   
   iii. Do the cattle share grazing with cattle from other herds?

   iv. See the **Contiguous Herds** option on the BVD dashboard (Figure 2) for information on the status on neighbouring herds. This shows the total number of contiguous herds, and the number with positive results. An absence of positive contiguous herds helps exclude boundary contact as a plausible source of infection. Where contiguous herds have had positive animals, further information on their dates of birth and removal is available by selecting the “Contiguous Herds” option on the dashboard. This information, along with the WOS of the PIs that have triggered the TASAH investigation, to determine whether or not the contiguous herd is a plausible source of infection. Note that transmission from a contiguous herd may occur by indirect pathways (see below), as well as by direct contact at boundaries.

b. Did any cattle leave the herd and subsequently return e.g. from shows, unsold from sale, or after contract rearing or leasing out?
   
i. Did these cattle have the opportunity to contact cattle of unknown status from other herds while out of the herd?
   
   ii. Were returning cattle quarantined prior to reintroduction to the main herd?
   
   iii. How long after return did they first have contact with the dams that produced PI calves?
2. **Indirect exposure** occurs when cattle in the herd under investigation acquire the virus without coming into direct contact with the animal shedding the virus. This typically occurs through movement or sharing of contaminated personnel, equipment and facilities. Consider:

   a. **Personnel**

      i. What visitors were on the farm during the window of susceptibility? This could include: farm employees and relief workers, knackery staff, AI technicians, hoof trimmers, weighing technicians, veterinary practitioners, advisors, nutritionists etc.

      ii. For those identified, how much contact did they have with the cattle generally and the dams that produced PI calves in particular (with more contact being associated with higher risk)?

      iii. What level of biosecurity/hygiene was applied to/demonstrated by these visitors on arrival (and departure)?
         1. Boots and clothing provided by the farmer for on-farm use?
         2. Routine disinfection of visitors’ boots, clothing, equipment?
         3. Hand washing?
         4. Knackery collection point located and operated to minimize contact with cattle?

      iv. What level of biosecurity did the farmer him/herself practice during this period in relation to contact with cattle (and potentially the virus) in other herds e.g.:
         1. Working with cattle in other herds (paid or unpaid)
         2. Attending shows, sales.

   b. **Equipment.** BVDV can potentially be transmitted on large or small items of equipment if not thoroughly cleaned and disinfected before use. Were any of the following borrowed/used during the window of susceptibility:

      i. Small items e.g. nose tongs, calving aid, drenching gun, dehorning or foot paring equipment.

      ii. Larger items e.g. trailers used to move cattle.

      iii. Shared facilities, particularly housing, yards and crushes.
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**STEP 4**

**Reviewing biosecurity and making recommendations to prevent re-exposure and onward transmission**

It will not always be possible to identify likely sources of infection, but this document provides a structured approach to investigating breakdowns, identifying the window of susceptibility for each dam that produced a PI calf and seeking to identify possible direct or indirect means by which exposure could have happened during this time.

Even where the source is not definitively identified, working through this process will identify weaknesses in biosecurity and allow recommendations to correct these to be made. Where PI animals are identified it is vital that they are removed as quickly as possible.

Prevention of onward transmission through Trojan dams. Herd investigations in previous years have highlighted the role of Trojan dams in introducing BVDV into previously free herds. As the programme moves towards eradication, it is increasingly important to prevent onward spread of infection by this route. To minimize the risk of the sale of Trojan dams from herds with positive results in 2019, herd owners will be advised by the BVD Helpdesk and in writing that they should not sell animals that were pregnant at the time of removal of the last PI(s) unless they are antibody negative within two weeks of sale. PVPs conducting TASAH investigations are requested to reinforce and supplement this advice. The testing of animals on the TASAH Sample List will generate both virus and antibody results, which may help inform this advice.

While this document is focussed on BVD, implementation of recommendations on biosecurity will typically produce wider benefits in relation to improving or maintaining herd health.

**Click here** for further guidance on biosecurity measures, including quarantine.

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## APPENDIX

<table>
<thead>
<tr>
<th>RESULT</th>
<th>INTERPRETATION (BVD VIRUS)</th>
<th>ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEGATIVE</td>
<td>Tested negative (most recent)</td>
<td></td>
</tr>
<tr>
<td>INDINEG 1, 2, 3, N</td>
<td>Dam that has produced 1, 2, 3, n negative calves (not pi)</td>
<td></td>
</tr>
<tr>
<td>EMPTY</td>
<td>No tissue in submitted sample (unsuitable for testing)</td>
<td>Re-test required*. Tissue or blood**</td>
</tr>
<tr>
<td>INVALID</td>
<td>Result not valid</td>
<td>Re-test required*. Tissue or blood**</td>
</tr>
<tr>
<td>NONCOMP35</td>
<td>Animal without any test result 35 days after date of birth</td>
<td>Re-test required*. Tissue or blood**</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>(1) Born before 1st January 2013 and has not been tested and has not calved OR (2) a calf that has been born less than 35 days ago without any test result.</td>
<td>(1) Test to clarify status (result required for Negative herd status if it remains in herd). (2) Test required by legislation.</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>Current positive result on database where initial result was not positive/inconclusive (e.g. Initial empty result)</td>
<td>Isolate; option to re-test after 3-4 weeks to confirm PI*. Consider removal without retest.</td>
</tr>
<tr>
<td>INCONCLUSIVE</td>
<td>Current inconclusive result on database where initial result was not positive/inconclusive (e.g. Initial empty result)</td>
<td>Isolate; option to re-test after 3-4 weeks to confirm PI*.</td>
</tr>
<tr>
<td>INIPOS</td>
<td>Initial test result is positive, no re-test result</td>
<td>Isolate; option to re-test after 3-4 weeks to confirm PI*. Consider removal without retest.</td>
</tr>
<tr>
<td>INIINC</td>
<td>Initial test result is inconclusive, no re-test result</td>
<td>Isolate; option to re-test after 3-4 weeks to confirm PI*.</td>
</tr>
<tr>
<td>PI</td>
<td>Initial and confirmatory positive (or inconclusive) result</td>
<td>Isolate and remove as soon as possible (&lt;5 weeks of first test)</td>
</tr>
<tr>
<td>DAMPI</td>
<td>Dam of an animal with a current positive (or inconclusive) result</td>
<td>Test to clarify dam status</td>
</tr>
<tr>
<td>OFFPI</td>
<td>Untested offspring of a dam with a current positive (or inconclusive) result</td>
<td>Isolate and remove as soon as possible</td>
</tr>
<tr>
<td>ALL NON-NEGATIVE</td>
<td>Total number of animals without a neg or INDINEG status</td>
<td>Reduce to zero through above steps</td>
</tr>
<tr>
<td>ALL</td>
<td>Total (and details) of all cattle currently in the herd</td>
<td>-</td>
</tr>
</tbody>
</table>

** Diagnostic gap