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Veterinary Laboratories Ltd
'The Quality Clinical Pathology Service'

Investigating nervous disorders in ruminants

The field and laboratory investigation of nervous disease in ruminants involves carefully assessment of the live animal and appropriate post-mortem sampling to help achieve a diagnosis.

When presented with nervous disease it is important to fully assess the animal clinically to differentiate between musculoskeletal disorders and true neurological disease. Subsequent neurological evaluation should then attempt to establish which area of the CNS or PNS is involved so that a list of differential diagnoses can be established.

Many diseases are site and age specific which help to narrow down the differential diagnoses. In addition the clinical and farm history is important as information may be available regarding animal movements, previous infectious conditions (e.g. Louping ill) or vaccination history that should be taken into account.

The following lists are of some of the causes of neurological disease in ruminants in the UK;

Cattle

- **BSE**
- **Louping ill virus (LIV)**
- **MCF**
- **BHV 1**
- **BVDV (CNS malformation)**
- **Thrombotic meningo-encephalitis *(Histophilus somni)***
- **Listeriosis**
- **Bacterial meningitis**
- **Neospora caninum**
- **Hypomagnesaemia**
- **Hypocalcaemia**
- **Nervous ketosis**
- **Vitamin A deficiency**
- **Lead poisoning**
- **Spinal cord compression (abscess/osteomyelitis)**

Small ruminants

- **Scrapie**
- **LIV**
- **Visna (MV/CAE)**
- **Listeriosis**
- **Bacterial meningitis**
- **Sarcosporidiosis**
- **Gid**
- **Hypomagnesaemia**
- **Hypocalcaemia**
- **Pregnancy toxaemia**
- **Swayback**
- ***Clostridium perfringens* enterotoxaemia type D (pulpy kidney/FSE)**

A variety of metabolic (e.g. hepatic encephalopathy) and degenerative conditions can affect all species. Some of these conditions are breed related.

This information sheet will cover post-mortem examination, sample selection and test interpretation for the common neurological diseases of ruminants.

Sampling from the live animal

In the live animal a number of samples can be collected to assist in establishing a diagnosis once clinical examination has narrowed the differentials and localised the lesion.

Metabolic diseases such as hypocalcaemia, hypomagnesaemia, nervous ketosis and pregnancy toxaemia can be confirmed by pre-treatment blood sampling and biochemical testing for calcium, magnesium and beta hydroxy-butyrate respectively. Cohort sampling especially for hypomagnesaemia can also be useful as post-mortem samples can be difficult to assess especially when faced with prolonged post-mortem intervals.

Should there be evidence to suggest hepatic encephalopathy assessment of liver function can be difficult in ruminants but evaluation of liver enzymes and other biochemical parameters (GGT, ALP, GLDH, bilirubin etc.) will assist in establishing a diagnosis. The detection of hyperammonaemia is not easily undertaken and bile acid evaluation is of limited value in ruminants. As such reliance on post-mortem examination and histopathology is important.

Other metabolic disorders such as swayback can be difficult to confirm in the live animal as blood copper levels may be within the normal range either due to copper deficiency occurring in utero (with congenital swayback) or some time previous to the onset of delayed swayback followed by supplementation. Low blood copper levels are therefore only suggestive but not conclusive in achieving a diagnosis.

Animals presenting with neurological signs and pyrexia may have a bacterial or viral infection and although non-specific an EDTA blood sample for a white blood cell and differential count can help to provide supportive evidence of an infectious condition.

More invasive techniques such as collection of CSF for cytology, biochemical assessment and culture can be undertaken if required.

Post-mortem examination and sampling for diagnosis

Many cases of nervous disease in ruminants can be further investigated by undertaking post-mortem examination. This may be an elective procedure (if so collection of ante-mortem blood samples is advised) or as an opportunistic investigation on presentation of a dead animal for post-mortem.

By the very nature of the CNS anatomy post-mortem examination is less frequently undertaken by practitioners but with good technique and practice brain and spinal cord can be removed for examination. It is, though, important to undertake a full post-mortem examination as lesions in other organs may provide additional information to assist in achieving a diagnosis and understanding the pathogenesis.

Assess the carcass grossly-look for ticks, evidence of ocular change (hypopyon as with MCF), skin abrasions on the hind legs indicative of ataxia/hind leg weakness etc. Please refer to the information sheet ‘Post mortem procedures for large animal species’ which describes post-mortem examination and sample collection for the viscera.

To remove the brain you will need a saw, bone forceps/snips and a T piece or bone chisel. As an alternative to a hand saw an oscillating saw (or DIY multi-tool) can be used but taking into account the necessary health and safety precautions of wearing protective eye wear and a face mask. The head can either be left attached to the carcass or removed. If a vice is available to steady the head this will assist in brain removal and a suitable one can easily be adapted/made. Make saw cuts as shown in the photographs through the calvarium (figures 1 and 2) and then insert the T piece or bone chisel into the occipital region cuts and gently lever the bone upwards. Remove the calvarium being careful to dissect the dura mater away from the brain.

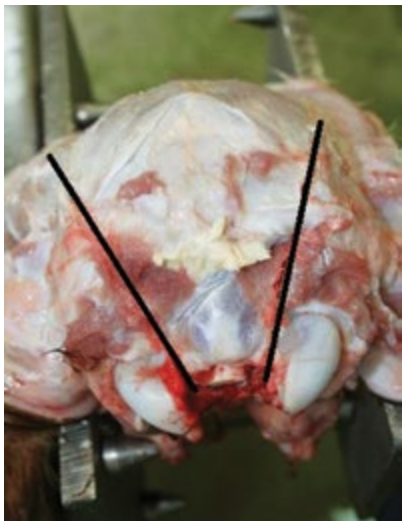

Fig.1. Position of saw cuts dorsally from the foramen magnum

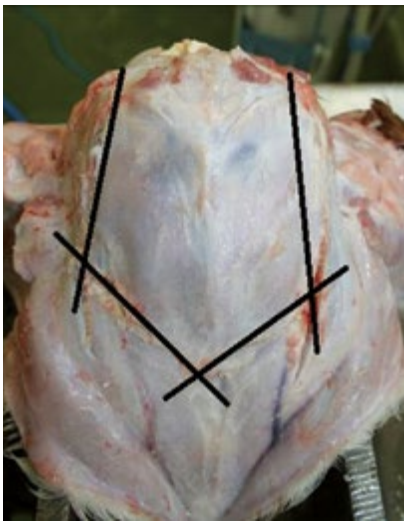

Fig.2. Position of saw cuts on the calvarium

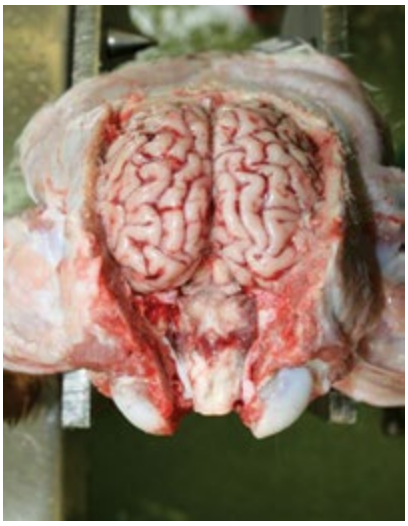

Fig.3. Calvarium removed exposing underlying brain. Note the severe cerebellar hypoplasia in this calf

With the brain exposed consideration should be given to bacteriological sampling in the case of meningitis. Inserting a swab between the cerebellum and cerebral hemispheres as indicated in figure 4 should suffice.

To remove the brain tip the head backwards and release the brain by cutting through the cranial nerves from the olfactory nerve backwards - the brain will naturally fall away as you do so. Once released place the brain on a clean surface and examine grossly. The next steps will depend upon your presumptive diagnosis and therefore sampling requirements.

If spinal cord disease is suspected then cord removal is required. Remove as much musculature as possible and using a saw cut the vertebral arches along the spine before releasing them with bone forceps/snips (with young animals-especially lambs-this procedure can be done using bone forceps alone) (figure 5).

Take care to avoid damage to the spinal cord. Again an oscillating saw will help with this procedure. Once exposed the cord can be removed by cutting the nerve roots as shown in figures 6 and 7.

An alternative method is to remove segments of cord from certain lengths of the spine. The target lengths of the cord are caudal cervical, mid-thoracic and caudal lumbar regions. The approach to this method is to make two parallel cuts transversely across the spine approximately 2-3 cm apart. This results in a short length of the spine containing the cord. By carefully inserting a small scalpel along the spinal canal between the dura mater and the vertebral canal wall it is possible to release the nerve roots and subsequently the section of cord can then be removed/pulled free.

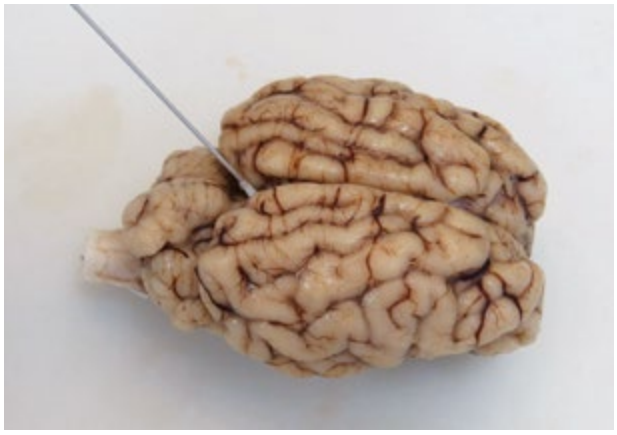

Fig.4. Site for swabbing meninges for bacteriology (between the cerebral hemispheres and cerebellum)

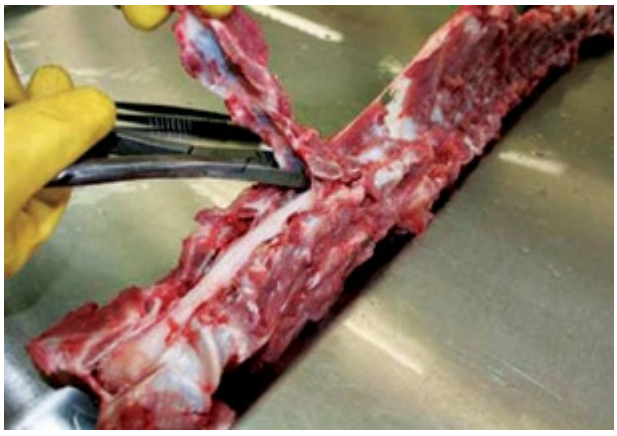

Fig.5. Exposing the spinal cord using bone forceps

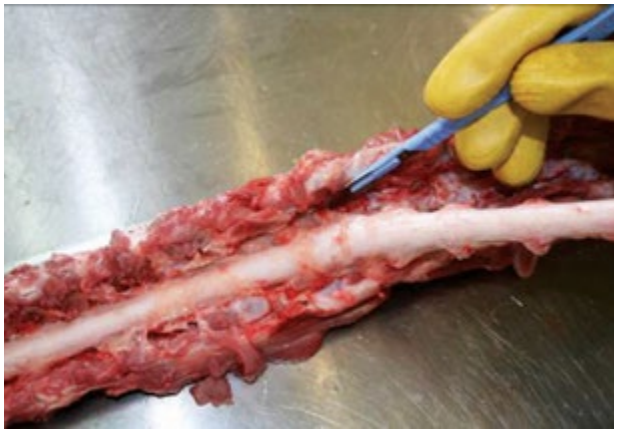

Fig.6. Releasing the spinal nerve roots during cord removal

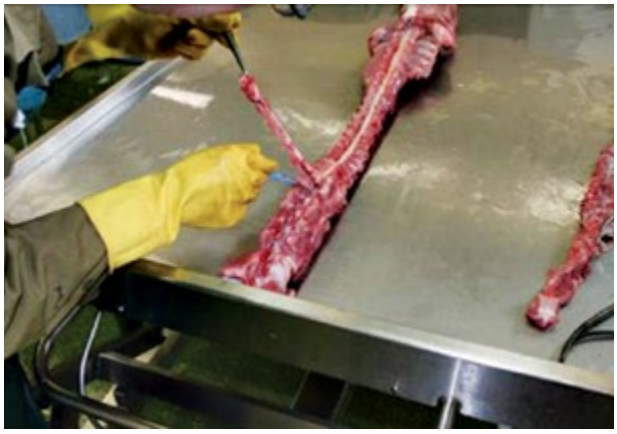

Fig.7. Progressive cord removal

Whichever method is used it is then useful to split the spinal column sagittally particularly if spinal abscessation/osteomyelitis is suspected to identify the affected area.

Fixation

For histopathology it is important to fix the brain in adequate quantities of formalin (ideally 10 times the volume of the tissue). Brain can be partly fixed and subsequently removed from fixative, wrapped in gauze and sent to the laboratory after 3-4 days where it will be examined by a pathologist and placed back in fixative before being processed. The spinal cord can be loosely coiled within a pot preferably after the dura mater has been opened to ensure rapid fixation-similar fixation and dispatch of the tissue can then occur. If sections of spinal cord are submitted then ideally individual segments of spinal cord should be labelled.

On receipt at the laboratory the CNS material is examined by a pathologist who will make a gross assessment looking for any overt lesions. Selective areas of the brain are trimmed for histological examination. As part of the examination, should CCN be suspected, the brain will be examined under ultraviolet light.

Cerebrocortical Necrosis Examination

Should you wish to examine the brain for CCN at the practice all you require is an ultraviolet light (Wood's lamp) and a dark room. Make 3 parallel incisions through the brain as shown in figure 8, expose the cortex and examine under an ultraviolet light to look for symmetrical autofluorescence of the cortex (see figure 9). This procedure can also be undertaken on fixed brain.

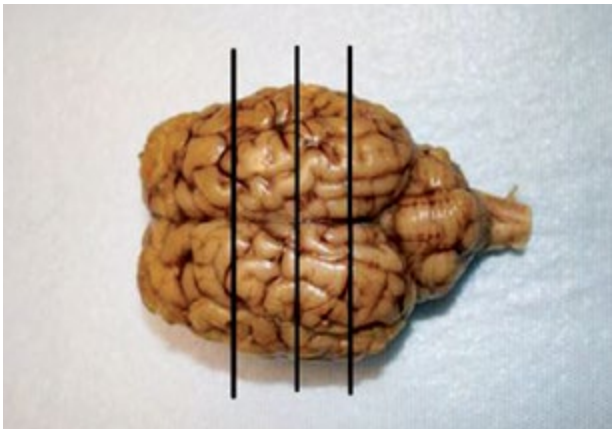

Fig.8. Dorsal view of brain showing sites for incision through the frontal, parietal and occipital regions of the brain for assessment for CCN.

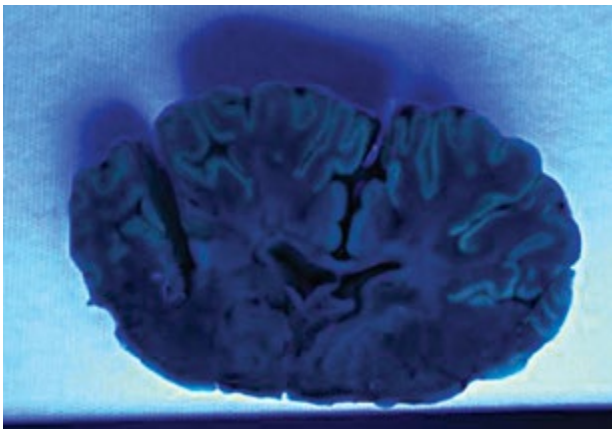

Fig.9. Extensive symmetrical cortical autofluorescence characteristic of CCN

Listerial encephalitis sampling

Listerial encephalitis has characteristic histological changes only necessitating the submission of fixed brain for histological examination.

However if you are considering listeriosis and wish to undertake bacteriology a sample of brainstem needs to be collected in a sterile fashion for culture. Figure 11 shows the area of brainstem that needs to be collected. Carefully remove the cerebellum and make 2 parallel cuts through the brainstem with clean instruments. Place the central portion of the brainstem in a sterile container and send to the laboratory with an ice pack for selective listerial culture.

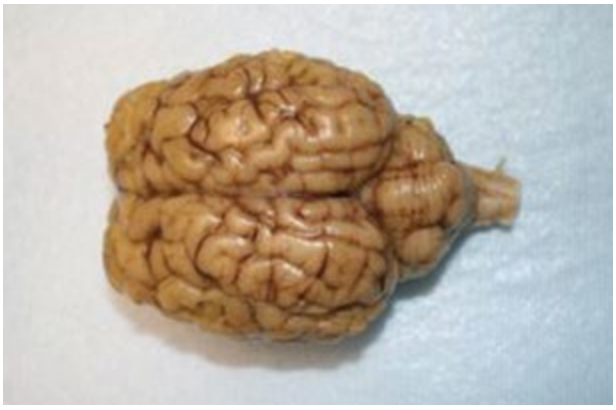

Fig.10. Dorsal view of brain

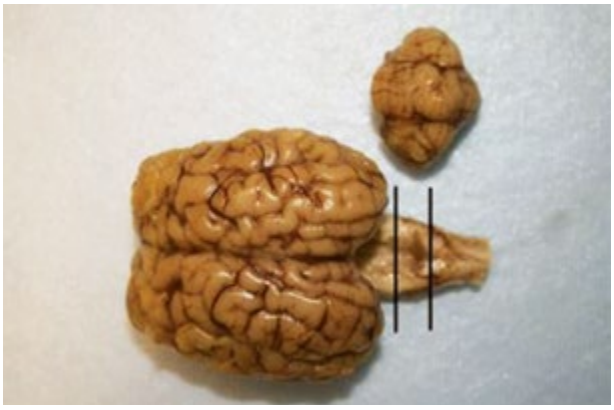

Fig.11. Cerebellum removed and sites of parallel cuts for brainstem sampling are indicated.

Aqueous humour sampling

Aqueous humour should be collected using either a syringe and needle, or Vacutainer (figure 12). Take care to avoid aspirating iris tissue (black deposits within the fluid) as this can affect magnesium estimations. Should such material be present, centrifuge the sample and remove the supernatant for testing. Vitreous humour can also be submitted. A variety of tests can be run including calcium, magnesium, beta hydroxy-butyrate and urea to assist in establishing a diagnosis in metabolic disease (Edwards and Foster 2009).

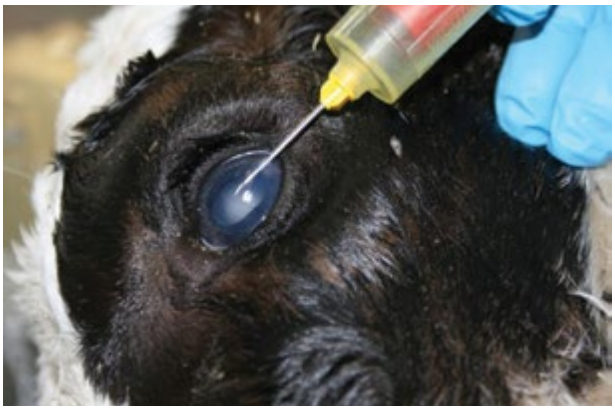

Fig.12. Sampling the aqueous humour using a vacutainer

Toxic, deficiency states and focal symmetrical encephalomalacia

For toxic causes such as lead or other heavy metals collect (and freeze at -20°C if required) 50g of liver and kidney.

To assess trace element status (as in swayback) liver can again be collected for evaluation. Fixed brain can also be used for copper estimation and is perhaps a more useful indicator of deficiency in cases of swayback which have already had a histological diagnosis.

A diagnosis of pulpy kidney/focal symmetrical encephalomalacia (due to *Clostridium perfringens* enterotoxaemia type D) can be suspected based upon the gross changes (pulmonary oedema, serous fluid with a fibrin clot in the pericardial sac, endocardial haemorrhage and brain swelling), glucosuria and the presence of large numbers of Gram positive bacilli in the small intestinal contents. Confirmation can be established through histopathology of the brain and clostridial toxin analysis of the small intestinal or caecal contents (collect approximately 10 g into a sterile container). Care should be taken in the interpretation of clostridial toxin results as low levels of some toxins are normally present within the intestine. These toxins are also labile so a negative result need not necessarily negate pulpy kidney as a diagnosis. The laboratory pathologists will be able to assist in interpreting these findings for you.

In addition to collecting brain and spinal cord it is strongly advised that representative portions of heart, lung, liver, spleen and kidney are also

collected as per the information sheet ‘Post mortem procedures for large animal species’. Any lesioned tissue should also be collected and fixed and other appropriate samples taken. These samples should be dispatched along with the fixed brain and spinal cord.

Summary

In summary the following samples can be collected for the investigation of nervous disease

- **Fixed brain and spinal cord**
- **Fixed major viscera**
- **Meningeal swab for bacteriology**
- **Brainstem for selective listerial culture**
- **50g of fresh liver and kidney-toxicology and trace element deficiency assessment**
- **Aqueous or vitreous humour**
- **Distal small intestinal/caecal contents-clostridial toxin analysis**

Although daunting, the careful assessment of the live animal, thorough post-mortem examination and collection of a relatively small number of sample types will help in the investigation and diagnosis of many neurological conditions of ruminants. Full neuropathological backup is available via the laboratory and we are happy to discuss cases with you to help achieve a diagnosis.

Reference

Edwards and Foster (2009). Use of ocular fluids to aid post-mortem diagnosis in cattle and sheep. In Practice 31: 22-25

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