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Enterotoxaemia

in Sheep, Goats and Cattle

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1. Introduction

Enterotoxaemia caused by *Clostridium perfringens (Clostridium welchii)* type D occurs throughout the world. It is one of the most important infectious diseases of sheep in Australia. Effective vaccines are available and widely used.

The disease is most common in lambs three to 10 months old. Important predisposing factors are the suckling of well-fed heavy milk producing ewes, grazing of lush rapidly growing pasture or cereal crops and diets containing high levels of grain.

Older sheep also may be affected, particularly those in good body condition being fed nutritious diets. In other species of ruminants enterotoxaemia occurs most frequently in goats of any age and calves. The diagnosis of the disease is confirmed infrequently in adult cattle.

A strong indication of a diagnosis of enterotoxaemia can be obtained from the dietary history, clinical signs and gross pathological findings. The detection of glycosuria by colorimetric test strips or reagent tablets provides further support. Laboratory confirmation of the diagnosis depends upon bacteriological and histopathological techniques. The former include the visualisation of *C. perfringens* in Gram stained smears and the detection of epsilon toxin in intestinal contents by mouse assay or immunological techniques. Pathognomonic histological lesions can be found in the brain.

2. Aetiology

C. perfringens type D is part of the normal flora of the alimentary tract of most ruminants. It cannot survive long in soil.

The ingestion of high levels of starchy food provides a suitable substrate for *C. perfringens* type D strains to multiply in the small intestine and produce considerable amounts of epsilon toxin. During logarithmic growth of the clostridia epsilon toxin is produced as a relative non-toxic prototoxin which is converted to its highly toxic principle by proteolytic enzymes in the intestine. The intestinal wall is normally impermeable to proteins, but epsilon toxin increases the permeability of the intestinal mucosa, allowing it to be readily absorbed. Epsilon toxin exerts its primary action on vascular endothelium.

3. Clinical Signs

3.1. Sheep

Acute, subacute and chronic forms of enterotoxaemia occur. Acute cases in lambs may show convulsions, frothing at the mouth and death. Older sheep may exhibit convulsions, muscle tremor, teeth grinding, salivation, rapid breathing, diarrhoea and bloat. Subacute and chronic forms are also known as focal symmetrical encephalomalacia (FSE). Most acute cases are found dead but some lambs will show convulsions, gasping and struggling prior to death. Animals with FSE show a variety of signs which include dullness, circling, incoordination, head pressing and opisthotonus.

3.2. Goats

The symptoms are similar to those in sheep but are frequently more prolonged with evidence of abdominal pain and diarrhoea. Chronic cases are more common, being characterised by anorexia, dullness and diarrhoea with faeces containing shreds of mucosa.

3.3. Cattle

As for sheep.

4. Gross Pathology

4.1. Sheep

Affected animals are usually in good body condition and autolysis can be rapid. There may be evidence of struggling, opisthotonus and diarrhoea. Gross pathological changes in acute cases include accumulation of serofibrinous fluid in the pericardial sac, pulmonary congestion and oedema, and petechiae and ecchymoses beneath the endocardium of either ventricle, and beneath other serous membranes especially the parietal peritoneum.

In sheep that have been dead for longer than four to six hours the kidneys are very autolytic or 'pulpy' and contain many cortical haemorrhages. Glycosuria may be present. The small intestine is usually flaccid, distended with gas, hyperaemic and the contents vary from mayonnaise-like to watery and blood stained.

In subacute or chronic cases gross lesions are generally restricted to the brain. Bilaterally symmetrical focal haemorrhagic lesions of malacia may be detected in the internal capsule, dorsolateral thalamus, mid-brain and cerebellar peduncles.

4.2. Goats

The changes are similar to those in sheep except that 'pulpy' lesions of kidneys are not prominent and lesions of the small intestine are frequently more severe. A chronic condition with catarrhal enteritis and anaemia has also been described.

4.3. Cattle

Affected cattle may have exhibited nervous signs prior to death. The gross changes observed in calves resemble those found in sheep except for rapid autolysis of the kidneys and visible brain lesions. However subcapsular renal congestion and haemorrhage may be apparent with blood clots up to 1 cm being found.

5. Histopathology

5.1. Sheep

The most consistent histological lesion in acute cases is perivascular proteinaceous oedema in the brain. Fresh sections of small intestine reveal emigration of neutrophils across the lamina epithelialis mucosa and numerous Gram-positive bacilli in association with the microvillous brush border. Kidneys from animals dead for longer than four to six hours have accelerated autolysis of the proximal tubular epithelium and interstitial cortical congestion and haemorrhage.

In the brains of chronic cases there may be bilaterally symmetrical foci of leucoencephalomalacia in the areas outlined above.

5.2. Goats

As for sheep.

5.3. Cattle

FSE may occasionally be seen in cattle, but its presence is not diagnostic of enterotoxaemia because of another symmetrical malacias of unknown aetiology. Congestion of the renal cortex may be present. The intestinal changes are similar to those in sheep and goats.

6. Collection of Specimens and Laboratory Examination

6.1. Procedure

(a) Collect a sample of urine; test for presence of ketone, glucose and protein.

(b) Remove whole brain and slice transversely at the internal capsule, optic chiasma, midbrain and middle cerebellar peduncles. This allows examination for macroscopic lesions of FSE. Immerse the brain in four times the volume of 10% buffered formol saline (Lillie and Fullmer, 1976). After fixation, examine haemotoxylin and eosin (H and E) stained sections prepared from these sites plus the cerebral and cerebellar cortices.

(c) Make and heat fix at least 12 smears of the inner surface of hyperaemic areas of small intestinal wall, taken at various levels; stain with Gram.

(d) Collect a 40 mL aliquot of the contents of the small intestine (including content from some hyperaemic regions) in a clean glass container for epsilon toxin detection using counter immunoelectrophoresis or toxin–antitoxin cross-protection tests in mice (see 6.1.1.). This sample should be kept cold and taken to the laboratory expeditiously; delays in transportation can lead to misdiagnosis. The practice of adding chloroform ($CHCl_3$) to intestinal content to preserve epsilon toxin is no longer recommended. These intestinal contents are often viscous and if so they may be diluted 1:2 with saline; because of the viscosity filtration is rarely used to prepare the inoculum. Transfer the intestinal contents to a centrifuge, place a small piece of cotton wool on the surface of the contents and centrifuge at 4000 *g* for 30 min. Decant the supernatant and use for the following tests (see 6.1.1.).

6.1.1. Counter Immunoelectrophoresis for the Detection of Epsilon Toxin

Overlay a glass plate 8.5 x 14 cm with agarose type IV (Sigma Chemicals, St Louis, USA) prepared in Tris-barbiturate buffer, pH 8.6 (Hornitzky *et al.*, 1989). Prepare a series of wells of 10 µL volume in 2 parallel rows 1 cm apart. Place the intestinal contents (neat, diluted 1:2 and 1:4), prepared as described in 6.1.(d), in the series of wells closest to the cathode, while 10 µL of horse anti-C. *perfringens* type D (Wellcome Diagnostics, Dartford, UK) is placed in the series of wells closest to the anode. Apply a constant current of 10 mA and variable voltage of 250 V for 60 min using a standard immunoelectrophoresis unit. Examine the plates after 60 min for precipitin bands between the wells. Place the plates in a humid environment at room temperature overnight, as some precipitin bands take some time to develop. As a control procedure place 10 µL of purified epsilon toxin (Wellcome Diagnostics, Dartford, UK) or known epsilon toxin positive intestinal contents in the wells nearest the cathode (Hornitzky *et al.*, 1989). Precipitin bands produced from the reaction between C. *perfringens* type D toxin and antitoxin are thin lines. Diffuse lines are non-specific reactions. The highest dilution at which toxin is detected provides a quantitative estimate of the amount of toxin present.

6.1.2. Mouse Toxin Assay

Due to animal welfare considerations this assay should only be used if facilities for counter immunoelectrophoresis (CIEP) are unavailable.

To 0.6 mL of the supernatant add 0.2 mL of C. *perfringens* type D antitoxin. These volumes are not critical and may be varied according to circumstances. Stand mixture for 30 min. Inject two mice slowly intravenously with 0.3 mL of supernatant and two with about 0.4 mL of the supernatant–antitoxin mixture. Observe mice for one hour and again after 24 hours. Deaths occurring within three minutes are usually non-specific. If mice survive when given supernatant plus type D antitoxin and the control mice die, it may be deduced that epsilon toxin is present in the original sample.

7. Diagnosis

Diagnosis of enterotoxaemia is based upon the following.

(a) Consideration of the nutritional and clinical history.

(b) Consideration of gross pathological and laboratory findings, including lesions detected in the sliced brain.
(c) Demonstration of large numbers of uniform clostridia (Gram-positive large rods with slightly rounded ends) *arranged in groups* in smears prepared from the tunica mucosa of the small intestine. However, in subacute or chronic enterotoxaemia very few clostridia may be detectable even though consistent lesions may be present in the brain.
(d) Examination of urine — glycosuria present in most cases.
(e) Demonstration of epsilon toxin in the small intestine of animals which have died recently.
(f) Examination of H and E stained sections of brain for perivascular oedema and FSE.
(g) Culture of small intestine for the isolation and typing of *C. perfringens* strains offers no great advantage. It is a relatively tedious process and many strains appear non-toxigenic although production of conditions *in vitro* suitable for toxin production may not have been realised.
(h) The degree of post-mortem autolysis present. Toxin may be produced by post-mortem clostridial proliferation in animals dying from other conditions.

8. Differential Diagnosis

8.1. Sheep

In young lambs *Escherichia coli* septicaemia closely resembles acute enterotoxaemia. However there is a greater tendency for pleural exudates to be present and Gram-negative rods will be in smears of heart blood and the organism isolated by culture. Selenium toxicity presents similar signs and may occur when weaners have been drenched with selenium-containing preparations. The clinical signs of FSE can sometimes be closely mimicked by tetanus, polioencephalomalacia and listeriosis. Various metabolic conditions and grain poisoning need to be considered in adult sheep as these frequently occur under the same conditions as enterotoxaemia.

8.2. Goats

As for sheep (incidence of *E. coli* septicaemia not known). The chronic form can be confused with salmonellosis and arsenic poisoning.

8.3. Cattle

As for sheep. In calves lead poisoning frequently produce clinical signs and gross lesions suggestive of enterotoxaemia. Bloat is frequently confused with enterotoxaemia during post-mortem examinations, particularly in animals which have been dead for a number of hours. Severe haemorrhage in the nasal mucosa characterises the former and CIEP should be used to confirm the latter.

9. Enterotoxaemia in Sheep, Goats and Cattle Caused by Clostridium perfringens Other than Type D

Where enterotoxaemia is suspected and epsilon toxin or consistent brain lesions cannot be demonstrated, pairs of mice may be injected intravenously with intestinal content and antitoxin prepared from types A, B, C and D; one pair should also receive intestinal content with no antitoxin added. Results should be treated with caution as the evidence for the involvement of types A, B and C in enterotoxaemia of sheep and cattle in Australia is largely circumstantial.

10. References

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