WAS IT CLOSTRIDIAL? HOW DO WE PROVE IT?

Michael Hardcastle, of Gribbles Veterinary, Auckland, outlines how to rule in or rule out the most commonly suspected clostridia.

CLOSTRIDIAL DISEASE IS frequently associated with sudden death syndromes in both production and companion animals in New Zealand, but has been notoriously difficult to prove.

Over the last 15 years there has been extensive research in this area which has led to fulfillment of Koch's postulates in a number of diseases. However there are a lot of suspected diseases which have still not been proven to occur in veterinary species.

There has been a recent surge in the popularity of clostridial toxin testing overseas and in New Zealand. However, the significance of toxin, gene or pathogen identification depends on clinical signs, gross and histological lesions, clostridial and veterinary species involved and sometimes the quantity of toxin identified.

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For example:

- *Clostridium perfringens* Type A is commonly found in faecal samples of normal production and companion animals therefore isolation is insignificant on its own.
- Alpha toxin is produced by all *C. perfringens* therefore isolation is insignificant on its own.
- Up to 20% of normal sheep and cattle can be positive for Epsilon toxin which causes Enterotoxaemia ("Pulpy Kidney Disease") most commonly caused by *C. perfringens* Type D.
- *C. septicum* and *C. sordellii* are the most commonly isolated post-mortem clostridia and are only considered significant in cases of Malignant Oedema in production animals in New Zealand.

Diagnostic testing includes:

- Bacterial identification (Culture, PCR, IHC, FAT).
- Toxin testing e.g. ELISA.
- Toxin gene testing e.g. PCR, qPCR.
- Histology.
- Mouse inoculation.

Some of these tests (e.g. immunohistochemistry (IHC), fluorescent antibody testing (FAT) and mouse inoculation) are not currently available in New Zealand.

Some toxin or IHC tests could be referred overseas, but these would be expensive and have a slow turnaround time. Considering all of this, the following is an attempt to list how the most commonly suspected Clostridia might be ruled in or out in the New Zealand context.

CLOSTRIDIUM PERFRINGENS TYPE A

This strain is mainly characterised by production of alpha toxin, although it sometimes also produces beta2, enterotoxin and a range of other toxins. It is considered to cause necrotic enteritis in poultry, and has also been associated with gastroenteritis in a range of other species – although, absolute proof of this is lacking. It is commonly found in the intestinal tract of healthy animals. Exceptions to this may occur in pigs, where the gene for beta2 has been frequently found in Type A strains isolated from cases of enteritis, and in dogs, where *C. perfringens* Type A netE and netF toxin genes have been more frequently found in cases of haemorrhagic gastroenteritis than in healthy dogs. Overseas, it is linked to 'yellow lamb disease', but to my knowledge that is not seen in New Zealand. Points to note:

- Gross lesions will include areas of haemorrhagic intestine and sometimes stomach in dogs.
- Histologically, there is haemorrhagic necrosis of the mucosa lined by large Gram-positive bacilli.
- *C. perfringens* can be cultured by routine methods, but a positive culture is not diagnostic given that non-pathogenic strains can be commonly found in the intestinal tract.
- There are PCR tests for some *C. perfringens* toxin genes, but the potency and effects of alpha toxin and enterotoxin in domestic animals are controversial and probably limited.
- Overall, the gross and histological findings combined with PCR may be suggestive of the diagnosis.

CLOSTRIDIUM PERFRINGENS TYPE C

This strain is characterised by alpha and beta toxins. It is found less often than Type A in the intestinal tract of healthy animals, and causes necrohaemorrhagic enteritis in mainly neonatal calves, lambs, foals and pigs due to the effects of beta toxin in the absence of trypsin digestion.

- Gross lesions will include areas of red/black small intestine.
- Histologically, there is mucosal to transmural necrosis with thrombosis, haemorrhage or fibrin in the lumen, along with many large Gram-positive bacilli.
- The previous comments regarding *C. perfringens* culture apply here.
- The beta toxin gene of *C. perfringens* Type C can be detected by PCR. If found, this is consistent with the diagnosis.
- The gross and histological findings alone may be suggestive of the diagnosis.

CLOSTRIDIUM PERFRINGENS TYPE D (ENTEROTOXAEMIA)

This strain is characterised by alpha and epsilon toxins. It causes disease in lambs, kids and occasionally calves, mainly presenting as neurological signs and/or sudden death in well-fed animals – classically, those fed concentrate or grain. It is also reported to cause chronic enterocolitis and diarrhoea in goats.

- There may be non-specific gross changes including fibrinous effusions, haemorrhage or oedema. The kidneys autolyse quickly after death in ruminants, and a diagnosis of enterotoxaemia cannot be based solely on 'pulpy' kidneys.
- The only diagnostic histological lesions of enterotoxaemia are found in the brain, where there can be regions of perivascular oedema or malacia after vascular permeability changes caused by epsilon toxin. This is worse in certain sites, and so it is best if the entire brain is submitted for histopathology.
- Goats may have gross colonic ulcers, with histological enterocolitis and sometimes necrosis with Gram-positive bacilli.
- The previous comments regarding *C. perfringens* culture apply here.
- PCR for the epsilon toxin gene is not currently available in New Zealand.
- Glycosuria can be seen in other conditions and is not pathognomonic.
- However, classical histological findings are diagnostic.

CLOSTRIDIUM DIFFICILE

This produces two toxins (A and B) and causes enteritis and/or colitis in horses, rabbits, piglets and a range of other species, often after antibiotic treatment or hospitalisation.

- Gross lesions will include areas of congested and haemorrhagic intestine.
- Histologically, there is mucosal necrosis with thrombosis, haemorrhage, fibrin and neutrophils in the lumen, along with short Gram-positive bacilli.
- *C. difficile* can be cultured by routine methods, but a positive culture is not diagnostic given that non-pathogenic strains can be found in the intestinal tract.
- Testing for *C. difficile* toxins is available by referral to a medical laboratory, but these tests are not validated in animals.
- The gross and histological findings may be suggestive of the diagnosis.
- Positive PCR for the toxin genes of *C. difficile* is consistent with the diagnosis.

CLOSTRIDIUM CHAUVOEI (BLACKLEG)

This is a disease of ruminants, mainly associated with lameness leading to sudden death, although it can also be involved in malignant oedema. The bacterium cause lesions of necrosis within cardiac and skeletal muscles; these are classically ascribed to trauma creating anaerobic conditions favourable to the growth of resident spores.

- Grossly, there will be haemorrhagic, crepitant and oedematous areas with a 'rancid butter' odour in muscle bellies. These will be easier to find in 'fresh' animals, may be small, and so may require extensive dissection to locate.
- Histopathology of affected areas shows haemorrhage, oedema, necrosis, inflammation and scattered Gram-positive bacilli. This can

Bacteria	Disease[a]	Species[b]	Definitive diagnosis requirements
Clostridium perfringens Type A	Haemorrhagic enteritis	**Dogs**	Clinical signs Histology Toxin (NetF)
	Enteritis/enterocolitis	**Pigs** **Horses**	Clinical signs Histology
Clostridium perfringens Type B	Lamb Dysentery	**Sheep**	Histology Toxin (CPB and ETX)
Clostridium perfringens Type C	**Neonatal Necrotising Enteritis**	**Horses** **Pigs** **Lambs** Calves	Histology Toxin (CPB)
Clostridium perfringens Type D	**Pulpy Kidney Disease**	**Sheep** Goats	Histology (brain)
		Cattle	Histology (brain)
Clostridium perfringens Type G	**Necrotic enteritis**	**Poultry**	Histology Toxin (NetB)
Clostridium difficile	**Enterocolitis**	**Horses** **Pigs** **Rabbits** Rodents	Histology Toxin (A + B)
Clostridium novyi Type B	**Blacks disease**	**Ruminants** Horses	Postmortem/Histology Culture/PCR
Clostridium novyi Type D *(Clostridium haemolyticum)*	**Bacillary haemoglobinuria**	**Ruminants**	Postmortem/Histology Culture/PCR
Clostridium piliforme	**Tyzzers disease**	**Rabbits** **Horses** **Cats** **Rodents** Ruminants	Histology or PCR
Clostridium chauvoei	**Blackleg** **Malignant oedema**	**Cattle** Sheep	Postmortem/Histology Culture/PCR
Clostridium septicum	**Malignant oedema**	**Ruminants** Horses	Postmortem/Histology Culture/PCR
Clostridium sordellii	**Malignant oedema** **Foal omphalophlebitis** **Postparturient metritis**	**Ruminants** **Horses** **Sheep**	Postmortem/Histology Culture/PCR
Clostridium tetani	**Tetanus**	**Ruminants** **Horses** Pigs Dogs	Clinical history Gram stains of wounds
Clostridium botulinum	**Botulism**	**Avian** Horses Ruminants	Clinical history
Clostridium spiroforme	**Enteric disease**	**Rabbits**	Histology Culture

a Clinical diseases in bold font are confirmed in Veterinary Species (Fulfil Koch's postulates).
b Species in bold font are most commonly affected by this disease

be difficult to distinguish from putrefaction if animals are not fresh.
- The gross and histological findings may be suggestive of the diagnosis.
- *C. chauvoei* culture is supportive of the diagnosis since it is not a post-mortem contaminant.

CLOSTRIDIUM SEPTICUM, *C PERFRINGENS* AND *C SORDELLII* AS PART OF MALIGNANT OEDEMA, OR GAS GANGRENE

These bacteria cause lesions of oedema and necrosis within muscle and adjacent tissues of ruminants, horses and pigs, generally after trauma allowing bacteria to be introduced into an anaerobic wound. *C. septicum* also causes abomasitis (braxy).
- Grossly, there will be haemorrhage, oedema and emphysema; this will be easier to find in fresh animals.
- Histopathology of affected areas shows the same changes along with necrosis, scant inflammation and scattered Gram-positive bacilli. This can be difficult to distinguish from putrefaction if animals are not fresh, but histology may be suggestive of the diagnosis.
- Bacterial culture from tissues of a fresh animal is also suggestive of the diagnosis, but these species can also represent post-mortem invasion, and so careful interpretation of culture results is required.

CLOSTRIDIUM NOVYI TYPE B

This species causes rapid death, mainly in sheep. Bacteria latent in tissues sporulate within anaerobic liver foci (generally secondary to fluke migration) and produce their own alpha and beta toxins.
- Grossly, there is marked subcutaneous congestion ('black disease'), oedema, cavitary effusions and haemorrhages, with a yellow area of necrosis found in the liver. This may require thorough dissection to locate.
- Histologically, bacteria are found at the margin of the necrotic focus.
- Histology and culture of *C. novyi* from a necrotic focus are consistent with the diagnosis.

CLOSTRIDIUM HAEMOLYTICUM (AKA *C NOVYI* TYPE D)

This species causes intravascular haemolysis in cattle and sheep. Bacteria latent in tissues sporulate within anaerobic liver foci (generally secondary to fluke migration) and produce a beta toxin.
- Grossly, there is a large area of necrosis found in the liver, peritonitis, anaemia, red/brown kidneys and 'port-wine' urine.
- Histologically, the liver is as *C. novyi*, and there is haemoglobinuric nephrosis.
- Histology and culture of *C. haemolyticum* from a necrotic focus are consistent with the diagnosis.

CLOSTRIDIUM TETANI (TETANUS)

Tetanus is mainly suspected in horses and dogs showing signs such as spastic paralysis and rigor leading to death. This is thought to occur after *C. tetani* living and dying in an anaerobic wound release tetanus toxins.
- There are no diagnostic gross or histological lesions in tetanus.
- The bacteria could theoretically be cultured from a contaminated wound.
- However, most cases are diagnosed on clinical grounds.

CLOSTRIDIUM BOTULINUM (BOTULISM)

In New Zealand, botulism is mainly suspected in waterfowl undergoing warm weather mass mortality and flaccid paralysis. The bacteria live and die in anaerobic stagnant ponds, and waterfowl ingest their toxins. Other species may ingest toxins in forage contaminated by carcasses. There are also reports of *C. botulinum* colonisation of the intestinal tract or anaerobic wounds (toxicoinfectious botulism).
- There are no diagnostic gross or histological lesions in botulism.
- The bacteria are rarely cultured from affected animals.
- Most cases are diagnosed on clinical grounds and by ruling out other causes of death. (VS)

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