**Clostridium perfringens** TYPES A AND D ASSOCIATED WITH ENTEROTOXEMIA IN AN 18-MONTH-OLD GOAT


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**ABSTRACT:** Postmortem examination of a Boer buck that died peracutely revealed bowel and liver diffusely congested and edematous. Kidney was apparently edematous. *Clostridium perfringens* type A was isolated from bowel and type D from kidney. Microscopic examination revealed large areas of necrosis in the renal cortex and medulla (pulpy kidney disease), hyperemia and centrilobular necrosis of the liver, necrosis of the small-intestine wall, pulmonary edema and congestion, intense hyperemia of the cerebellum, hyperemia and edema of the brain.

**KEY WORDS:** goats, enterotoxemia, pulpy kidney disease, *Clostridium perfringens* type A, *Clostridium perfringens* type D, histopathology.

**CONFLICTS OF INTEREST:** There is no conflict.

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INTRODUCTION

An 18-month-old Boer buck in a 50-head commercial beef goat herd from São Paulo, Brazil, was found in acute distress and recumbent, and died the next day. The animal had only presented liquid feces some days before but had not experienced any previous health problems. All the animals had been vaccinated against clostridiosis with a commercial vaccine containing inactivated culture of *Clostridium chauvoei* and toxoids of *C. septicum, C. novyi, C. perfringens* types B, C and D, and *C. sordelli*. Vaccination was performed four months before the death; a booster dose used to be applied to the primovaccinated animals about 30 days after the first vaccination, and then all the herd was vaccinated every six months.

Postmortem examination revealed good body condition and no obvious external lesions. The small intestine was diffusely necrotized with presence of gas the suprascapular lymph node was enlarged and congested as the liver, kidneys were congested but only the left one was edematous, both kidneys presented loss of areas in the cortical and medullar zones. The heart and lungs were also congested. Despite the prior vaccination, a presumptive diagnosis of enterotoxemia was made.

Samples of heart, lung, liver, kidneys, small intestine, cerebellum and brain were collected into 10% buffered formalin and subjected to the Pathological Anatomy Laboratory of Instituto Biológico for histopathological analysis. Bowel content and the left kidney were also subjected to General Bacteriology Laboratory of Instituto Biológico for bacteriological culture and polymerase chain reaction (PCR) procedures.

Due to diarrhea, an occasional coccidial infection was suspected, which could have contributed to pathogenesis in this case, but unfortunately, parasitological tests were not performed.

The *C. perfringens* species is a very heterogeneous group of organisms regarding their metabolic byproducts, toxins and pathogenic potential. For practical classification purposes, the species is divided into five types, from A to E, based on their ability to produce any of the four major lethal toxins (alpha, beta, epsilon and iota) (10). Type A is the most frequently occurring *Clostridium* in mammals, birds and in the environment; it produces enteric disease generally mild, with minimal damage noted in the intestinal mucosa and, in addition to enteritis, it produces gas gangrene; in the Western United
States, it causes hemorrhagic abomasitis in young ruminants, often accompanied by severe diarrhea; in the Pacific Northwest of USA, a condition called yellow lamb is associated with \textit{C. perfringens} type A (7). Type D is perhaps the best known pathogenic \textit{C. perfringens} type, being widely regarded as the causative organism of fatal enterotoxemia of sheep or “overeating disease”. It appears to have a worldwide distribution but is not a common intestinal commensal. It produces epsilon-toxin, an angitoxin that damages endothelial cells, which is almost exclusively responsible for the host pathology and subsequent death. The toxin is produced in the gut by abundantly growing bacterial cells and is triggered by some feeding factors and absorbed into the systemic circulation. The epsilon-toxin is resistant to digestive enzymes; in fact, these enzymes convert the freshly secreted less active prototoxin into the fully toxic form. Clinically, when large amounts of epsilon-toxin are produced in the gut, its absorption into the systemic circulation increases capillary permeability in many organs and tissues, including intestinal mucosa. This increases its absorption rate and consequently the systemic effects leading to extensive renal damage, hyperglycemia, hypertension and edema in various organs, including the brain (10). Lesions of \textit{C. perfringens} type D infection consist of multisystemic hemorrhages, particularly of serosal surfaces; pericardial effusion is present along with mild gastroenteritis (7). Pulpy kidney, another common name for type D enterotoxemia, is derived from one of the hallmark lesions in affected sheep, a result of postmortem autolysis, which occurs rapidly in hyperemic, toxin-damaged tissue (7, 12). Although the classical pulpy kidney has been reported to be absent in calves and goats, in the present report it was possible to identify this characteristic (Figure 1).

Fragments of organs were processed for paraffin inclusion. The histological sections (5µm thick) were stained with hematoxylin-eosin and subjected to microscopic examination. Both kidneys revealed several hemorrhagic foci in the renal parenchyma, large areas of coagulation necrosis in cortex and medulla, affecting glomeruli, renal tubules and collector ducts (Figure 2). The liver presented intense hyperemia, degeneration and necrosis of the centrilobular area. There was necrotizing enteritis of the small intestine and severe pulmonary edema and congestion. The central nervous
system presented extensive areas of hyperemia in the cerebellum, and in the cerebral cortex, there was intense perivascular and perineuronal edema.

Ultrastructural examination of brain tissue from animals inoculated with epsilon-toxin revealed that tight junctions in the vascular endothelium degenerate (5), causing perivascular astrocyte processes to swell and rupture. Hemorrhage is not a hallmark of typical type D-induced disease in sheep or cattle, although hemorrhagic areas in the small intestine and petechial hemorrhages of the endocardium can be present, as can subendocardial hemorrhage around the mitral valve. Disease in goats frequently presents a hemorrhagic enterocolitis, which can be chronic; it is often associated with lactation and high food intake (12).

Microbiological culture was performed as described by Baldassi (1). Macerated tissues (kidney and small intestine) were inoculated into tubes with cooked meat medium (Difco®) and maintained at 37°C for 48h, then supernatant was plated on 5% defibrinated sheep blood agar at 37°C for 48h under anaerobic conditions. A large number of *Clostridium perfringens* isolates were cultured from the bowel content and from the macerated kidney. Polymerase chain reaction analysis, with primers described by Meer and Songer (8), of the isolates identified two different genotypes. The bowel strain was type A and the kidney strain was type D with production of epsilon-toxin besides alpha-toxin. This fact shows the importance of typing *C. perfringens* strains from different tissues origin because some strains might have a specific tissue tropism, and they can act jointly in the process of illness pathogenesis.

Clostridial toxins have been identified by serum neutralization on laboratory animals (mice or guinea pigs) using specific antisera. This toxin-typing requires a continuous supply of laboratory animals and the employment of monovalent antibody for diagnostic sera, and it is difficult to find and extremely expensive. Compared with this classical technique, the PCR method has been shown to be much more rapid, with results obtained in a few hours, and it is much more reliable. The prophylaxis of enterotoxemia in animals is achieved with vaccination; the PCR technique can thus become a first-choice tool for the identification and typing of the *C. perfringens* strains which cause these diseases. In turn, this simplifies the development of vaccines adapted to the epidemiological situation (6). Miserez *et al.* (9) described the detection of alpha and
epsilon-toxins from diseased animals by means of PCR and the results were confirmed by the mouse model biological assay, correlating them with the PCR analysis. Overeating appears to play an important role in the pathogenesis of enterotoxemia. In addition to dietary factors, other unknown prerequisites seem to be necessary for the development of enterotoxemia in sheep and goats and some outbreaks of enterotoxemia type D have been reported in goats under extensive grazing systems without known diet change. In goats, an accidental overdose of netobimin, cold weather stress and a concomitant infestation with coccidia were suggested as possible predisposing factors in an outbreak of caprine enterotoxemia (16).

Enterotoxemia caused by Clostridium perfringens type D (pulpy kidney disease) is a disease of great economical and sanitary importance for sheep and goat farming worldwide (10), and it is probably the most important cause of sudden death in goats of different ages. Several factors have been cited as predisposing to the occurrence of pulpy kidney disease, with the most important including sudden dietary changes and a reduction in intestinal transit. The persistence of C. perfringens in the environment is the result of previous cases of enterotoxemia (11).

In goats, enterotoxemia is more frequently associated with enteritis; diarrhea and hemorrhagic enterocolitis are the most prominent clinical signs and postmortem findings, respectively (13). Although the disease and its lesions have been demonstrated to be produced by C. perfringens type D (14), there is no conclusive evidence that epsilon-toxin is solely responsible for the effects in this species. As C. perfringens type D produces another major exotoxin (alpha-toxin) in addition to several minor toxins (10), the clinical signs and pathological findings of enterotoxemia in goats may be due either to the effect of epsilon-toxin alone or to this toxin in combination with some of these other toxins.

Vaccination history is frequently used by animal owners and veterinarians to rule out infections by C. perfringens. However, the quality of C. perfringens vaccines varies greatly between countries and manufacturers, and vaccines are not always correctly transported, stored and/or administered. In addition, individual variation in antibody responses between animals occurs frequently in both sheep and goats (4). Sheep are protected against the disease when vaccines of high immunogenic power and adequate
immunization strategies are used, and remain protected for a year when a booster dose is applied 28–42 days after the first vaccination (14). In goats, however, conventional vaccination produces lower and shorter-lived titers than in sheep (3) and the animals require booster doses every three or four months throughout their life after the first double vaccination (15).

In the present report, the affected buck was in a good body condition, as in an outbreak reported by Baldassi et al. (2) due to *Clostridium perfringens*. In a herd of 60 goats, 15 died suddenly, and the affected animals were mainly adult females in high milk production. These ones had received feed supplementation with high level of protein. Some animals presented diarrhea and also recumbency, opisthotonos, convulsion and intense vocalizing.

Enterotoxemia morbidity rate is variable; however, in general, it does not exceed 10% of the herd but its lethality is high and usually kills 100% of the affected animals. In the present case, the following were recommended as additional preventive measures:

1- Decrease in food volume, and increase in the frequency of feeding
2- Increase in animal movement
3- Application of a booster dose every three or four months throughout their life after the first double vaccination with a vaccine containing *C. perfringens* types A and D.

The present paper details some observations about the lesions and epidemiological aspects in a *Clostridium perfringens* type D infection in a goat, providing a better understanding of the infection in this animal species.
Figure 1. Advanced autolysis of the kidney - “Pulpy kidney” (tissue in formol).

Figure 2. Histological features: hyperemia, degeneration and renal tubules necrosis in the renal cortex.
REFERENCES


