

Clostridial Diseases Diagnosed in Herbivores in Southern Brazil

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ABSTRACT

Background: The genus *Clostridium* includes a group of Gram-positive, anaerobic bacteria which producing endospores and produce toxins when encounter conditions favorable to their development. These toxins can be produced and absorbed in the intestinal lumen, as occurs in cases of enterotoxemia (*Clostridium perfringens*), or are produced in areas of tissue necrosis after bacterial infections, as seen in tetanus (*C. tetani*), blackleg (*C. chauvoei*) and bacillary hemoglobinuria (*C. haemolyticum*), or in infections by *C. chauvoei*, *C. novyi* and *C. septicum* frequently associated with cases of malignant edema. The aim of this research was relates the epidemiological and clinicopathological aspects of the clostridiosis observed in the region of influence of the Setor de Patologia Veterinária of Universidade Federal do Rio Grande do Sul.

Materials, Methods & Results: The necropsy records were reviewed from January 1996 to December 2011 to identify the cases of clostridiosis that were diagnosed. In the period, 4.689 necropsies were performed by the (SPV-UFRGS). A total of 135 cases (2.88%) were associated with clostridiosis. The most prevalent clostridiosis included tetanus (48.15%) in horses, cattle, sheep and goats; botulism (17.04%) in cattle and enterotoxemia (22.96%) in goats. Additional diseases were blackleg (5.93%) in cattle, necrotic myositis/malignant edema in horses and sheep and bacillary hemoglobinuria in cattle, both with 4 cases each (2.96%).

Discussion: Tetanus, enterotoxemia, and botulism were the most prevalent clostridiosis diagnosed at SPV-UFRGS and together accounted for approximately 90% of cases in the period 1996-2011. As for blackleg, bacillary hemoglobinuria, and necrotic myositis/malignant edema, together they represented slightly less than 10% of the clostridioses in the period. The most significant clostridiosis in the period studied was tetanus, affecting cattle, sheep, and horses. There was a large number of cases distributed over many years; probably this occurrence was associated with these species' (sheep and horse) greater sensitivity to the toxin. In cattle the cases of tetanus in cattle were observed in various outbreaks distributed only in two years, the death of the animals was associated with the contamination of anthelmintic bottles with *C. tetani* spores. Botulism is an important clostridiosis for bovines. In all the outbreaks of botulism, the cases were concentrated in the hottest periods of the year: summer, late spring, and early fall, as stated in previous reports of botulism. Cases of enterotoxemia were important and observed only in goats. Outbreaks of enterotoxemia were attributed to excessive consumption of concentrated feed, and the disease affected vaccinated and unvaccinated goats alike. Blackleg was apparently less significant in the area of influence of the SPV-UFRGS, to the contrary of what was previously reports, when it was considered the most important clostridiosis in cattle in Rio Grande do Sul. It is possible that the prompt recognition of this disease by veterinarians in the field reduced the frequency of requests for diagnostic services. The two less frequent clostridiosis were bacillary hemoglobinuria and necrotic myositis/malignant edema, which occurred in cattle; horses, and sheep, respectively. All the clostridiosis showed a significant presence in the livestock necropsies, but only three (tetanus in horses, sheep and cattle; botulism in cattle, and enterotoxemia in goats) affected an elevated number of animals.

Keywords: tetanus, botulism, enterotoxemia, blackleg.

Descritores: tétano, botulismo, enterotoxemia, carbúnculo sintomático.

INTRODUCTION

The genus *Clostridium* includes a group of Gram-positive obligate anaerobic bacteria which are capable of producing heat-resistant endospores [2]. These bacteria are found in soil, dust, water, and the intestinal contents of animals [10,13] and produce toxins when they encounter conditions favorable to their development. These toxins can be produced and absorbed in the intestinal lumen, as occurs in cases of enterotoxemia by *Clostridium perfringens* [2], or are produced in areas of tissue necrosis after bacterial infections, as seen in tetanus (*C. tetani*), blackleg (*C. chauvoei*) and bacillary hemoglobinuria (*C. haemolyticum*) [2]. The toxins can also be produced in food and organic matter of animal or vegetable origin which are in a state of decomposition and are subsequently ingested pre-formed, as occurs in botulism (*Clostridium botulinum*) [2].

In Brazil, botulism is responsible for great economic losses, due to the number of animals that die every year [9], among the clostridiosis that affect cattle, this is the main disease affecting livestock in the Southeast and Central-West regions [4]. In the southern of Rio Grande do Sul the blackleg is the most frequently clostridiosis [14]. Enterotoxemia is the most frequent clostridiosis in sheep and goats [11,3]; while tetanus mainly affects horses [14]. Together, clostridiosis are responsible for considerable economic losses in livestock across Brazil [16].

The current article relates the epidemiological and clinicopathological aspects of the clostridiosis observed in the region of influence of the Setor de Patologia Veterinária da Universidade Federal do Rio Grande do Sul (SPV-UFRGS).

MATERIALS AND METHODS

A retrospective and prospective study was conducted comprising the 16-year period (January 1996-December 2011) using the protocols of the SPV-UFRGS. The epidemiological data and clinicopathological findings from diagnosed cases of clostridiosis were analyzed. During the necropsies, samples of viscera were collected and subsequently refrigerated for bacteriological analysis, toxin research and bioassay tests on mice. Visceral samples were fixed in a buffered 10% formalin solution for 24 to 48 h and processed by routine histological techniques. Slices 5 µm thick were colored using the hematoxylin and

eosin (HE) stain technique and, when necessary, were colored using special techniques for bacteria such as the Brown-Hopps method and Warthin-Starry's silver stain method.

In cases of botulism, samples of ruminal, gastric, and intestinal contents as well as portions of liver were kept refrigerated and delivered to the Laboratório de Doenças Infecciosas at UNESP-Araçatuba for bioassay testing on mice and detection of botulism toxin. In cases where botulism was suspected, samples of the food and water provided to the animals were also forwarded for testing for the botulism toxin. In cases where enterotoxemia was suspected, samples of the intestinal contents (small intestine, cecum, and colon) were forwarded to LANAGRO in Minas Gerais in order to identify the epsilon-toxin produced by *C. perfringens*. Samples of blood serum and the edema fluid from the animals' wounds were used to conduct the bioassay test in mice for the tetanus toxin, and were sent for the bacterial culture of this agent. Samples of the liver, skeletal muscles, and the edema fluid were sent for bacterial culture in cases where bacillary hemoglobinuria was suspected, or blackleg and necrotic myositis/malignant edema, respectively.

RESULTS

From the total of 4,689 necropsies which were conducted on animals during the period of the study, 135 (2.88%) were associated with clostridiosis. Bovines, equines, caprines and ovines were affected. The most prevalent clostridiosis included tetanus (65 animals), enterotoxemia (31 goats) and botulism (23 cattle). Other less-frequently observed clostridiosis were blackleg (8 cattle), necrotic myositis/malignant edema (4 animals) and bacillary hemoglobinuria (4 cattle). The distribution of the clostridiosis diagnosed at SPV-UFRGS are presented in Figure 1 and in Table 1.

The 65 cases of tetanus represented 1.39% of the total and 48.15% of the clostridiosis. Mortality in the outbreaks of tetanus varied between one and 100 animals and there were cases practically every year, principally in cattle and horses, but in lesser quantities in sheep and goats. Botulism was observed in 23 cases (0.49% of the necropsies and 17.04% of the cases of clostridiosis) and affected cattle. Enterotoxemia, diagnosed in 31 (0.66%) necropsies, represented 22.96% of the clostridiosis and affected only goats. There were 8 cases of blackleg, which accounted for 0.15% of

the necropsies and 5.93% of the clostridiosis. Only 4 cases of bacillary hemoglobinuria in cattle and 4 cases of malignant edema/necrotic myositis in horses and sheep were registered, and represented 0.09% of the necropsies and 2.96% of the clostridiosis. The distribution of the cases of tetanus, botulism, enterotoxemia, blackleg, bacillary hemoglobinuria and necrotic myositis/malignant edema by animal species is shown in Figure 2.

All outbreaks of botulism occurred during the hottest times of the year, especially in summer, but also at the end of spring or the beginning of the fall. The outbreaks of tetanus were homogeneously distributed among the four seasons of the year, as was bacillary hemoglobinuria, which presented the same number of cases in hot and cold periods. Outbreaks of enterotoxemia, blackleg, and necrotic myositis/malignant edema were concentrated in the winter. The distribution of the clostridioses among the different seasons is shown in Figure 3.

The source of botulism contamination in cattle was associated with osteophagy in one outbreak, and food waste from a restaurant in another outbreak, but the other outbreaks were related to supplying soybean paste contaminated with the toxin.

In the majority of cases of tetanus in horses, the point of entry was not identified. Nevertheless, in some cases, some perforating injuries, limb fractures, inadequate shoeing, and wounds were identified as possible points of entry for the agent. In sheep and goats, the cases of tetanus were associated with ear tagging, castration, and tail docking. The large majority of cases of tetanus in cattle occurred as outbreaks which affected large numbers of animals on several farms over the same period. All the outbreaks of tetanus which occurred in 2001 were related to the administration of

an antihelminthic contaminated with the toxin. The origin of outbreaks occurring in 2009 was not proven, but in all the properties the same medications were being administered before the cases appeared.

The outbreaks of enterotoxemia in goats were associated with the consumption of large quantities of concentrated feed and affected animals that were vaccinated or not vaccinated against enterotoxemia. The cattle affected by blackleg were between 6 months and 1.5 years of age and had never been vaccinated against the agent. In the cases of bacillary hemoglobinuria, the cattle were kept in low-lying floodplains in regions where *Fasciola hepatica* was present. Cases of necrotizing myositis / malignant edema were observed in horses caused by *C. septicum* inoculated with the administration of intramuscular injectable medication. However, in the cases in sheep, no injury or point of entry for the agent was identified. The clinic-pathological findings of the main clostridiosis and the affected species which were diagnosed in the period are shown in Table 2.

All the tissue samples from cattle with clinical symptoms of botulism which were sent for biological confirmation and soroneutralization in mice showed negative results. The samples of water and feed given to the cattle, which were suspected of contamination with botulism toxin, only one (the outbreak among the animals that ate restaurant waste) was positive for type C of botulinum toxin. Samples of the intestinal contents of goats suspected to have enterotoxemia showed the presence of epsilon-toxin produced by *C. perfringens*. In the samples of necrotic myositis/malignant edema in horses and sheep, there was no aerobiotic growth; however, after 48 h, in an anaerobic environment, the growth of countless β -hemolytic colonies characteristics of *C. septicum*.

Table 1. Distribution of cases of clostridial diagnosed in the Setor de Patologia Veterinária (UFRGS) by animal species in the period January 1996 to December 2011.

Clostridiosis	Cattle		Horse		Goat		Sheep		Total	%
	Cases		Cases		Cases		Cases			
	Nº	%	Nº	%	Nº	%	Nº	%		
Tetanus	24	40,68	28	93,33	1	3,13	12	85,71	65	48,15
Botulism	23	38,98	-	-	-	-	-	-	23	17,04
Enterotoxemia	-	-	-	-	31	96,88	-	-	31	22,96
Blackleg	8	13,56	-	-	-	-	-	-	8	5,93
Bacillary hemoglobinuria	4	6,78	-	-	-	-	-	-	4	2,96
Necrotic myositis/malignant edema	-	-	2	6,67	-	-	2	14,29	4	2,96
TOTAL	59	43,70	30	22,22	32	23,70	14	10,37	135	-

Table 2. Clinicopathological findings of the main clostridiosis and affected species diagnosed by the Setor de Patologia Veterinária (UFRGS), for the period January 1996 - December 2011.

Disease	Species	Clinical symptoms	Necropsy findings	Histopathology
Tetanus	Horse	IP: 5 days to 3 months, EP: 3 days to 2 weeks. Difficulty in feeding, urinating, defecating, and movement. Colic, rigid gait, stiffness of pelvic and thoracic members and neck musculature, recumbency, trismus, prolapse of the 3rd eyelid, spastic tetraparesis	Some animals pallid, with necrosis and rupture of some muscles.	No microscopic alterations
	Cattle	IP: 10 to 20 days, EP: 24 to 48 hours. Stiffness of pelvic and thoracic member musculature, rigid gait, position at wide stance, wide-open eyes, erect ears, base of tail raised, nystagmus, prolapse of the 3 rd eyelid, lateral recumbency, tetanic spasms, opisthotonus	Subcutaneous tissue in the scapula, neck, and withers regions with areas of edema, hemorrhage and necrosis	Hemorrhage and edema between muscle fibers associated with areas of necrosis and infiltration of neutrophils with agglomerations of Gram-positive bacilli with terminal spores
	Sheep and Goats	IP: 15 days. Stiffening of limb musculature, position at wide stance, lateral recumbency, trismus, sialorrhoea, and opisthotonus	Purulent green contents where bacteria enter and proliferate	Areas of hemorrhage, necrosis, edema, and large quantity of bacilli
Botulism	Cattle	Weakness of pelvic and thoracic members, difficulty moving, flaccid paralysis, reduction of muscle tonus in the tongue, difficulty swallowing and breathing, falls, sternal recumbency, animals alert and feeding	Congested lung	Areas of hyaline necrosis in skeletal muscle fibers
Blackleg	Cattle	EP: Animals found dead. Two animals exhibited claudication, lack of coordination, cessation of rumination, recumbency, increased volume of pelvic and thoracic members with crepitation	Increased volume of thoracic and/or pelvic members, with crepitation, serosanguinolent edema, hemorrhage and emphysema of the subcutaneous and muscular tissue. At times, serosanguinolent fluid in the lung and pericarditis and necrotic myocarditis, in one animal.	Necrohemorrhagic myositis with large quantity of emphysema and edema associated with Gram-positive bacteria. In one case, there was also fibrinopurulent myocarditis with Gram-positive bacteria.
Enterotoxemia	Goat	EP: Animals found dead, or with evolution of 12 hours. Apathy, depression, anorexia, colic, contraction of abdominal musculature, groaning, dark green or blackish liquid and fetid diarrhea.	Dehydration, hydropericardium, hydrothorax, ascites, distended intestinal loops with liquid contents; cecum and colon with serosa red and dark-green, liquid contents dark-green or reddish with necrotic residue and flakes of fibrin, mesentery lymph nodes enlarged, reddish kidneys with diminished consistency, reddish lung with edema	Cecum and colon with fibrin and necrotic residue and bacterial colonies in the mucosa; congestion, hemorrhage, edema, and inflammatory mono-nuclear infiltration in the submucosa. Necrosis and hyperplasia of germinative centers of the lymphoid follicles (Peyer patches, lymph nodes and spleen). One goat exhibited perivascular hyaline eosinophilic material in the caudal nucleus and thalamus.
Necrotic myositis/malignant edema	Horse	Difficulty walking, reluctance to move, colic, increased volume of the right pelvic member	Increased volume of the right pelvic member and ventral abdominal region, with crepitation and pitting edema, with serosanguinous edema and gas bubbles in the subcutaneous and muscular tissue. Skeletal muscles with areas of hemorrhage and serosanguinous edema, crepitant and appeared dry when cut. Congested lung with edema. One animal presented serosanguinous fluid in the pericardial sac.	Skeletal muscle: vacuolization, hyaline and floccular necrosis associated with edema, hemorrhage and large quantity of Gram-positive bacilli, areas of emphysema and infiltrate of neutrophils and monocytes.
	Sheep	EP: 1 day. Claudication, apathy, hyperemic mucosae, permanent recumbency	Extensive area of hemorrhage and edema in the subcutaneous tissue and musculature of the right thoracic member	Skeletal muscle: gas bubbles, fibrin, hemorrhage, discrete infiltrate of neutrophils and large quantity of Gram-positive bacilli
Bacillary hemoglobinuria	Cattle	Sudden death with no observation of symptoms. In one case, apathy, isolation from the herd, and sanguineous discharge from the nose and rectum were observed	Congestion and jaundice of the mucosae, hemorrhage of the serosae, dark red urine, liver with multiple points of anemic infarct associated with vascular thrombosis, <i>F. Hepatica</i> in bile ducts	Liver with coagulation necrosis and large quantity of peripheral Gram-positive bacilli with polymorphonuclear infiltrate. Cylinders of hemoglobin and hyaline droplets in the renal tubular epithelium. Lung with congestion, edema, hemorrhage, and fibrin's thrombi

IP: Incubation period; EP: evolution period.

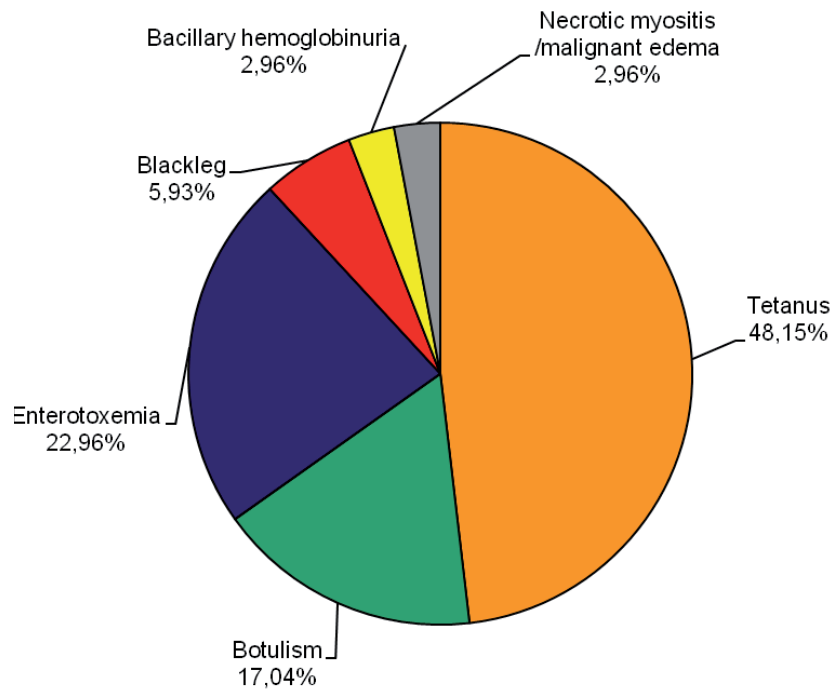


Figure 1. Distribution of cases of clostridial diagnosed in the SPV-UFRGS in the period 1996 -2011.

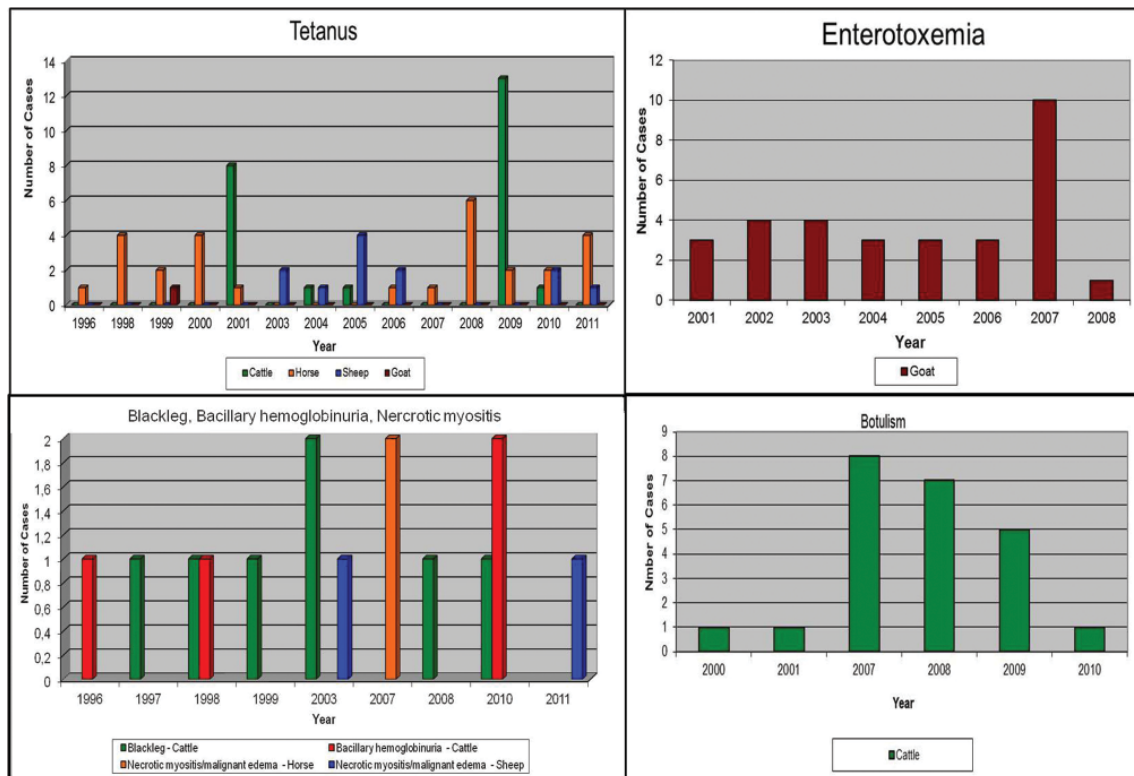


Figure 2. Distribution of cases of tetanus, botulism, enterotoxemia, blackleg, bacillary hemoglobinuria and necrotic myositis/malignant edema by animal species and year of occurrence.

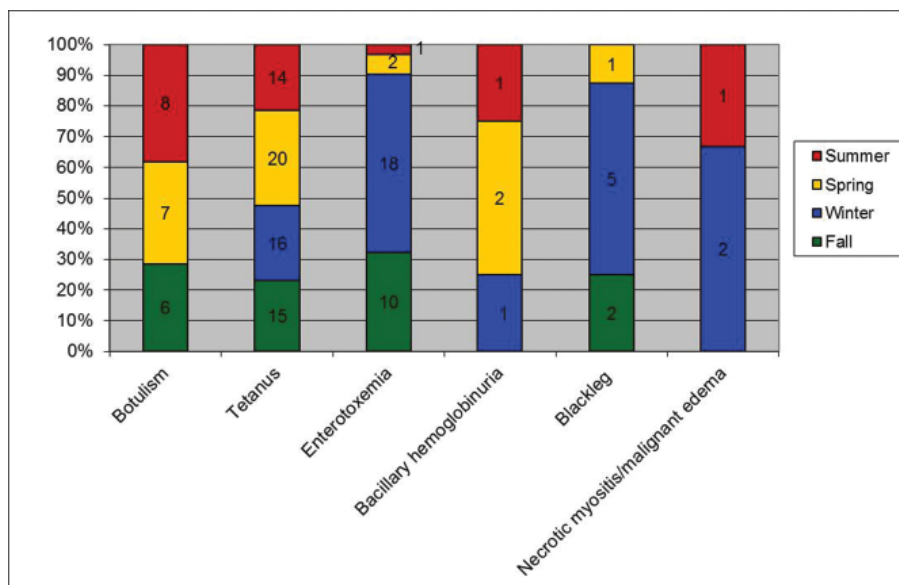


Figure 3. Distribution clostridiosis diagnosed in the SPV-UFRGS in the period 1996 -2011 by the seasons.

DISCUSSION

Tetanus, enterotoxemia, and botulism were the most prevalent clostridiosis diagnosed at SPV-UFRGS and together accounted for approximately 90% of cases in the period 1996-2011. As for blackleg, bacillary hemoglobinuria, and necrotic myositis/malignant edema, together they represented slightly less than 10% of the clostridioses in the period.

Tetanus was the most significant clostridiosis in the period studied, and affected cattle, sheep, and horses. In these animals, there was a large number of cases distributed over many years; probably this occurrence was associated with these species' greater sensitivity to the toxin [2,13]. Sheep and goats are also species that are sensitive to tetanus toxin [2]; however, differently from the sheep, in goats the prevalence of the disease was extremely low, a fact which could be related to the clinico-epidemiological diagnosis of the disease, which is widely known by veterinarians. On the other hand, the diagnosis of tetanus as the most frequent clostridiosis in bovines contradicts the literature, which shows blackleg as the most frequent clostridiosis in the state [14,16]. In the period studied, the cases of tetanus in cattle were observed in various outbreaks distributed only in the years 2001 and 2009, and affected many animals in herds on various properties. These data also contradict observations that bovines are less susceptible to the toxin [14]. In the outbreaks which took place in 2001, the death of the animals was associated with the contamination of anthelmintic bottles

with *C. tetani* spores [6]. The outbreaks that occurred in 2009 exhibited epidemiological characteristics very similar to those occurring in 2001, as some medications or vaccines had been used similarly in herds on different properties which were monitored by SPV-UFRGS. This fact was also described by other diagnostic laboratories in the state [12]. The cases of tetanus in horses, sheep, and goats were associated with contamination of perforating injuries, without climatic influence on the number of cases observed, while in cattle, they were associated with specific triggering factors, since they occurred in two large isolated outbreaks in two distinct periods. In the area of influence of the SPV-UFRGS, tetanus is a significant clostridiosis for sensitive species such as horses, sheep, and goats, in which cases are constantly distributed.

Botulism is an especially important clostridiosis for bovines. In Brazil, the majority of cases of botulism in cattle have been linked with mineral deficiency and the ingestion of bones and carcasses in the fields [8]; however, of the outbreaks included in this study, this association was only observed once. In the other outbreaks, the disease was associated with feed contamination, principally soybean paste. In all the outbreaks of botulism, the cases were concentrated in the hottest periods of the year: summer, late spring, and early fall, as stated in previous reports of botulism [1,5]. It is suggested that the elevation in temperature allied with ideal conditions for anaerobiosis can boost bacterial proliferation and the production of the toxin. Only one sample of the

feed provided to the cattle outbreak were positive for botulism toxin in the mouse bioassay, but it was not possible to identify the type of the toxin. In this cases, the diagnosis was mainly based on the history and clinical observations in association with the exclusion of other diseases, which has been frequent in bovine outbreaks, as the laboratory diagnostic exhibits low sensitivity [14].

Cases of enterotoxemia were important in the area of influence of the SPV-UFRGS, were observed only in goats and distributed uniformly over the years. In the present study, outbreaks of enterotoxemia were all attributed to excessive consumption of concentrated feed, a known predisposing factor for proliferation of *C. perfringens* in the intestine [2]. Vaccinated animals with high degrees of protection usually do not develop the disease [17]; however, in the present study, the disease affected vaccinated and unvaccinated goats alike. It is known that there are variations in the response to immunization associated with individuals, vaccine intervals [7] and adjuvants [17]. Alterations in the necropsy concentrate on the large intestine, in accordance with reports of this being the main alteration observed in goats [14]. The diagnosis was confirmed by the detection of the epsilon-toxin in the animals' intestinal contents in the majority of outbreaks.

Blackleg was apparently less significant in the area of influence of the SPV-UFRGS, to the contrary of what was previously observed, when it was considered the most important clostridiosis in cattle in Rio Grande do Sul [14,16]. It is possible that the prompt recognition of this disease by veterinarians in the field reduced the frequency of requests for diagnostic services. The disease exhibited constant distribution throughout the period under study, but in the form of sporadic cases. All the affected cattle were less than two years of age and were not vaccinated for *C. chaouvei*, known predisposing factors to development of the illness [14]. The two less frequent clostridiosis were bacillary hemoglobinuria and necrotic myositis/malignant edema, which occurred in cattle; horses, and sheep, respectively. The influence of the climate on the occurrence of these two diseases was not observed. The cases of bacillary hemoglobinuria registered here present epidemiology identical to that described previously: sporadic cases in animals of good physical condition, kept in low-lying floodplain fields where *Fasciola hepatica* was present [16,14]. The cases of necrotic myositis/malignant edema were sporadic and related to bacterial contamination of wounds, as described by Coetzer

& Tustin [2]. In the present study, the only species of *Clostridium* isolated in bacterial cultures from cases of necrotic myositis was *Clostridium septicum*, which is frequently associated with cases of the disease [15]. The clinical symptoms observed in the outbreaks of clostridiosis described in this study were similar to those described in the literature and are presented in Table 2.

All the clostridiosis showed a significant presence in the livestock necropsies, but only three (tetanus in horses, sheep and cattle; botulism in cattle, and enterotoxemia in goats) affected an elevated number of animals. Cases of tetanus, necrotic myositis/malignant edema and blackleg were associated with improper management situations, but could be controlled through simple adjustment of hygienic and sanitary measures such as disinfecting needles, using commercially recognized veterinary products, and maintaining the animals' vaccine schedules. The majority of cases of botulism in cattle were associated with the contamination of feed provided to the animals; only one case was linked to osteophagy and mineral deficiency. To avoid the large majority of cases, proper handling of soy industry by-products, as well as frequency of acquisition (perishable feed) and forms of storage (in ventilated and clean environments) are necessary to avoid feed contamination. In the cases of enterotoxemia, correct control of vaccine programs and reduced quantity of concentrated feed are measures which can be taken to avoid fatalities. As shown, the creation of adequate measures for handling the animals would have been sufficient to guarantee the health and performance of the majority of the herds of livestock affected by the outbreaks of clostridiosis described herein.

CONCLUSION

When clostridiosis's casuistic is analyzed, our results showed a significant presence in the livestock necropsies. Three clostridiosis was most important and affected an elevated number of animals (tetanus in horses, sheep and cattle; botulism in cattle, and enterotoxemia in goats). Tetanus, necrotic myositis/malignant edema and blackleg were associated with improper management situations, but could be controlled through simple adjustment of hygienic and sanitary measures. Botulism cases were associated with the contamination of feed provided to the animals. Thus, adequate measures for handling the animals ensure the health and performance of the majority of the herds of livestock affected by the outbreaks of clostridiosis.

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