AXIOM
Veterinary Laboratories Ltd
'The Quality Clinical Pathology Service'

Post Mortem Sampling for the Investigation of Nervous Disease in the Pig

The field and laboratory investigation of nervous disease in pigs involves careful 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. Glasser's disease) or vaccination history that should be taken into account in assessing likely differentials prior to necropsy and guiding the investigation.

Central nervous system diseases broadly fall into four categories in pigs-developmental, infectious, intoxications and deficiencies. Examples of these include;

Developmental-congenital tremor syndrome

Infectious-

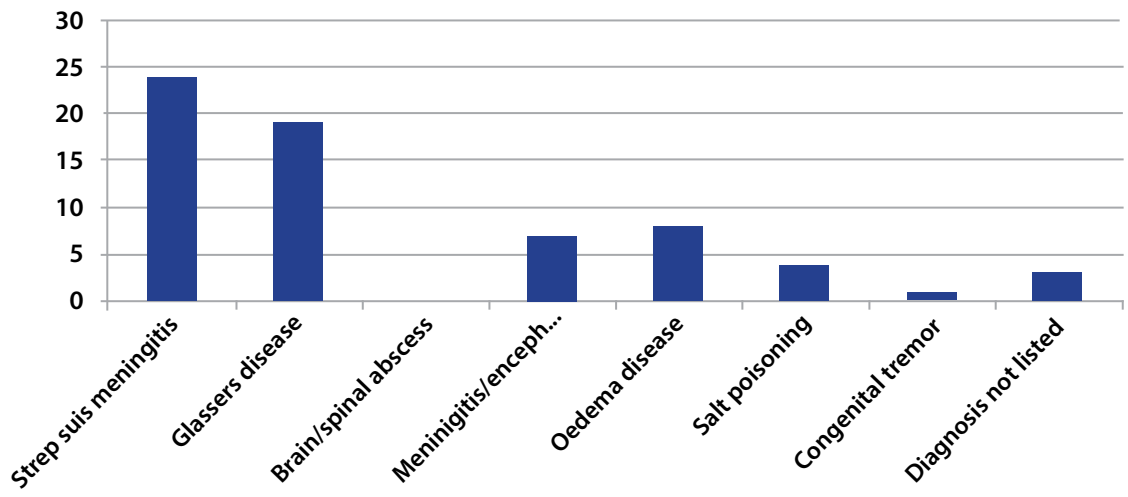
Viral - Aujeszky's disease, CSF, Teschen disease, enterovirus, HEV, PCV2, EMCV etc.
Bacterial - *Streptococcus suis*, *Haemophilus parasuis*, spinal abscess etc.

Intoxications-

Chemical - salt poisoning/water deprivation, selenium toxicity etc.
Bacterial - oedema disease and tetanus.

Deficiencies-vitamin A, and pantothenic acid.

The following graph shows the APHA VIDA information for 2012 providing an outline of the common causes of nervous disease seen in pigs in the UK.



Before you start...

Health and Safety

Before embarking on any post mortem whether on farm or in dedicated facilities you should consider the health and safety aspects not only for yourself but also of those who may be assisting or having to deal with the carcass waste.

Personnel protective wear should include suitable gloves, apron or overall, wellington boots, a cut proof glove (to be worn on the non-cutting hand), and a face mask and hard hat if required. This will significantly reduce the likelihood of personnel injury or acquisition of zoonotic infection. Should you be presented with a potential zoonotic disease ensure you are familiar with any procedures that may be required to reduce infection to yourself and others.

Working in the correct environment and with suitable tools/instruments will make the post mortem easier. Consider what is available to you and always opt for the most suitable facilities.

Post Mortem Instruments

The following photograph provides a guide as to the instruments needed to undertake a full post mortem. They are available through veterinary suppliers (or garden centres for the loppers) and should be dedicated for post mortem work.

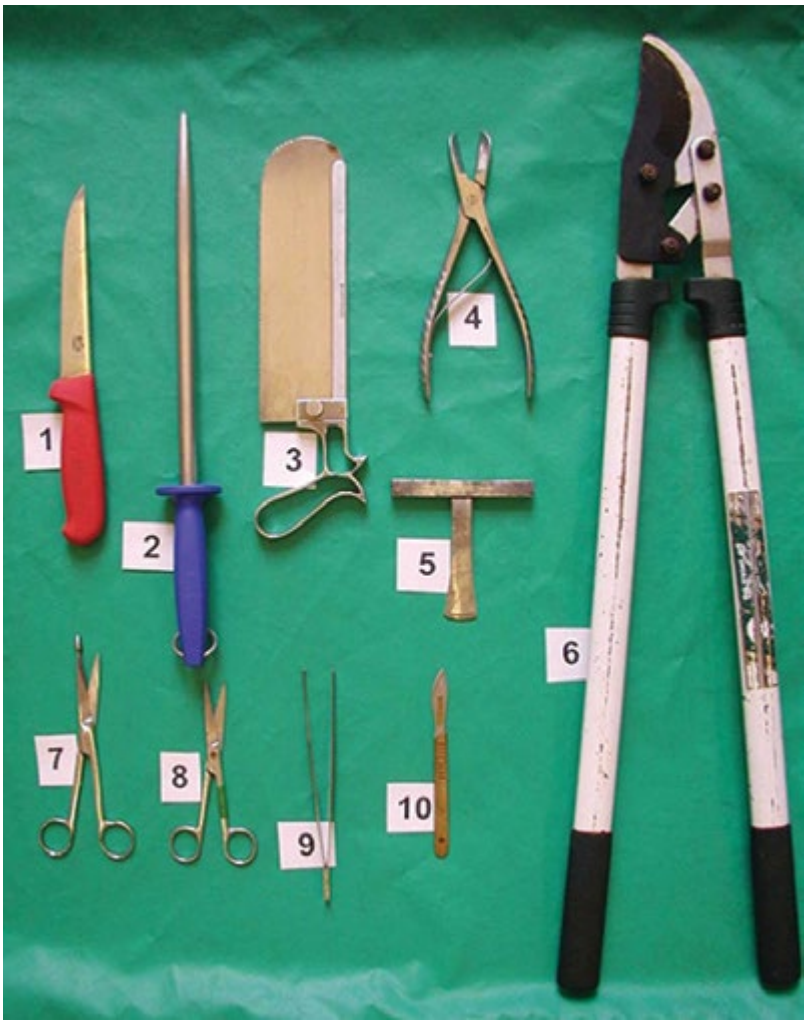


Fig. 1 PM equipment

1. Post mortem knife(PM40 knives - Swann Morton are an alternative)
2. Steel
3. Saw (in addition a butchers saw is useful for larger species)
4. Bone cutters
5. T piece (for inserting into saw cuts in cranium to apply leverage)
6. Garden Loppers
7. Gut scissors
8. Sharp pointed scissors
9. Rat tooth forceps
10. Scalpel

In many cases the brain can be removed whilst the head is still attached to smaller carcasses but for larger animals the head can be removed and either steadied between your knees or placed in a modified vice as shown in figure 2. They will make the task of brain removal much easier and are a worthwhile investment.

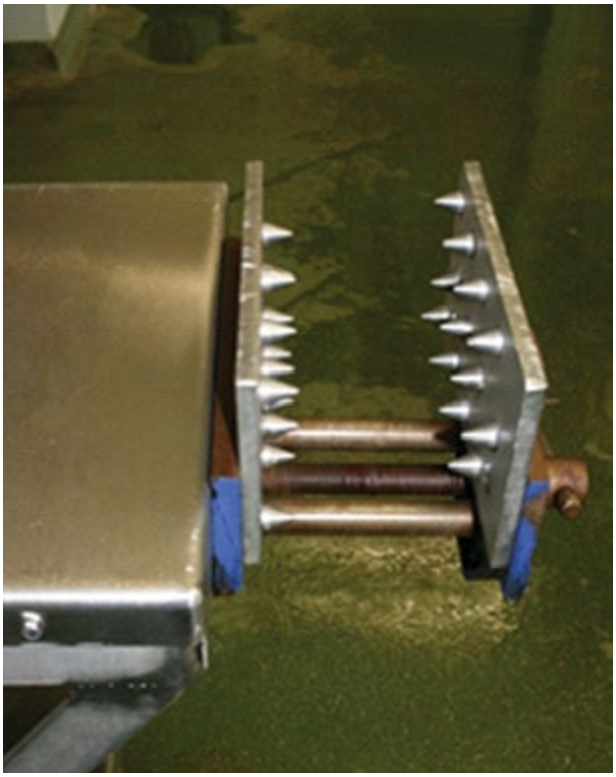
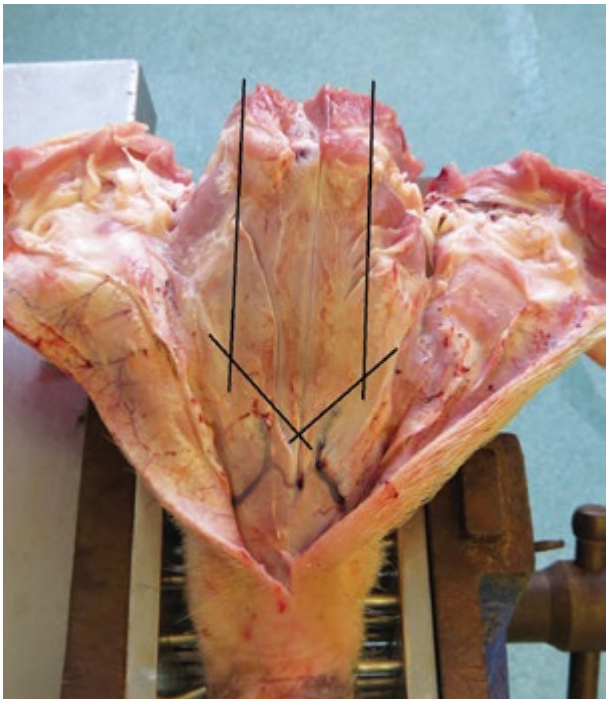


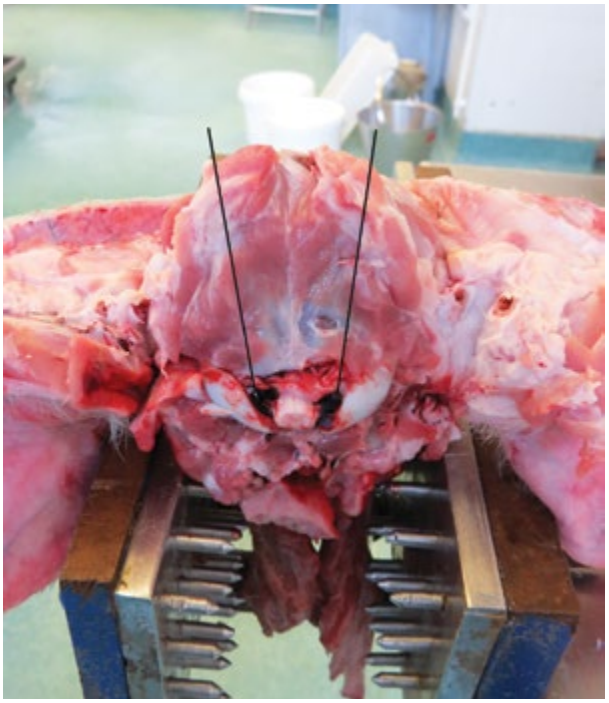
Fig.2

Post-mortem examination and sampling for diagnosis

Post-mortem examination should be an integral part of any nervous disease investigation in pigs. This may be an elective procedure or as an opportunistic investigation on presentation of a dead animal for post-mortem. Ideally up to 3 animals should be examined and these should preferably be animals early on in the course of the disease and not receiving any medication.

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.





Figs.3 and 4 Position of saw cuts on the calvarium

To remove the brain you will need a saw, bone forceps/snips and a T piece or bone chisel (see figure 1). As an alternative to a hand saw an oscillating saw (or 'DIY multi-tool') can be used however you must ensure the necessary health and safety precautions of wearing protective eye wear and a face mask are adhered to as pathogens such as *Strep. suis* have zoonotic potential. 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.

Skin the head to expose the calvarium. Make saw cuts as shown in the photographs through the calvarium (figures 3 and 4) 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. Note – the tentorium cerebelli between the cerebellum and the cerebral hemispheres needs to be cut/ removed (Fig.5).

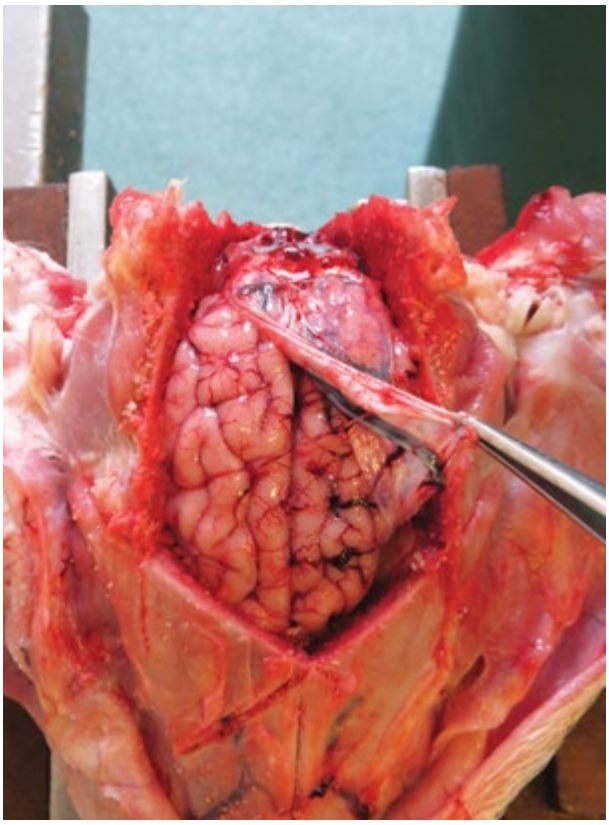


Fig.5

See section on bacteriology sampling (below) at this point before proceeding.

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 this procedure can be done using bone forceps alone) (Fig.6).

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 figure 7.

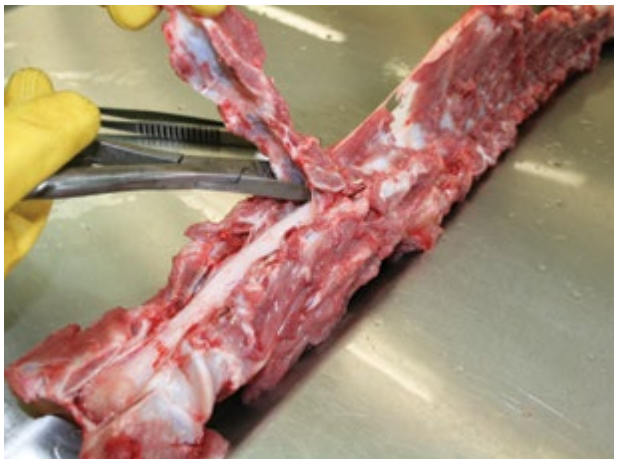


Fig.6

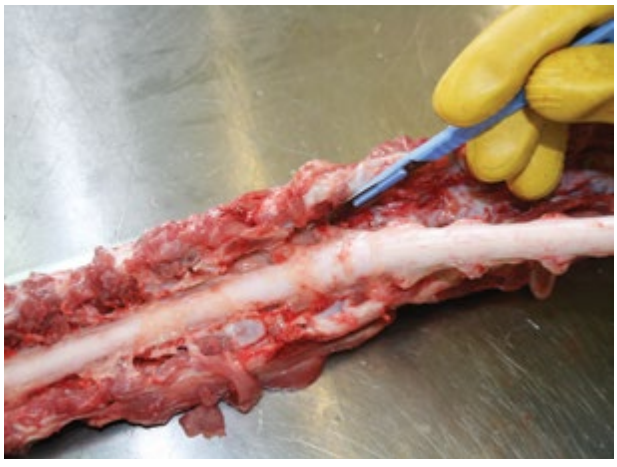


Fig.7

An alternative method is to split the spinal column sagittally particularly if spinal abscessation/osteomyelitis is suspected to identify the affected area. Following removal of the head hang the carcass by one hindleg and using a butcher's saw cut the spinal column longitudinally (sagittally). The weight of the free half of the carcass facilitates this procedure (Fig.8).

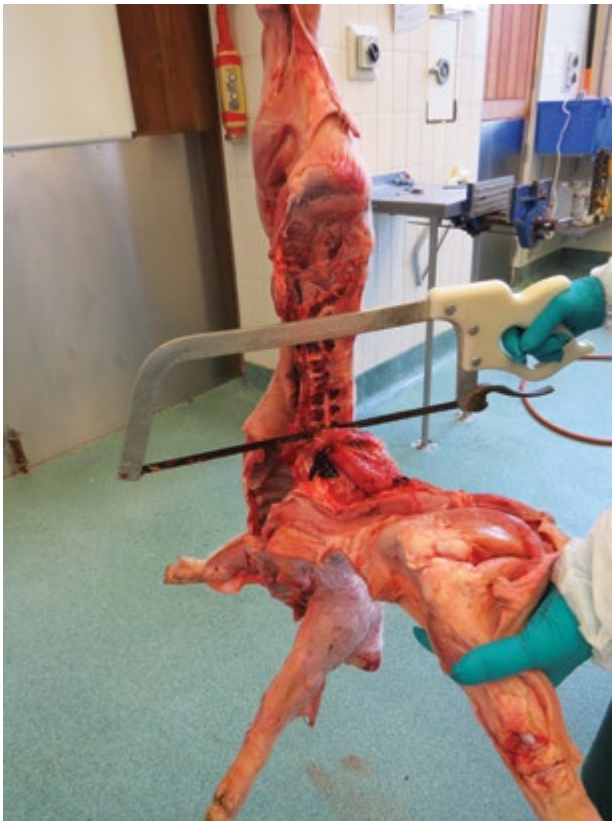


Fig.8

Fixation for Histopathology

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.

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.

Bacteriology sampling

As bacterial meningitis is a common cause of nervous disease in pigs consideration should be given to sampling for bacterial culture. It is possible to collect cerebrospinal fluid (CSF) prior to removal of the head. Cutting immediately behind the head/ears through the cervical muscles it is possible to expose the spinal cord still within the dura mater where it exits the foramen magnum. Spray this area with methylated spirits and then insert a needle through the dura mater to aspirate CSF in a sterile fashion. Place the CSF in a sterile container (a vacutainer is used here for illustration) for submission to the laboratory (Fig.9).

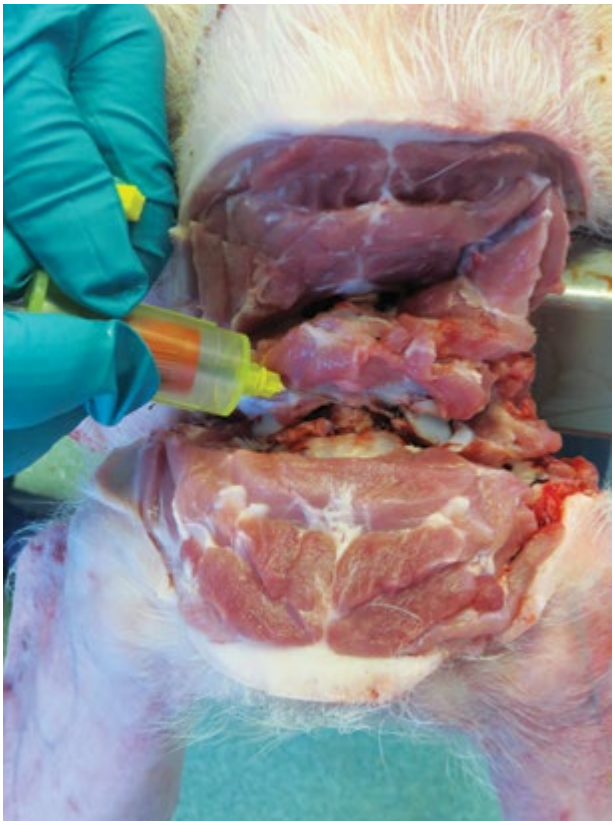


Fig.9

During the post mortem once the calvarium has been removed and the brain exposed a swab can be taken for routine bacteriology by inserting between the cerebral hemispheres and cerebellum. Place the swab in transport media. Prompt submission of swabs to the laboratory will aid isolation of relevant pathogens (Fig.10).

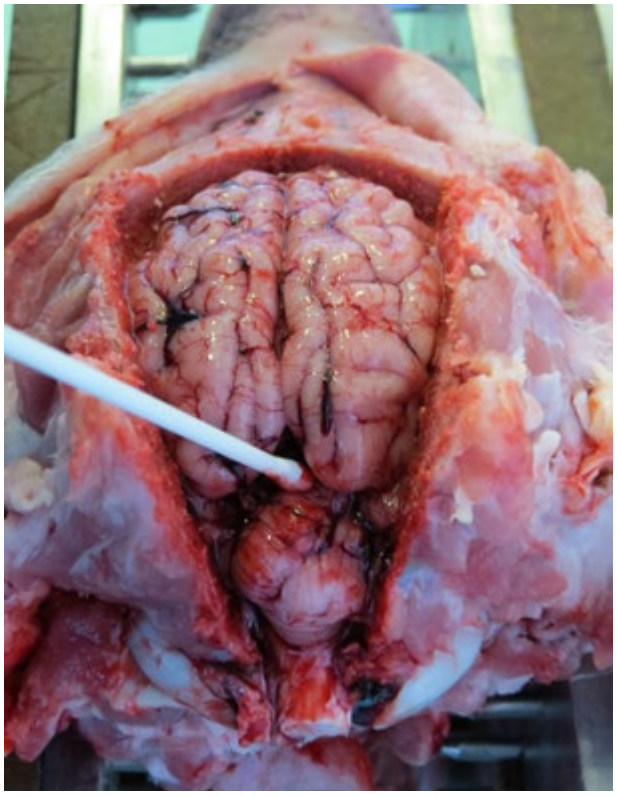


Fig.10

Virology sampling

In the majority of cases samples are not taken specifically for virology. Concurrent PRRSV infection however maybe a significant factor in the development of bacterial infection. In the case of post-mortem examination collection of fresh spleen for PCR is advisable. It may be more useful to make an assessment of the PRRSV status of the group as a whole by blood sampling 10 pigs and submitting serum for a pooled PCR.

For specific CNS virology testing the collection of fresh proximal spinal cord, rostral cerebrum and the lateral lobe of the cerebellum is required. This would only normally be undertaken in specialist laboratories.

Summary

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

1. Fixed brain and spinal cord
2. Portions of fixed major viscera – liver, lung, heart, kidney, lymph node, spleen and lesioned tissues (stomach and large intestine/mesocolon for oedema disease)
3. Meningeal swab/CSF for bacteriology
4. Caecal contents-bacterial culture (oedema disease)
5. Fresh lesioned tissue (e.g. lung) or swabs – bacteriology
6. 50 g of fresh liver and kidney-toxicology
7. (Fresh spleen/serum blood for PRRSV PCR)
8. Fresh proximal spinal cord, rostral cerebrum and the lateral lobe of the cerebellum - virology

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 pigs. Full neuropathological backup is available via your laboratory and pathologists are happy to discuss cases with you to help achieve a diagnosis.

Further reading

Done, S (1995) Diagnosis of central nervous system disorders in the pig. *In Practice*, 17:7 318-327

DVD Prosection guide for pigs, Edinburgh University.

(www.ed.ac.uk/schools-departments/vet/studying/cpd/learning-materials/overview)

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