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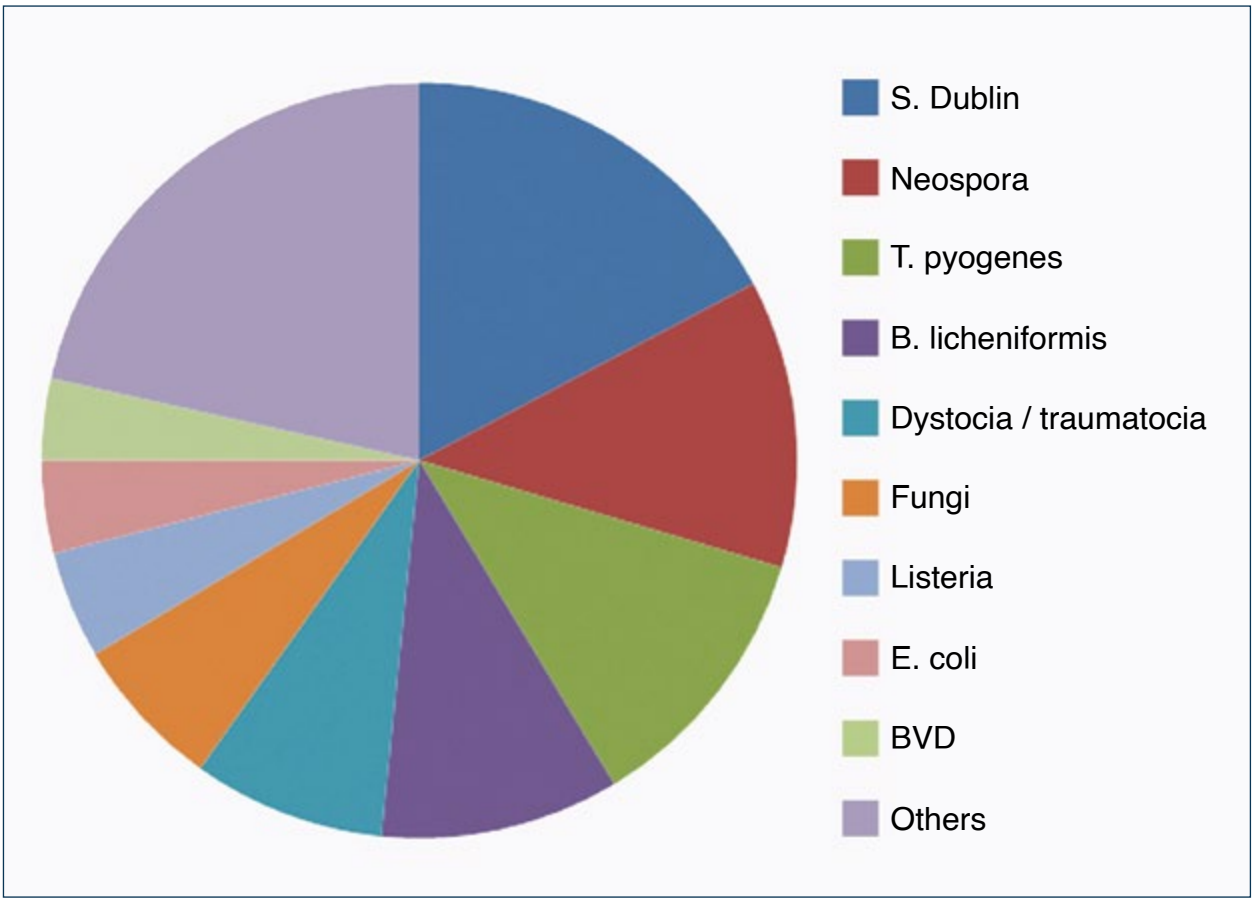
Veterinary Laboratories Ltd
'The Quality Clinical Pathology Service'

Bovine Abortion Sampling Protocol

The causes of bovine abortion are numerous with bacterial, fungal, protozoal and viral agents being implicated. The pie chart below helps to illustrate the relative frequency of the common abortion pathogens in the UK and is derived from APHA VIDA data for 2018-2020. Causes of fetopathy associated with stillbirth are also included.



Infection of the placenta/foetus usually follows haematogenous spread of infection which is often clinically silent but some abortions may be preceded by maternal illness (e.g. salmonellosis or IBR). Establishing a definitive diagnosis is important in helping to put into place interventions to reduce reproductive morbidity. In addition, other disease problems on the farm may be reduced through the identification of abortion agents. The diagnostic rate for bovine abortion is relatively low but it should be borne in mind that other environmental and genetic factors will also play a part in the overall abortion profile in a herd.

Many of the causes of abortion can be established with the collection of a limited number of samples. Freshly aborted placenta and foetuses will provide the best material for sampling and more than one case in a herd may need to be sampled to help achieve a diagnosis. Mummified foetuses are of limited value diagnostically and it would be better to await another case rather than sample such a foetus. The intervention level is set at 5% for bovine abortion but different farmers will have their own levels at which action will be taken. Currently all abortions need to be reported to APHA who will judge whether they need sampling for brucellosis

(a notifiable/reportable disease) which, because of the UK's current disease free status, often means no testing is required/undertaken.

When presented with an abortion on a farm, collect any relevant clinical history about the dam herself and the herd as a whole – there maybe a history of *Salmonella* Dublin, for example, that should raise suspicions and could limit your testing requirements. Consider changes in management, including feeding, as this again may provide clues to possible causes. If there is a history of neosporosis, farm/ breeding records may suggest vertical transmission along certain dam lines. Use all the information available to you to construct a clear picture of the reproductive disease patterns.

If a fresh foetus and placenta is available clean these with tap water to remove excess bedding and faecal material. Separate the placenta from the calf and examine it for evidence of inflammation and necrosis. Remove a cotyledon and surrounding membrane, section in half placing one portion in a sterile container and the other in formal saline.

Ideally weigh the calf and obtain a crown-rump length to assess whether the approximate gestation age corresponds to the size of the foetus particularly if no service dates are available. Placental insufficiency, for example, will result in retarded in-utero growth with a dysmature foetus.

Lay the calf in lateral recumbency; inspect the skin for any lesions (e.g. raised plaques can be seen in cases of mycotic abortion); reflect the fore and hind limbs and open the abdomen and thorax by cutting from the pubis cranially and along the costo-chondral junctions. Reflect the rib cage dorsally by snapping the ribs at the costo-vertebral end – this then exposes thoracic and abdominal viscera (see ovine abortion protocol for photographs).

Using a vacutainer and needle (or similar) collect foetal fluids either from a body cavity or heart. Then collect foetal stomach contents in a similar fashion. If stomach contents are not available collect fresh liver or lung for culture. From the point of view of additional testing collect a portion of fresh spleen (or thymus) and kidney and place in individual sterile pots – these tissues can be used for PCR analysis for BVDV and *Leptospira spp* respectively.
Testing for neosporosis is reliant on the detection of the organism and pathology associated with infection. Fresh midbrain and placenta should be collected. In addition a portion of heart (the interventricular septum or ventricular free wall) should be fixed in formalin and ideally the brain removed (this is sometimes semi-liquid – provided the brainstem is solid enough to be placed in formalin it will be OK) and fixed after midbrain has been removed for Neospora PCR. See the instructions on brain removal in the Nervous disease sampling protocol.

If the dam is available collect whole clotted blood - this can be submitted at the same time as the foetal samples and retained for further testing if required. A convalescent blood sample collected 2-3 weeks later will allow paired serology to be undertaken for BVDV and BHV1 but results need to be interpreted with caution as antibody titres may be falling dependent upon the time of infection in relation to the abortion.

For assessing the *Neospora* status of the dam a single clotted blood sample at the time of abortion is all that is required – a positive result indicates infection with the likelihood that the foetus is also infected but in itself does not confirm a diagnosis of *Neospora* associated abortion. Leptospira serology can be a useful tool in diagnosing abortion (and milk drop) in herds. Tests based on IgM antibody are used as levels are elevated following recent exposure and decrease over a 6 month period – so if high titres are obtained in a single sample at the time of abortion it suggests recent exposure and as such may implicate the organism in the abortion. PCR on foetal kidney is the definitive test but tissue must be fresh and not autolysed as this adversely affects the recovery rate of the organism's DNA.

Summary of samples for testing;

Placenta – cotyledon – □ fresh, □ fixed in formalin.

Foetus –

- **Foetal fluids**
- **Foetal stomach contents or liver**
- **Fresh spleen or thymus**
- **Fresh kidney**
- **Fresh midbrain**
- **Fixed heart and brain**
- **Maternal blood samples – acute and convalescent**

Appendix – Stillbirth investigations

Although not technically an abortion many farmers perceive stillbirth as the same problem. In some cases it can be due to abortifacient agents but there are a number of other causes to bear in mind when presented with such cases.

In many ways investigating these cases is more difficult as there is often poor history (many animals are not observed closely so the timing of foetal death is not precise) and a wider range of management factors are involved. The following list covers some of the areas to be considered:

1. Dry cow management – diet (including access to minerals), exercise, body condition, pre-calving environment, 'psychological' factors, group size, recent movements/mixing etc.
2. Dam age.
3. Sire usage – with regards to foeto-maternal disproportion.
4. Obstetrical interference.
5. Evidence of early placental separation.
6. Foetal dysmaturity.
7. Peri-parturient disease – infectious or metabolic.
8. Herd/dam history of abortion agents.
9. History of poor newborn calf viability.

It is very important to undertake a full post-mortem examination of the calf and placenta as this may provide clues to underlying causes. Dystocia can often be identified on gross examination – changes seen may include:

1. Swollen, firm, protruding tongue.
2. Submandibular oedema and haemorrhage.
3. Bruising -along the horizontal rami of the mandible, lateral cheek area, over the calvarium, and less frequently over the elbows and lateral stifle.
4. Poor lung inflation - either patchy or near total atelectasis.
5. Fractured ribs or bruising along lateral ribcage and ecchymotic/petechial pleural haemorrhage.
6. Excess foetal stomach contents often containing meconium. Occasionally large amounts of gas are present in the abomasum indicative of terminal gasping.
7. Meconium staining of coat.
8. Haemorrhage surrounding caudal brainstem.
9. If an assisted calving assess any bruising at points where traction may have been applied.

To further the investigation (depending on the history/findings) assessing management, diet (including using metabolic profiles), calcium/ magnesium/energy metabolism and the possibility of iodine deficiency could be pursued.

Iodine deficiency during pregnancy is thought to be implicated in stillbirth. It will result in goitre if of a prolonged nature. Goitrogenic substances will also induce similar change so if on gross post mortem examination an enlarged thyroid is suspected questioning regards possible exposure to goitrogenic plants such as *Brassica spp* is necessary. If there is no history then assessment of the gland histologically and checking the iodine status of the dam (or a cohort of individuals on the same diet) is recommended.

As a rough guide if the thyroid gland weighs more than 30g, it is likely to be abnormal. A more accurate assessment is possible by weighing the calf and the gland. An abnormally enlarged thyroid gland is present if the ratio of body weight (kg) to thyroid weight (g) is <2.5.

The following photographs show the dissection of the thyroid gland.

Figure 1. Stillborn calf.

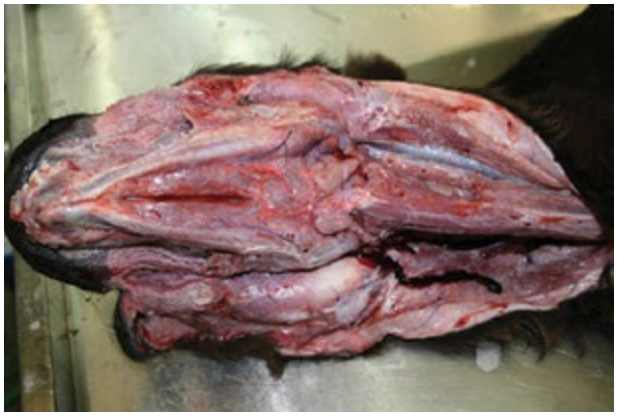


Figure 2. Reflect the skin of the ventral head and neck.

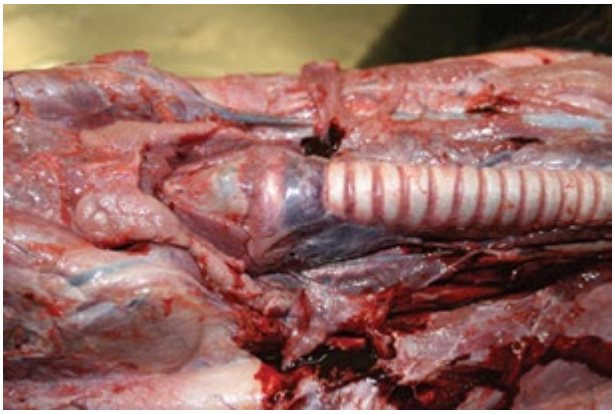


Figure 3. Dissect the ventral cervical muscles from the larynx and trachea.

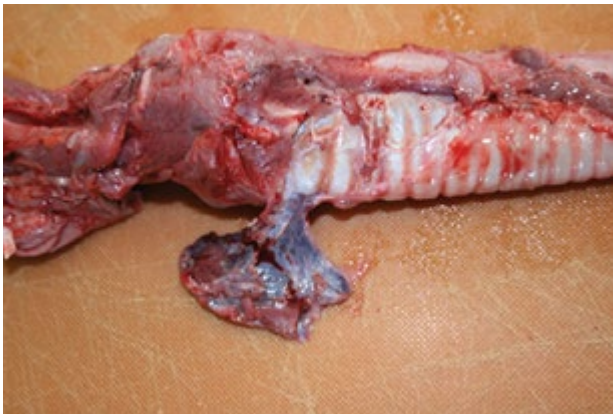


Figure 6. Carefully dissect off the thyroid ensuring all the gland is removed and excess surrounding tissue is cut away.

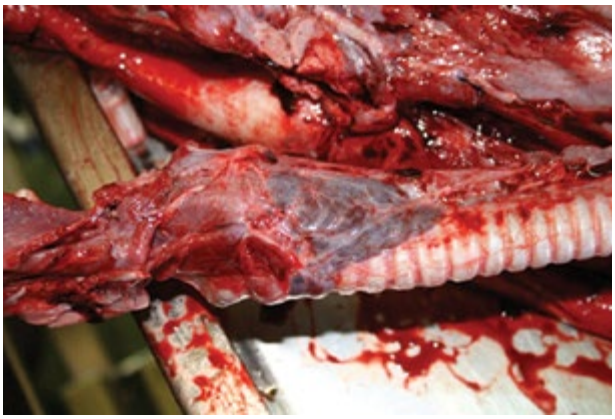


Figure 4. Free the tongue, larynx and proximal trachea to reveal the thyroid gland on either side of the larynx.

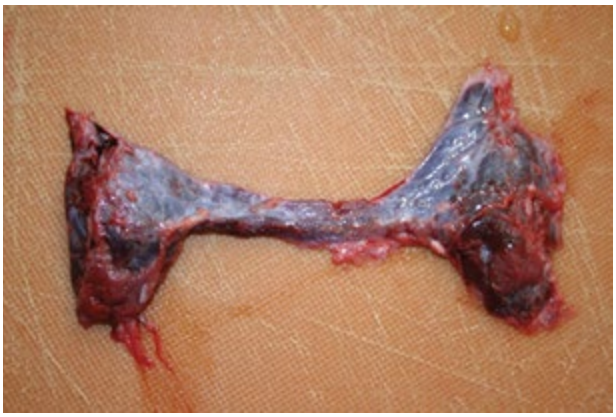


Figure 7. Normal bilobed thyroid gland with narrow isthmus.

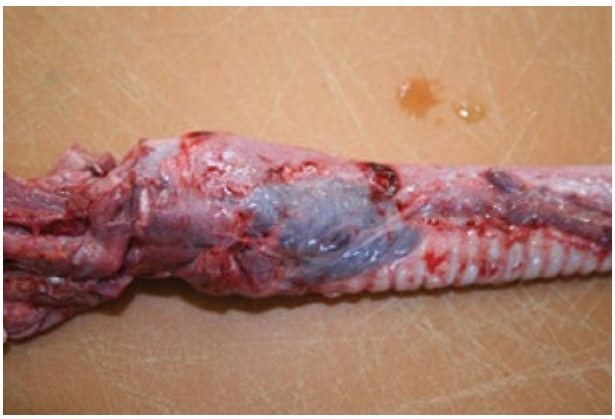


Figure 5. Lateral larynx/proximal trachea showing thyroid gland.

Once dissected weigh the gland and then cut in half through the narrow isthmus. Place one half in a sterile container for iodine analysis (if required) and place the other half in formol saline. Preference would be to undertake histology first to identify any hyperplastic change before assessing iodine levels.

If goitre is detected histologically then either iodine analysis can be undertaken (advice will be provided by the pathologist) or plasma inorganic iodine of the dam or cohort would be advised.

Histopathological assessment of other tissues in cases of stillbirth is recommended if no obvious cause is seen on gross examination.

The following tissues should be collected into formol saline;

1. **Heart (interventricular septum or ventricular free wall)**
2. **Lung (cranial, middle and caudal lobe)**
3. **Liver**
4. **Kidney**
5. **Brain**
6. **Placenta**

In addition collect:

1. **Foetal fluid**
2. **Fresh placenta**
3. **Fresh thymus/spleen (BVDV)**
4. **Fresh midbrain (Neospora)**

The Manor House, Brunel Road, Newton Abbot, Devon TQ12 4PB
Tel: 01626 357776 • dsfarm@axiomvetlab.co.uk

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