Maximising the Chance of Achieving Diagnoses on Bovine Abortions and a Diagnostic Algorithm Based on Western Canadian Bovine Diseases

Prairie Diagnostic Services Inc.

Reproductive loss is one of the major problems in cow-calf operations in Western Canada. Although achieving the diagnosis of abortion is undoubtedly important, diagnostic rates had been low traditionally. This article offers some insights to help veterinarians to maximize the chance to achieve the diagnosis of abortion based on diseases that are most common in Western Canada. A diagnostic algorithm is also presented to aid veterinarians to understand how a diagnosis is established and how to choose proper diagnostic tests.

1. Collect a thorough list of tissues.

Providing sufficient diagnostic materials is a key for successful diagnostic investigation. Of these the most important is placenta and every effort should be made to collect it. Also, in abortion storms, one fetus may not representative. At least two or better three abortions should be examined. If it is uncertain whether the abortion is only part of the background abortion rate of ~ 2%, the first one or two can be frozen if snowbanks are available or weather is still cold enough to do so.

Necropsy and tissue collection in fetuses is quite standard and a well-trained technologist can perform this task with supervision of veterinarians or pathologists. The Bovine Fetus submission form on the PDS Inc. website (https://pdsinc.ca/Resources/Forms.aspx) provides a good guidance of tissue collection and veterinarians are strongly recommended to consult this resource. Although it looks exhaustive, with exercise, the procedure can be usually completed within 30 minutes. It is understandable that not all tissues and procedures can be achieved. For examples, sometimes the placenta is not expelled or consumed by the cow or other animals; or some tissues are scavenged. However, following the procedures as much as possible should increase the probability of arriving at a diagnosis.

2. A good diagnostic plan

There are certain diseases/conditions that PDS diagnoses more commonly based on the time of gestation. Our recommended simplified diagnostic strategies below are based on these findings and can help alert you to the timing of the majority of the conditions in Western Canada. Please keep in mind, however, some diseases can occur in both trimesters such as IBR.

	Common diseases	Recommended diagnostic strategies
Second trimester	IBR; various fetal bacterial septicemia; *Neospora*	• Histopathology • Bacterial culture (Abomasal fluid, and one of the followings Lung, liver, or kidney) • Pre-authorize 2 diagnostic tests at pathologists' discretion if needed
Third Trimester	Vitamin A and/or E deficiencies; trace mineral deficiencies; various chronic non-viral infection, with possible placentitis	• Histopathology • Vit A and E analyses + mineral panel #1 (Mg, Mn, Fe, Co, Cu, Zn, Se, Mo [offered in PDS Inc.]) on liver • Bacterial and fungal culture (lung and abomasal fluid) • Pre-authorize 1 diagnostic test at pathologists' discretion if needed

Reproductive losses in the first trimester do not usually reach the diagnostic laboratories for various good reasons – fetuses can be reabsorbed or too small to be recognized on the ground even if aborted.

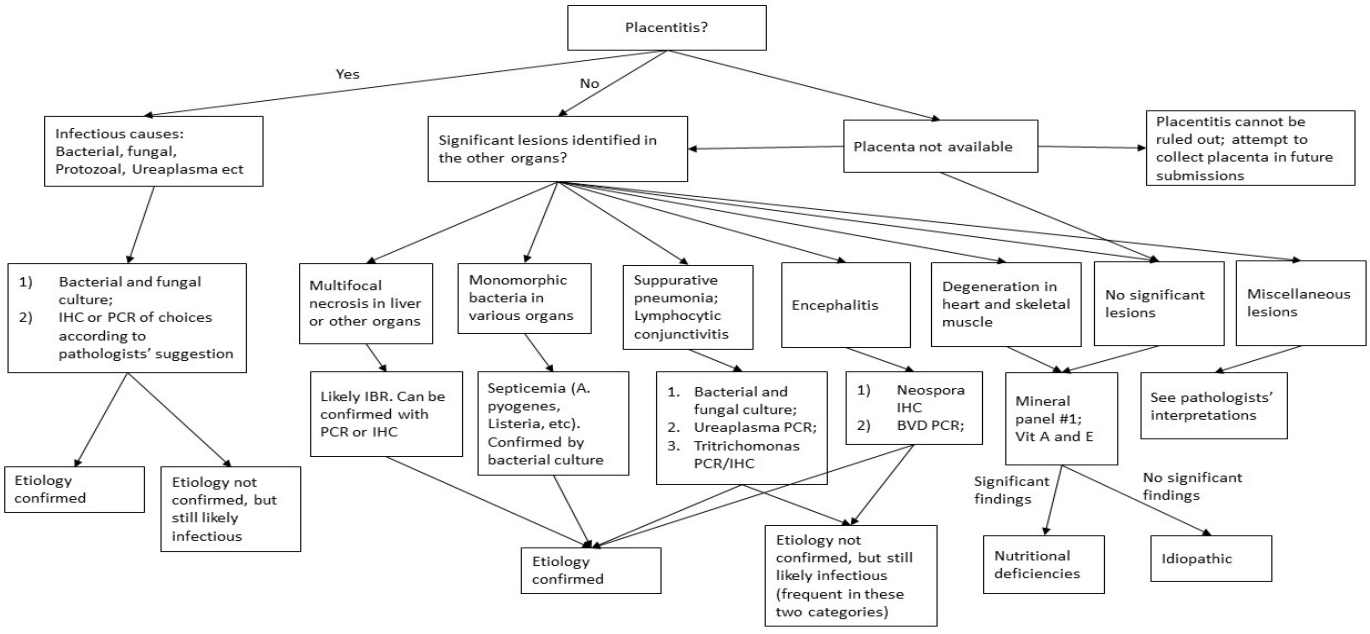
In the second trimester, IBR, various fetal bacterial septicemia and to a lesser degree *Neospora* are the diseases most frequently diagnosed in Western Canada. Diagnoses of bovine herpes virus 1 (aka. IBR) and bacterial septicemia are relatively straight forward, because of the presence of obvious lesions and the availability of good adjunct tests. The diagnosis of *Neospora* abortion can be challenging because the lesions and organisms are usually scattered. The presence of non-suppurative encephalitis can be a hint for the involvement of *Neospora*, which can be confirmed by IHC. One needs to understand the presence of such a lesion may be missed on just several sections of brain, and the amount of antigen within inflammatory foci may be too little to be detected by IHC. Thoracic fluid of the fetuses can be used as a sample for ELISA to detect antibodies against *Neospora* and BVDV. However, the sensitivity of this method is also a concern. As a result, a positive reaction can confirm fetal exposure to *Neospora* or BVDV, but a negative result cannot rule it out.

In the last trimester, Vitamin A and/or E deficiencies are frequent laboratory findings. Whether deficiencies of these two vitamins alone are the causes of abortion can be difficult to verify. Squamous metaplasia of salivary ducts (may be seen in Vit A deficiency) and myocardial and/or skeletal muscle degeneration (may be seen in Vit E deficiency) are not usually present. However, these deficiencies may not have to induce morphological changes in order to trigger abortion. Further, their deficiencies are frequently found in combinations with various trace mineral deficiencies and can be an indication of a bigger problem such as inadequate protein and/or energy intake. Thus, in our laboratory, Vit A and/or E deficiencies are usually interpreted as significant findings, regardless whether they accompany morphological changes.

Subacute to chronic non-viral infections, often with placentitis, associated with fungal infection, various bacterial and protozoal infections are also frequent diagnoses in the last trimester. Their diagnoses are relatively easy unless the placenta cannot be collected for diagnostic purposes. If the placenta is not available, but these infections are suspected, the lung and abomasal fluid can be a good diagnostic alternative as suggested in the table and diagnostic algorithm. Umbilicus can also be useful to include as fixed and fresh tissue. Unfortunately, these alternatives are not a perfect substitute, as some infections do not invade the amnion or the fetus.

3. A diagnostic algorithm

Below is a diagnostic algorithm. Again, this flowchart is designed to cover the more common scenarios and diseases and is not meant to be comprehensive. The algorithm shows the ways different diagnoses are established. With this algorithm, veterinarians can interpret various diagnostic findings and come up with a conclusion. In more complicated cases, veterinarians are recommended to contact the diagnosticians in the laboratory.



4. Submitting samples to PDS Inc.

Complete a PDS submission form. PDS Web Client portal (http://pdsserver.usask.ca/webclient) streamlines completion of the submission form.

- The main "HISTORY" field is the primary location to record vaccination history, treatments, and other notes for herd and individual animal. Gross necropsy notes should be recorded here as well.
- It is important to make sure all fields are filled out. Commodity, Production stage, Primary systems affected and animal age.
- Identifying sample type(s) and quantity on the main page of the submission form, is a required field to create a successful submission. When your sample type is not listed on the suggested samples area you can find all pathological sample types under the dropdown list found with each "sample type "description. "Fresh or Fixed" sample quantities should be listed in this "Samples Sent" field and a description of sample type on the Necropsy form.
- Clearly indicate your testing requests on the submission form and/or pre-authorize adjunct tests.
    - To optimize the turnaround time for an entire case, veterinary practitioners can specify on the Necropsy/Histology submission form which, if any, adjunct tests that they would like to have performed; or, alternatively, practitioners can pre-authorize some relevant tests and let the veterinary pathologist decide whether those tests are warranted. Some examples for pre-authorization are: "PCR for IBR, if needed", "mineral panel #1, Vitamin A and E, if suitable" and "bacterial culture, if indicated". This would reduce the number of telephone calls made by veterinary pathologists to veterinary practitioner seeking permission to proceed with adjunct testing. The response to these requests may take several days and further delay the timely completion of the report.
- Select the Necropsy/Histology form (found on the bottom righthand corner of the main submission form) to open a second page to use to identify type of fresh tissues being submitted. This is also the best location to direct your requests for adjunct lab test(s).
- After you have "SUBMITTED" the form on-line, print the finalized form with the barcode and include with samples.

Alternative to Web Client form is to complete Bovine Fetus submission form found at https://pdsinc.ca/Resources/Forms.aspx.

5. Correctly package the fixed and fresh tissues

The basic principle: All tissues for histologic examination should be placed in one or two, formalin-filled, submission jar(s) and each of the fresh tissues should be placed in separate, clearly labeled leakproof bags**.**

It is counterproductive to place tissues for histologic examination in many separate submission containers. Veterinary pathologists are trained to histologically identify the submitted tissues. When the tissues submitted for histologic examination are placed in separate containers, each of these will be opened at PDS and the tissues pooled into a single container for routine processing. The opposite applies to the fresh tissues. Place each tissue in a separate, clearly labeled bag to avoid cross contamination and facilitate future identification with adjunct testing.

In winter, freezing artifact can severely hamper the histologic examination of the formalin-fixed tissues. In cold weather, we recommend fixing the tissue in 10% formalin until the tissue is completely fixed (24-48 hrs or longer depending on the size of the tissue) and then transferring the formalin-fixed tissue to 70% isopropyl alcohol (rubbing alcohol) for transport to prevent freezing artifact.

With the information provided in this article, PDS Inc. hopes diagnostic plans for bovine abortion can be easier and clearer to veterinarians.

If you have any questions, contact PDS Inc. at (306) 966-7316.

Calving season is coming, let's be prepared for it!!