

Guide to sample collection for confirmation of equine grass sickness (EGS)

1. Ganglia

Confirmation by histology of autonomic ganglia is the best method of diagnosing EGS, however, if circumstances mean that only ileum can be collected, please do take this sample and send to us – many thanks in advance!

The easiest ganglia to locate post mortem are the intervertebral ganglia of the sympathetic chain. This can be found after placing the horse flat on either side and removing the uppermost rib cage, using loppers (e.g. garden loppers) just above the sternum and approximately 10-15cm from the vertebrae (Fig 1), or by hanging the cadaver vertically by the fore or hind legs and pulling the lungs and heart out of the way. There is a chain on each side and they are equally easy to find. If bleeding occurs, use swabs to clear the view. It is a white band approximately 8mm wide along the bodies of the thoracic vertebrae (Fig 1). Approximately 20-30cm should be collected (this should contain 4-5 small ganglia). As far as possible, attached tissue e.g. fat should be dissected off before placing the intact piece in formalin. If possible, a second piece should be placed in a tube containing RNA Later.

Please note: The amount of formalin should be at least 10x the volume of the tissue to ensure complete preservation of the ganglia.

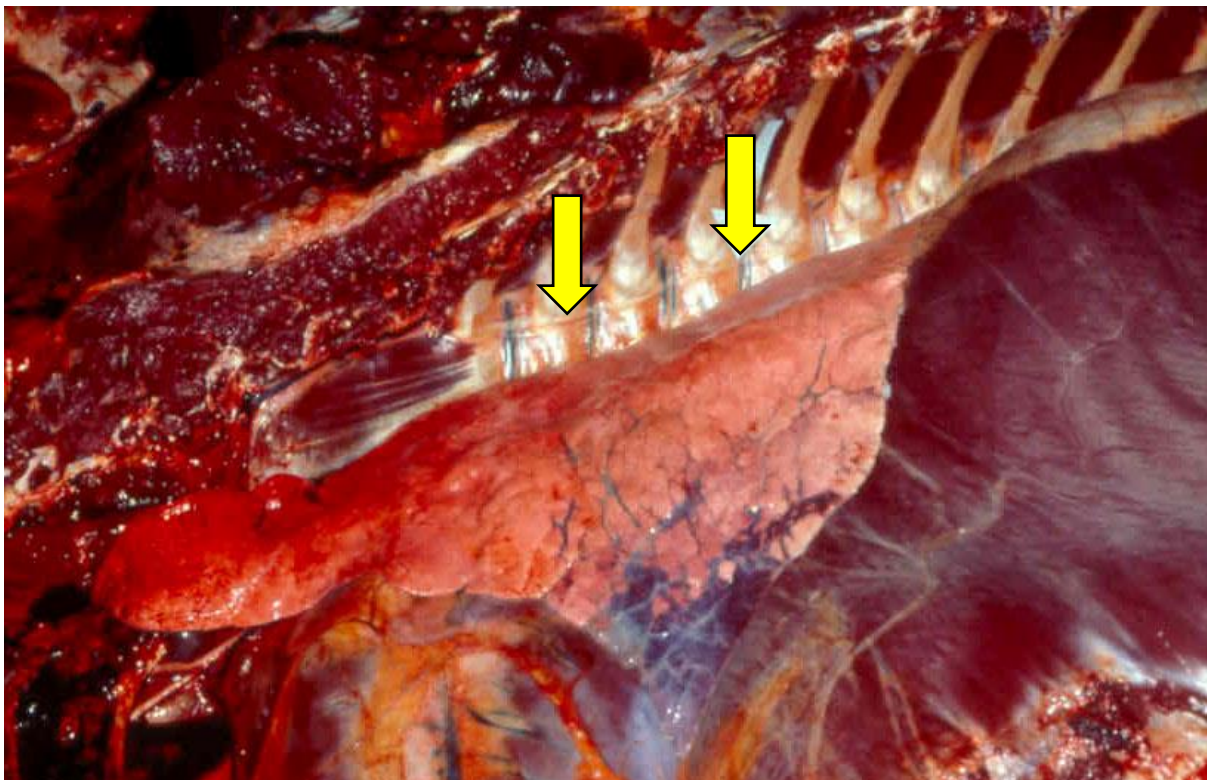


Fig 1. Lateral view of the thorax with the left side of the ribcage removed. The thoracic chain is the pale line running along the bodies of the vertebrae (yellow arrows).

2. Ileum

In addition to ganglia, or if ganglia cannot be collected, a small complete circle of ileum **not more than 1cm long**, from an area approximately 5-15cm from the end of the ileum should be collected (Figs 2 and 3), i.e. just before the ileocaecal opening. The intestinal tract should first be removed and the blind-ending caecum located by following the small intestine distally until the caecum is reached. The ileum enters the inner aspect of the caecum near the top.

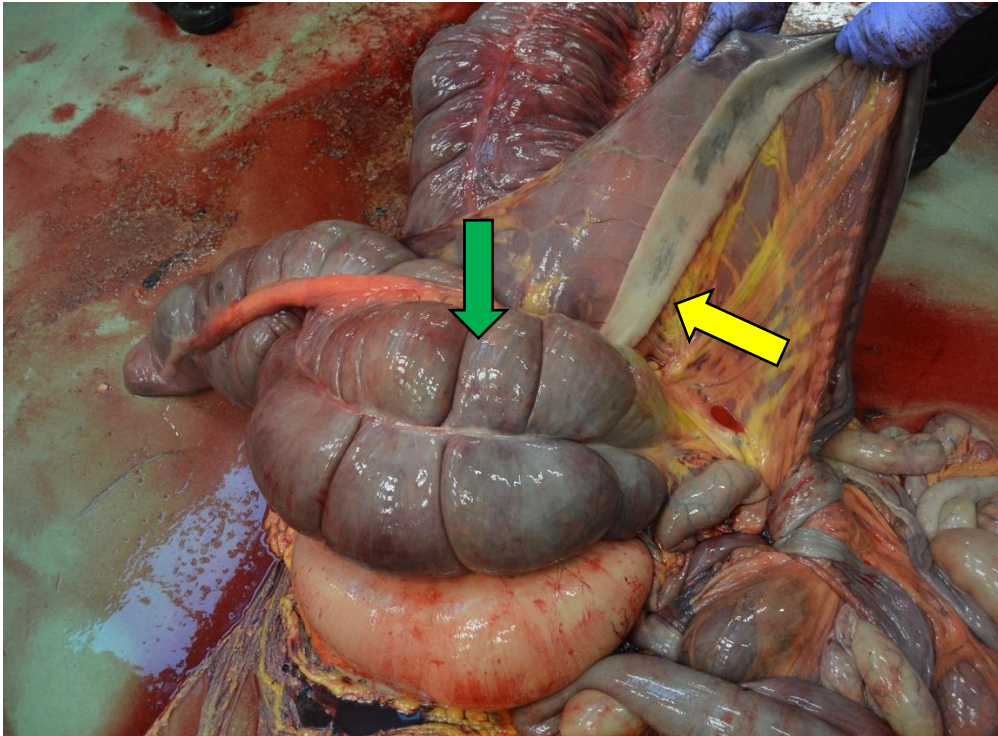
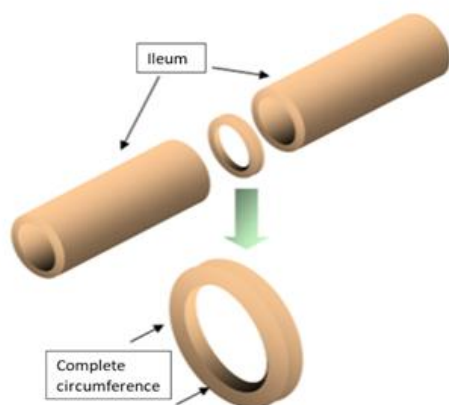


Fig 2. The intestinal tract has been removed. The end of the ileum (yellow arrow) enters the inner aspect of the top of the caecum (green arrow).



If circumstances only allow a small abdominal incision, make the cut in the midline just in front of the pelvis and feel for the top of the caecum on the horse's right side. The ileum can be felt as a thick tube entering the inner aspect of the caecum. It should be placed in at least 10x its volume of formalin. If there is a lot of food material in the piece of ileum collected, it can be gently agitated in the formalin to dislodge it. This aids fixation.

Fig 3. Diagram of sampling of terminal ileum. A complete circumference should be collected, not more than 1cm long.