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Intramural vascular edema in the brain of goats with Clostridium perfringens type D enterotoxemia

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Keywords:	Clostridium perfringens type D, enterotoxemia, brain, goats, microangiopathy, intramural edema

Abstract: Enterotoxemia caused by Clostridium perfringens type D is an important disease of sheep and goats with a worldwide distribution. Cerebral microangiopathy is considered pathognomonic for ovine enterotoxemia and is seen in most cases of the disorder in this species. These lesions are, however, rare and poorly described in goats. In this paper, we describe the vasculocentric brain lesions observed in 44 cases of caprine spontaneous C. perfringens type D enterotoxemia. Only one goat had gross changes in the brain, which consisted of mild cerebellar coning. However, 8/44 (18%) of the cases showed microscopic brain lesions, characterized mainly by intramural vascular proteinaceous edema, a novel and diagnostically significant finding. The precise location of the edema was better observed with PAS and Gomori's stains. The areas of the brain most frequently affected were cerebral cortex, corpus striatum (basal ganglia) and cerebellar peduncles and both arterioles and venules were involved. Most of the goats of this study showed lesions in the intestinal tract (enteritis, colitis and typhlitis), although pulmonary congestion and edema, hydrothorax, hydropericardium and ascites were also described. This is the largest study to date of neuropathological changes in naturally-occurring cases of caprine type D enterotoxemia, and it describes the main features of the cerebral microangiopathy in this species. Although the intramural edema found, for the first time, in these caprine cases is useful for the diagnosis of enterotoxemia when observed, its absence cannot exclude the disease because, in goats, it is only present in a small number of cases.

Full-length manuscript

Intramural vascular edema in the brain of goats with *Clostridium perfringens* type D enterotoxemia

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Abstract

Enterotoxemia caused by *Clostridium perfringens* type D is an important disease of sheep and goats with a worldwide distribution. Cerebral microangiopathy is considered pathognomonic for ovine enterotoxemia and is seen in most cases of the disorder in this species. These lesions are, however, rare and poorly described in goats. In this paper, we describe the vasculocentric brain lesions observed in 44 cases of caprine spontaneous *C. perfringens* type D enterotoxemia. Only one goat had gross changes in the brain, which consisted of mild cerebellar coning. However, 8/44 (18%) of the cases showed microscopic brain lesions, characterized mainly by intramural vascular proteinaceous edema, a novel and diagnostically significant finding. The precise location of the edema was better observed with PAS and Gomori's stains. The areas of the brain most frequently affected were cerebral cortex, corpus striatum (basal ganglia) and cerebellar peduncles and both arterioles and venules were involved. Most of the goats of this study showed lesions in the intestinal tract (enteritis, colitis and typhlitis), although pulmonary congestion and edema, hydrothorax, hydropericardium and ascites were also described. This is the largest study to date of neuropathological changes in naturally-occurring cases of caprine type D enterotoxemia, and it describes the main features of the cerebral microangiopathy in this species. Although the intramural edema found, for the first time, in these caprine cases is useful for the diagnosis of enterotoxemia when observed, its absence cannot exclude the disease because, in goats, it is only present in a small number of cases.

Keywords: *Clostridium perfringens* type D, enterotoxemia, brain, goats, microangiopathy, intramural edema.

Enterotoxemia caused by *Clostridium perfringens* type D is an important disease of sheep and goats of worldwide distribution.[19,31] *C. perfringens* type D can be a normal inhabitant of the intestine of several animal species, including goats.[22,32] Occasionally, when the intestine is altered by sudden changes in diet or other not yet well understood factors, *C. perfringens* type D proliferates in large numbers and produces toxins which are then absorbed to the systemic circulation and/or act locally in the intestine to produce disease.[15,19]

C. perfringens has been conventionally classified into five types (A, B, C, D and E), according to the production of four so-called major toxins: alpha, beta, epsilon (ETX) and iota. However, a new typing schedule has recently been proposed, which includes two additional types (i.e. F and G) based on the production of enterotoxin and necrotic enteritis beta-like toxin, respectively.[17] Several other toxins, such as perfringolysisn and beta 2 may be produced by different strains of *C. perfringens,* although they are not used in the classification of this microorganism.[20]

Strains of *C. perfringens* type D encode, at the minimum, alpha and epsilon toxin.[15,19] ETX is the main virulence factor of this toxinotype, although it is possible that one or more additional toxins act synergistically with ETX to produce disease.[15] Most clinical signs and pathological changes of naturally-occurring enterotoxemia have been reproduced by intravenous administration of ETX in sheep and goats.[4,7-9] Reverse genetic experiments have also demonstrated that ETX is required for enterotoxemia type D to occur in goats.

Field cases of enterotoxemia in goats are considered to occur in three clinical forms, namely peracute, acute and chronic.[2,18,25] An excess of protein-rich, pericardial, pleural and/or ascitic fluid, interstitial and/or alveolar pulmonary edema, and sub-epicardial and sub-endocardial hemorrhages are frequently observed in the peracute form of the disease, although cases in which no gross or microscopic lesions are found may also occur. In the chronic form, fibrinohemorrhagic enterocolitis is the most consistent lesion.[1,25] A combination of lesions found in the peracute and chronic forms of the

disease is frequently seen in the acute cases.[25] Gross findings suggestive of enterotoxemia can be found during post-mortem examination, although none are pathognomonic.[22]

Descriptions of histological changes in enterotoxemia of goats are scant and, with few exceptions, are based on experimental disease.[6,7,22,23,26,28,30] Cerebral microangiopathy, characterized by perivascular and intramural leakage of proteinaceus, eosinophilic fluid in certain areas of the brain is considered pathognomonic for ovine enterotoxemia and is seen in most ovine cases of the disease.[3,10] In goats with type D enterotoxemia only perivascular, but not intramural, edema has been described. This change is, however, considered to be unusual in goats, with only one published report describing it in two confirmed spontaneous cases of caprine enterotoxemia.[23]

This paper describes the histological lesions in the brain and its microvasculature of 44 cases of naturally-occurring enterotoxemia produced by *C. perfringens* type D.

MATERIAL AND METHODS

Case selection

Forty-four cases were selected from the archives of the California Animal Health and Food Safety Laboratory System (San Bernardino and Davis branches). The inclusion criteria were those previously described as the gold standard for the diagnosis type D caprine enterotoxemia. They were: (1) a characteristic history and clinical signs, including one or more of: i) sudden death, ii) colic and/or iii) diarrhea; (2) suggestive pathological findings, including one or more of: i) colitis, ii) hydropericardium, iii) hydrothorax, iv) ascites and/or v) pulmonary edema; and (3) detection of ETX toxin in the intestinal contents.[21,32] The cases occurred between January, 2000 and March, 2017. Four goats (2 males and 2 females) with diagnoses other than enterotoxemia (ketosis, mycoplasmosis, coccidiosis and enteritis caused by *Clostridium difficile*), which had negative results for epsilon toxin ELISA in the intestine, were used as controls. Details of signalment and clinical history are shown in Table 1. Briefly, the goats studied

were between 2 weeks and 6 years of age at the time of death, 24 were female and 20 male, and several breeds were represented.

Gross and microscopic pathology

The necropsy records of all cases were reviewed, with special emphasis on signalment, clinical history and gross findings in the central nervous system. At the time of necropsy, samples from multiple organs, including heart, lung, liver, pancreas, rumen, reticulum, omasum, abomasum, small and large intestine, kidney, spleen, lymph nodes and brain from all animals, were collected and fixed by immersion in 10% neutral buffered formalin for 24-48 hours. The brain was then cut into ~ 1 cm thick, coronal sections, and blocks were collected from all those areas where pathological findings in sheep and goats with enterotoxemia had been previously reported,[6-8,22,26-28] including cerebral cortex, corpus striatum (basal ganglia and internal capsule), thalamus, hippocampus, midbrain at the level of anterior colliculi, cerebellum, and medulla oblongata. All the tissues were paraffin-embedded, sectioned at 4 µm, and stained with hematoxylin and eosin. For this study, selected brain sections were also stained with periodic acid–Schiff (PAS) to evaluate the integrity of the basement membranes of blood vessels affected by edema and to further characterize the vasculocentric edema. In addition, to assess the integrity of the tunica adventitia (containing collagen type III) of the blood vessels, Gomori's reticulin stain was performed. All brain sections were examined independently by two pathologists (JO and JMV), with special emphasis on vascular lesions and the topographical distribution of these morphological changes.

Immunohistochemistry

For this study, duplicate sections from all areas of the brain sampled were examined immunohistochemically for the presence of injured axons (amyloid precursor protein [APP], the most sensitive, early marker of axonal injury), astrocytic reaction (glial fibrillary acidic protein [GFAP]), the water channel protein, aquaporin-4 (AQP4), plasma albumin, and a microglial/macrophage marker (ionized calcium binding adaptor molecule 1 [Iba1]), as previously described (Garcia et al. 2015). Briefly, the following primary antibodies were used: a mouse monoclonal antibody anti-APP (clone 22C11,

Chemicon; Millipore, Billerica, MA), a rabbit polyclonal antibody (Pab) anti-GFAP (Dako, Carpinteria, CA), a rabbit Pab against AQP4 (Millipore, Burlingame, CA), a goat Pab against rat albumin (Cappel, West Chester, PA), and a rabbit Pab against Iba1 (Wako Pure Chemical Industries, Osaka, Japan). Rabbit, mouse, or goat IgGs were used as primary antibodies in negative control sections. The Dako EnVision+ horseradish peroxidase (HRP) kit (Dako) was used for APP, according to the instructions of the manufacturer and standard operating procedures of the California Animal Health and Food Safety Laboratory, UC Davis. For GFAP, an avidin-biotin-peroxidase method (Vectastain Elite Kit; Vector Laboratories, Burlingame, CA) was used according to the manufacturer's instructions and standard operating procedures of the Veterinary Medical Teaching Hospital, UC Davis. Serum albumin, AQP4, and Iba1 were incubated with a streptavidin-conjugated peroxidase tertiary (Pierce, Pasadena, CA), according to the instructions of the manufacturer. For APP and GFAP, immunoreactivity was visualized using the chromogen 3-amino-9-ethylcarbazole (AEC K4001; Dako). Albumin, AQP4, and Iba1 were visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB). After chromogen incubation, all sections were counterstained with hematoxylin, cleared, and mounted.

C. perfringens and *C. perfringens* toxins ELISA

Samples of small and/or large intestinal content from all the goats were analyzed for CPA, CPB and ETX by a monoclonal antibody-based capture ELISA (BioX, Brussels, Belgium), according to the manufacturer's instructions. In addition to the three toxins mentioned, this ELISA also detects the presence of *C. perfringens*. Briefly, the samples were added to the wells of the ELISA plates to which CPA, CPB or ETX monoclonal antibodies had been bound and the plates were incubated for 60 minutes at room temperature. A washing step was performed and horseradish peroxidase conjugated with anti-CPA, anti-CPB and anti-ETX antibodies was added to the plates before another hour of incubation at room temperature. Following an additional wash step, a substrate/chromogen solution was added to each well and the plates were incubated for 15 minutes at room temperature. The results were read in an ELISA reader (Pharmacia, Uppsala, Sweden) with a 450 nm filter. Positive or negative readings were obtained according to the instructions of the manufacturer. CPA, CPB and ETX were used as

positive controls. This technique was previously found to detect as little as 25 MLD_{50}/ml of ETX in intestinal content of sheep and goats.[29]

RESULTS

The gross and histological lesions observed in the 44 goats with type D enterotoxemia are summarized in Table 1 and Fig. 1a. Although this study focuses on brain pathology of caprine enterotoxemia, a brief description of the most significant gross lesions observed in other organs is included. Grossly, the most common lesion was hemorrhagic and/or necrotizing inflammation of different regions of the intestinal tract (enteritis, colitis and/or typhlitis). The presence of fluid in cavities (i.e., ascites, hydrothorax and/or hydropericardium) was the second most frequent finding, followed by pulmonary congestion and edema (Fig. 1a). Only one goat in the study presented gross changes in the brain, which consisted of mild, diffuse swelling of the cerebrum, with the caudal portion of the cerebellum extending into the foramen magnum (cerebellar coning) (Fig. 2).

Microscopically, 8 (18%) out of the 44 goats with type D enterotoxemia showed lesions in the brain. They were observed in 5 males and 3 females, and most (n=7) were 1 year of age or younger (Table 1). The cerebral cortex (n=6), basal ganglia of the corpus striatum (n=6), and cerebellar peduncles (n=5) were the most frequently affected areas, followed by the thalamic nuclei (n=1) (Fig. 1b). Microscopic lesions in the brain were almost exclusively vasculocentric, with some involvement of the surrounding neuropil. Both arterioles and venules were injured, the former predominantly in the cerebral cortex and basal ganglia, and the latter in the cerebellar peduncles. In those goats in which vascular lesions were observed, only a very small number of vessels were affected, always less than 5% of the total number per section. The distribution of the microscopic lesions in the brain of the 8 affected goats is shown in Table 2.

In HE-stained brain sections of these 8 goats, the vascular wall was mildly to moderately distended with lakes of extravasated eosinophilic, homogeneous, proteinaceous fluid (Fig. 3a). Special stains and IHC (PAS, Gomori and GFAP) demonstrated that the high-protein edema was located between the muscularis and adventitia of the vessel wall, indicating mural involvement instead of perivascular edema (Figure 3b, 3c, 3d). The extravasated fluid was PAS-positive (Fig. 3b) and this edema sometimes also extended into the surrounding neuropil. GFAP immunostaining revealed strong immunolabelling of astrocyte foot processes around the adventitia (Fig. 3d), with these processes often being separated from the vascular wall by edema fluid. AQP4-immunoreactive granules were coarse and numerous in foot processes surrounding microvessels, especially where there was evidence of plasma protein extravasation and intramural edema, with similar robust immunolabelling of astrocytic processes in the surrounding neuropil (Fig. 3e). In blood vessels showing large mural lakes of eosinophilic, proteinaceous material, this leaked fluid was strongly immunopositive to albumin (Fig. 3f). Iba1 immunohistochemistry identified macrophages/microglial cells in the edematous spaces of vessels, and numerous APP-immunoreactive injured axons were found in the neuropil adjacent to these vessels.

In the control group of goats, most of the brain vessels did not show significant histological changes. However, in some areas (mostly in the brainstem), the presence of extravasated erythrocytes surrounding the vessel was noted. This finding has been previously described as a common post-mortem artefact.[5] In addition, a small amount of faintly eosinophilic granular or globular material surrounding the vessels was occasionally observed (Fig. 3a, inset). This perivascular material did not react positively to PAS or albumin immunostaining (Fig. 3b, 3f; insets) or cause any disruption to the vessel wall in Gomori-stained sections (Fig. 3c; inset). Accordingly, these findings were interpreted as artifacts or peri-mortem changes and not genuine vascular lesions. In control brains, GFAP and AQP4 immunopositivity was expressed as fine granularity of astrocytic processes and, more particularly, where their foot processes were in contact with the external surface of blood vessels (Fig. 3d, 3e; insets).

DISCUSION

Enterotoxemia caused by *Clostridium perfringens* type D ETX occurs frequently in sheep and goats, and occasionally cattle.[6,13,23,25] Although many of the clinical signs and post-mortem lesions of this disease were previously reproduced in goats by intravenous inoculation of ETX,[24] or by intraduodenal inoculation of whole cultures of *C. perfringens* type D,[12] brain lesions in goats with spontaneous type D enterotoxemia have only been reported in two animals with confirmed disease.[24]

Of the 44 goats with type D enterotoxemia in this study, gross lesions were seen most commonly in the intestinal tract, with only one goat showing macroscopic changes in the brain of cerebellar conning. However, microscopic lesions were observed in the brain of 8 (18%) of the 44 goats examined. These vascular lesions consisted of eosinophilic, homogeneous, proteinaceous fluid that dissected and expanded the vessel wall (intramural edema). In routinely formalin-fixed tissues, there is usually mild, often diffuse, immunostaining of many structures with antibodies directed against endogenous albumin, a pattern which is not found in the same perfusion-fixed material. However, when endogenous albumin is used as a surrogate immunohistochemical marker of increased vascular permeability in the brain, as in the present study, and the vascular injury is substantial, the pooled perivascular lakes of extravasated albumin are strongly immunopositive and correspond to the distribution of PAS-positive material. In these brains, the robustly albumin immunostained, high-protein, blood vessel-associated edema could be clearly differentiated from the much weaker, artefactual immunoreactivity of surrounding neural elements. In areas surrounding blood vessels affected by intramural edema, there was markedly increased expression of the major water channel protein in the brain, AQP4, especially in astrocytic foot processes. AQP4 regulates the movement of water in the brain and, in vasogenic edema, it appears that, while excess water enters the extracellular space independently of AQP4, this water is cleared from the brain via AQP4 channels in astrocytic foot processes.[33,34] By contrast, AQP4 water channels in astrocytic foot processes are active in the formation of cytotoxic edema.[14]

The microangiopathic lesions have been previously described in the brain of sheep and goats with type D ETX disease and interpreted as perivascular edema, based on findings observed with routine stains (i.e., hematoxylin and eosin). However, in the present study, using special stains, it was clear that the fluid accumulation was mainly within, and not around, the vascular wall, suggesting that mural edema is the most common manifestation of ETX-induced vascular injury in goats. In our study, the most useful stains to show the intramural location of the edema and, also to differentiate real edema from artifacts, were PAS and Gomori. We therefore recommend using these stains in suspected cases of enterotoxemia where equivocal vascular changes are observed. Mural edema was also recently reported in the brains of sheep experimentally inoculated with *C. perfringens* type D, where specials stains were also performed.[8]

Mural edema was mainly found in the cerebral cortex (6/8), basal ganglia of the corpus striatum (6/8), cerebellar peduncles (5/8) and, less frequently, in the thalamic nuclei (n=1) in the present study. In the corpus striatum, microscopic lesions were found in the internal capsule, in contrast to type D enterotoxemia in sheep, in which the basal ganglia is the most vulnerable part of the corpus striatum.[6-8,23,24] In this study, we also found many animals with vascular lesions in the cerebral cortex, a pattern not previously recognized in caprine enterotoxemia, but occasionally seen in sheep. In our study, 7 of 8 goats (88%) presented with lesions in the cortical grey matter and/or central gray matter/brainstem nuclei, and 6/8 (75%) in white matter (6/8). This neuroanatomical distribution of lesions in goats differs from an experimental study of ovine enterotoxemia, which showed a predominance of edema in white matter.[8] The predilection of white matter for the development of edema after vascular damage in sheep was attributed to its sparse cellularity and larger extracellular spaces, permitting the more rapid accumulation, and spread, of edema fluid.[11] Importantly, in those goats in which vascular lesions were observed, only a very small number of blood vessels were affected (< 5%), thus mandating a thorough examination of all susceptible brain regions in order to detect the characteristic microangiopathy in cases of suspected type D enterotoxemia.

ETX is a member of the aerolysin family of pore-forming toxins.[16] In mice and rats, ETX binds to specific receptors on the microvascular endothelium in the brain, causing vascular endothelial degeneration. One domain of ETX binds to endothelial receptors, where it utilizes lipid rafts and caveolin to oligomerize into a pre-pore on the surface of the cell membrane. Another ETX domain then inserts into the membrane lipid bilayer and forms an active pore.[20] ETX can be transiently prevented from exerting its deleterious effect by prior administration of the prototoxin, presumably by competitive inhibition of endothelial receptors.[16] The ETX-induced endothelial cytotoxicity appears to be due, in part at least, to a rapid decline via pores in cytoplasmic potassium levels, with a concomitant influx of sodium and chloride ions. This process, which seems to involve ATP depletion, leads to cell necrosis.[20] A similar sequence of pathophysiological events is probably also operative in goats and sheep with acute *C. perfringens* type D intoxication.

In conclusion, this study showed that a thorough histologic examination of the brain should be performed in goats with a suspected diagnosis of enterotoxemia, even in the absence of gross lesions, as intramural edema can be found in some cerebral vessels and constitutes a very useful finding for diagnostic purposes. However, the brain lesions in goats are usually mild and found less commonly than in sheep, so their absence cannot exclude the disease.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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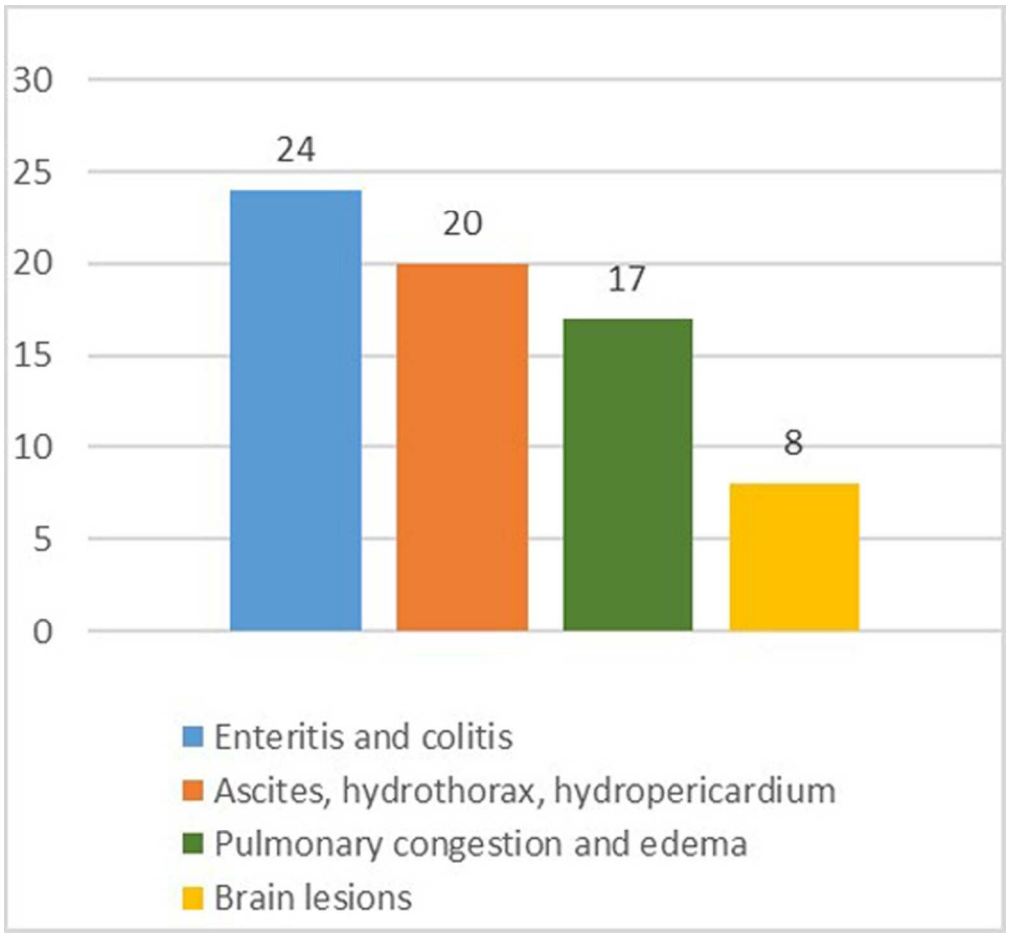
FIGURE LEGENDS

Figure 1. Gross and microscopic lesions in 44 goats with *C. perfringens* type D enterotoxemia. Fig. 1a. Summary of the gross and histologic lesions (n=44). Fig. 1b Distribution of brain lesions (n=8)

Figure 2. Goat nº 1. Diffuse swelling of the cerebrum with the caudal portion of the cerebellum extending into the foramen magnum (cerebellar coning).

Figure 3 (a-f): Brain of goats with type D entertotoxemia (main image) and of controls (insets). Intramural and perivascular edema in blood vessels. **3a.** The material expanding the wall is homogeneous and brightly eosinophilic in a ETX-damaged vessel, but pale and globular in vessels from control group (inset). Hematoxylin and eosin. **3b.** The eosinophilic material described in 3a is strongly PAS-positive in vessels from the study group, but not in the control group (inset). PAS stain. **3c.** The edema is located between the muscularis and adventitia in the ETX-injured vessel, indicating mural rather than perivascular edema. No wall disruption is observed in brains from control group (inset) Gomori´s stain. **3d.** Strong immunoreactivity identifying astrocyte foot processes is observed around the adventitia. In control group, no disruption of the arterial wall is observed and the foot processes extend much closer to the vessel lumen. GFAP, hematoxylin counterstain. **3e.** Astrocytic foot processes in an ETX-injured vessel are strongly immunopositive to AQP4, with similar robust immunoreactivity in astrocytic processes in the surrounding neuropil. By contrast, there is much weaker AQP4 immunopositivity in control blood vessels (inset). **3f.** Extravasated plasma albumin is strongly immunopositive around an ETX-damaged vessel. In an uninjured, control vessel, albumin immunoreactivity is confined to the vessel lumen (inset), but there is mild artefactive immunolabelling of the surrounding neuropil in these immersion-fixed brains.



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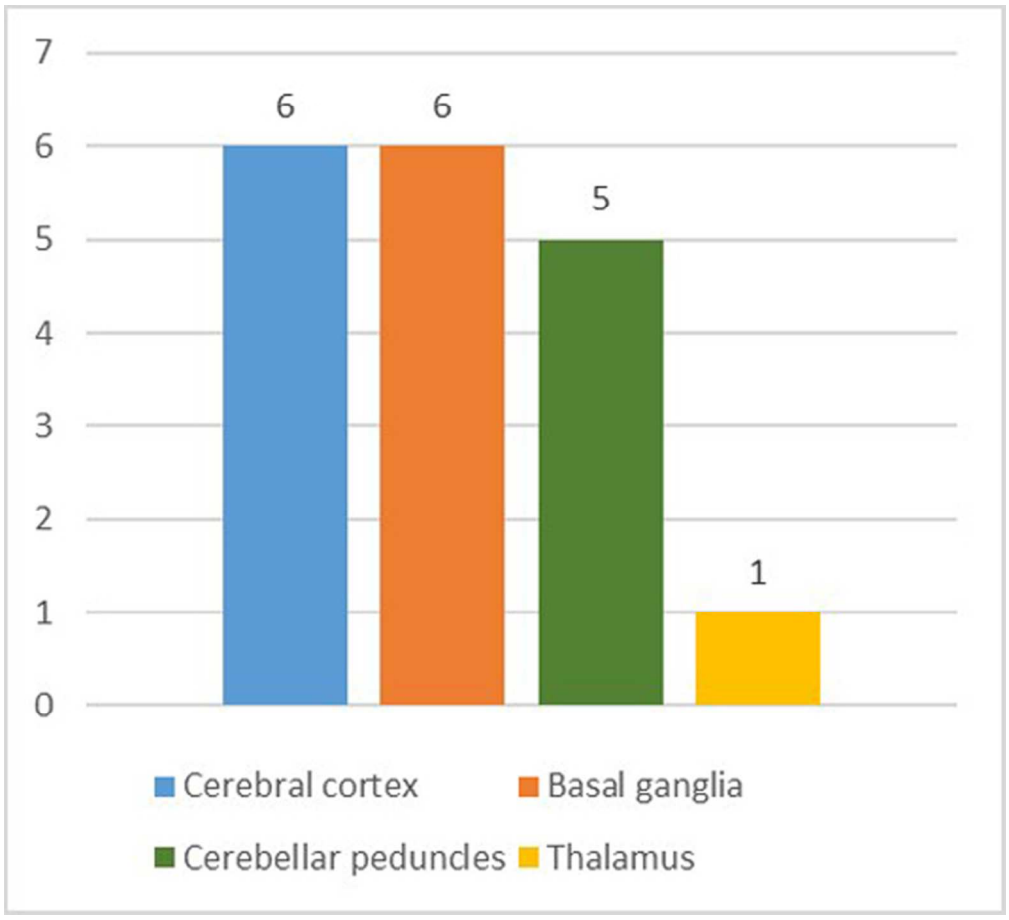

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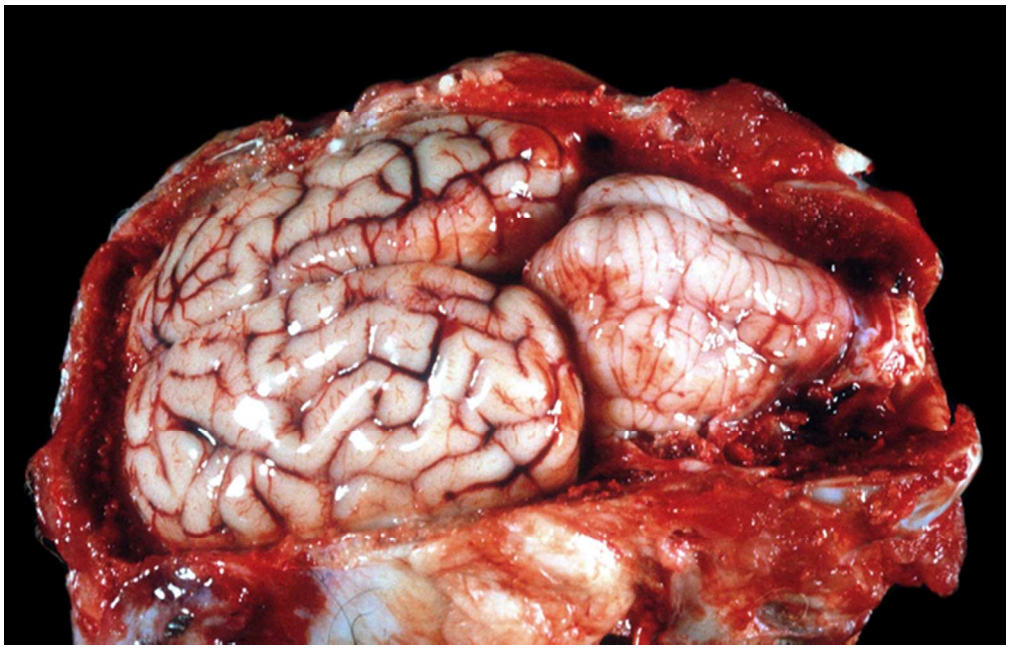
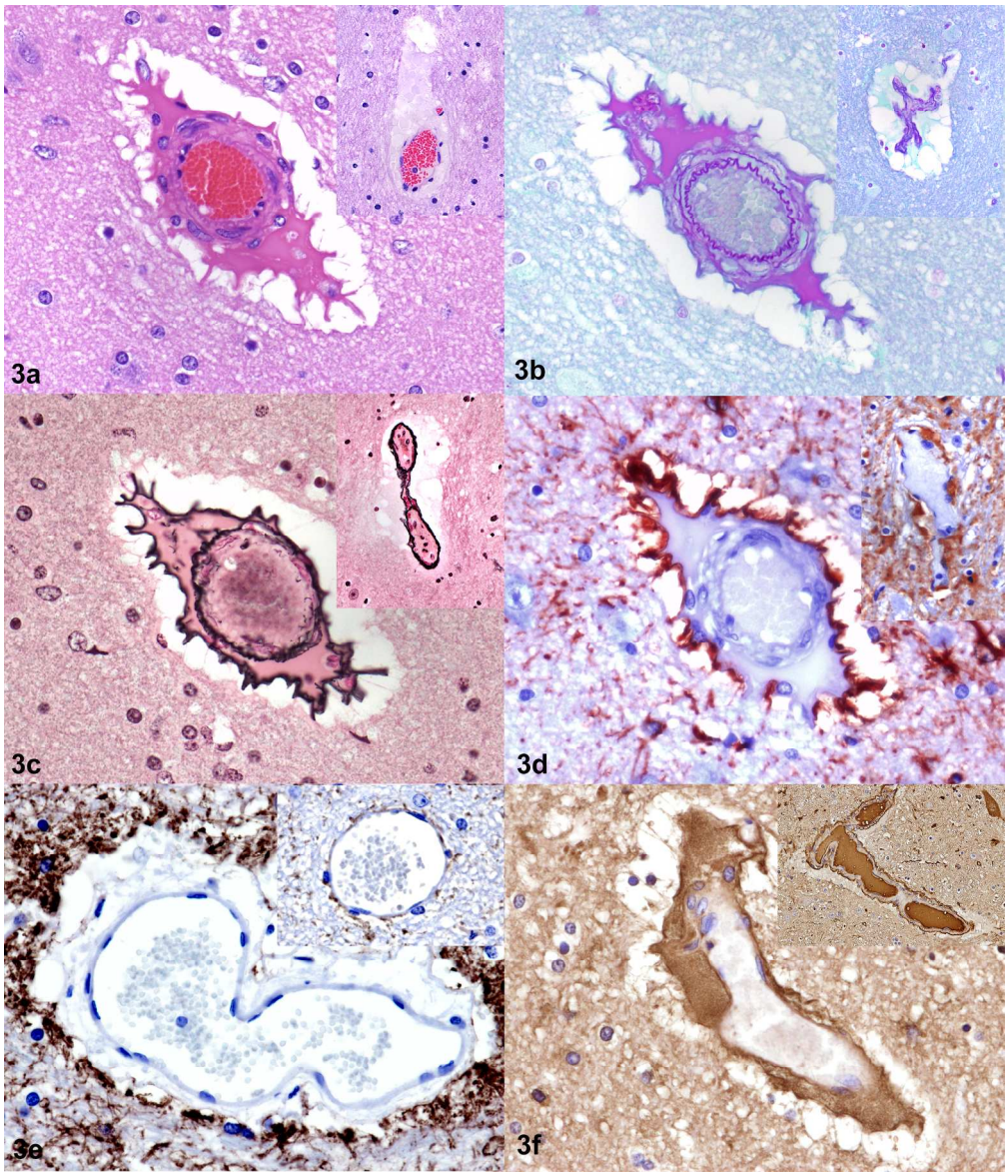


57x36mm (300 x 300 DPI)



209x244mm (300 x 300 DPI)

Table 1. Signalment and summary of gross and microscopic lesions in 44 goats with *C. perfringens* type D enterotoxemia (Goats 1 through 44) and in 4 control goats (Ct-1 through Ct-4).

				Lesions			
Goat	**Breed**	**Sex**	**Age**	**Intestine (Et/Co)**	**Lung (PC/PE)**	**Fluid (As,Ht,Hp)**	**Brain (Edema)**
1	Boer	M	4 m		+	+	+
2	N/R	M	2 m	+	-	-	+
3	Nubian	M	1 y	+	-	+	+
4	Anglonubian	F	2 m	-	+	-	+
5	N/R	F	3 y	+	-	+	+
6	Anglonubian	M	3w	-	+	+	+
7	Boer	F	7 m	-	-	-	+
8	Alpine	M	3 m	-	+	-	+
9	Boer	M	8w	+	-	-	-
10	Angora cross	F	2 y	-	-	-	-
11	Alpine	F	1 y	+	+	+	-
12	Crossbreed	M	6w	+	-	+	-
13	Saanen	M	1 m	-	-	-	-
14	Saanen	M	3m	-	+	+	-
15	Boer	F	2w	-	-	+	-
16	Nigerian buck	M	2w	-	-	+	-
17	La Mancha	F	3 y	+	+	-	-
18	La Mancha	F	4y	-	+	+	-
19	Pygmy	M	3 y	+	-	+	-
20	Boer	M	2 w	+	+	+	-
21	N/R	F	3 y	-	+	+	-
22	Saanen	F	3 m	-	+	-	-
23	Boer	M	3 w	+	-	-	-
24	Alpine Goat	M	6 w	+	-	+	-
25	Saanen	M	4 w	+	-	-	-
26	Anglo nubian	F	1 m	-	+	-	-
27	Anglonubian	F	2 y	-	+	+	-
28	Angora	M	5 w	+	+	+	-
29	PYGMY	F	4 m	+	-	-	-
30	Anglo nubian	F	3 m	-	-	-	-
31	Alpine	M	3 w	+	-	-	-
32	Boer	M	2 w	+	-	+	-
33	Saanen	F	2 m	+	-	+	-

34	N/R	F	4 y	-	+	-	-
35	N/R	F	5 y	-	+	-	-
36	N/R	F	6 y	+	-	-	-
37	N/R	F	6 w	+	-	+	-
38	Saanen	F	3 y	+	-	-	-
39	Nigerian Dwark	M	3 w	+	+	-	-
40	N/R	F	3 y	+	-	-	-
41	Boer	F	4 y	-	-	-	-
42	Anglonubian	F	5 y	+	-	-	-
43	La Mancha	M	8 w	-	-	+	-
44	N/R	F	3 y	+	-	-	-
Ct-1	La Mancha	F	8 y	-	-	-	-
Ct-2	Nigerian Dwarf	M	4 m	-	-	-	-
Ct-3	Alpine	F	6 w	+	-	-	-
Ct-4	La Mancha	M	7 w	+	-	-	-

[1] N/R: Not-reported; w: weeks; m: months. y: years. [2] M: male; F: female; Et: enteritis; Co: Colitis; As: ascites; Ht: hydrothorax. Hp: hydropericardium.

Table 2. Distribution of mural and perivascular edema in the brain of 8 goats with *C. perfringens* type D enterotoxemia

	Cerebral Cortex				Basal ganglia		Thalamic nuclei		Cerebellar peduncles	
	GM[1]		WM[2]							
Goat	A[3]	V[4]	A	V	A	V	A	V	A	V
1	-	+	-	-	+	-	-	-	+	+
2	-	-	+	-	+	+	-	-	-	-
3	+	-	+	+	-	+	+	-	-	+
4	+	+	+	+	+	-	-	-	-	+
5	+	-	-	-	+	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	+
7	-	-	-	-	+	-	-	-	-	-
8	+	+	-	-	-	-	-	-	-	+

[1]GM: Grey matter. [2]WM: White matter. [3]A: Arterioles [4]V: Venules