Mortality caused by gastrointestinal nematodes in beef cattle submitted to an inadequate sanitary protocol

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The aim of this study was to describe the epidemiological, clinical, and pathological findings of two outbreaks of gastrointestinal nematode infections in beef cattle kept under an inadequate sanitary protocol. An outbreak of trichostrongylosis occurred in Amambai, Mato Grosso do Sul (MS), from May to July 2016. The herd consisted of 3,000 Nellore cows, and of these, fifteen died after showing emaciation and diarrhea and remaining in recumbency. At necropsy, the abomasum showed edema in the folds, white, raised areas, multifocal to coalescent, and small ulcers. Histopathology showed larvae compatible with trichostrongylid nematodes were present inside the abomasum glands. Trichostrongylus axei was identified in the abomasum. The hemonchosis outbreak began in October 2018, in a breeding and finishing property in Santa Rita do Pardo, MS. Of 4,000 cattle aged 8 to 18 months, 673 became ill and 117 died. Clinical signs were emaciation, weakness, dehydration, submandibular edema, and soft stools. At necropsy, large numbers of nematodes were found in the abomasum; they were morphologically classified as Haemonchus placei. Both outbreaks were caused by failures in the parasite control protocol. However, in Outbreak I, frost and immune stress caused by lack of food may have contributed to the deaths. In Outbreak I, the main failure in the devermication protocol was the use of anthelmintics without carrying out an efficacy test. In Outbreak II, there was no parasite control protocol in the rearing and finishing property, and in the breeding property, there was resistance to the anthelmintic used (ivermectin). The two outbreaks highlight the importance of gastrointestinal nematode control protocols in cattle and demonstrate that infections by T. axei and H. placei can be lethal for this species.

INDEX TERMS: Mortality, gastrointestinal nematodes, beef cattle, sanitary protocol, Trichostrongylus axei, Haemonchus placei, parasitic control, anthelmintics, cattle, haemonchosis, triconstrogiliasis.
INTRODUCTION

Gastrointestinal nematodes (NGIs) are often found in mixed parasitic infections of cattle and are associated with decreased weight gain, milk production, reproductive performance, carcass quality, immunity, and feed conversion, leading to economic losses (Borges et al. 2012, Pfükenyi & Mukaratirwa 2013, Lopes et al. 2017).

*Haemonchus placei* parasites infect the abomasum of cattle and cause damage to the host due to its hematophagous habit and to the physiological changes in the abomasum resulting from the mucosal lesions that arise out of this eating habit. Affected cattle present anemia, hypoproteinemia, excessive iron loss, submucosal edema, bristly hair, anorexia, and abomasal mucosal lesions (Lopes et al. 2017). In the 1970s and 1980s, there were outbreaks of worm disease in cattle that could result in mortality of up to 10% of calves (Bianchin et al. 1993). In the current production system, deaths attributed to hemoncose are not common in this species, although they are still reported (Carmo et al. 2011, Hossa et al. 2013).

*Trichostrongylus axei* are found, respectively, inside mucosal glands and in the lumen of the abomasum (Lopes et al. 2017). Infections are usually asymptomatic, but can result in diarrhea, dehydration, and hypoproteinemia if there are large numbers of these helminths (Uzal et al. 2016). To date, deaths have been observed only when there is an association with other nematodes in severe mixed parasitic infections (Oliveira et al. 2017).

The main way to control these endoparasitosis is still through the administration of anthelmintics at times of the year when there are fewer larvae in the environment and greater infection in animals (Bianchin et al. 1993). Failures in sanitary protocols for parasite control are related to difficulties in handling, the choice of active ingredient and mode of application, and resistance to antiparasitic drugs (Bianchin 1991, Baiak et al. 2018). The majority (80%) of producers still use anthelmintic dosages in inappropriate categories and at epidemiologically unrecommended moments, in order to optimize management with other activities, especially mandatory vaccination against foot-and-mouth disease (Bianchin 1991, Soutello et al. 2007), which is held in most of Brazil in the months of May and November. In addition, the evaluation of the effectiveness of products is not yet routine in properties and, considering the serious scenario of resistance to avermectins in Brazil, the use of ineffective molecules can result in reduced productivity (Borges et al. 2012) and risk to animal health.

Considering the scarcity of information regarding the mortality of cattle in cases of natural infections by *T. axei* and *H. placei*, the aim of this study was to describe the epidemiological, clinical, and anatomopathological findings of two outbreaks: one of trichostrongylosis and the other of hemoncose, in cattle submitted to an inadequate sanitary protocol.

MATERIALS AND METHODS

**Outbreak I (trichostrongylosis).** The cases occurred in Amambai, Mato Grosso do Sul (MS), in a cow-calf operation with 3,000 permanent Nellore cows, six to seven years old. The animals were divided into lots and kept in a rotational grazing system in Brachiaria brizantha cv. Xaraés, Brachiaria brizantha cv. Piátã. Most of them were in the first third of pregnancy; some were empty and there were two paddocks of cows with calves at their feet. Cows received commercial mineral supplementation (Bellfós 80®) during the wet season and protein salt during the dry season. The calves received supplement in the form of creep feeding. Devermations were carried out in May and November, using ivermectin, abamectin, and albendazole, alternately. The last devermation performed before the outbreak occurred in May 2016 and the active ingredient used was albendazole at a dose of 1ml/40kg bodyweight.

Clinical and epidemiological data were obtained during an interview with those responsible for handling the cattle and during an inspection performed by veterinarians from the “Laboratório de Anatomia Patológica” (LAP) and “Laboratório de Parasitologia Veterinária” (Pathological Anatomy and Parasitology laboratories) of the “Universidade Federal do Mato Grosso do Sul” (Federal University of Mato Grosso do Sul, UFSM), on the property where the case occurred. During visits, affected cow were randomly selected for physical examination and collection of feces samples.

The Body Condition Score (BCS) was visually assessed, and scores from 1 to 5 were assigned according to Machado et al. (2008). Fecal samples were collected directly from the rectal ampoule in plastic bags or from the large intestines of necropsied animal, identified and stored under refrigeration to count eggs per gram of feces (OPG), according to the technique described by Gordon & Whitlock (1939), with modifications (1:25 sensitivity).

One cow were necropsied and fragments of various organs were collected, stored in a 10% formalin solution, routinely processed for histopathology, and stained with hematoxylin and eosin (HE). The abomasum and a small part of the duodenum were collected during the necropsy and separated by means of double ligatures; part of the total content of these organs was sieved (0.297 mm/tyler 48) and the solid part was fixed in 70% alcohol. Recovery of adult or immature forms embedded in the abomasal and duodenal mucosa was performed by digestion in a 3% hydrochloric acid solution at 36°C for 24 hours (Wood et al. 1995, Verbruyssse et al. 2001).
Outbreak II (hemoncosis). The outbreak occurred in Santa Rita do Pardo (MS). The property was intended for the rearing and finishing of cattle and had a herd composed of a total of 13,000 male and Nellore cattle, of which 4,000 were aged between eight and 18 months and 9,000 were aged between 18 and 24 months, all coming from one of three properties owned by the same owner, in which the breeding process was carried out. The cattle were separated into lots of 400 to 500 animals and kept in paddocks of approximately 50 to 100 hectares (ha), in a rotational grazing system with good availability of B. brizantha, in which they remained for seven to 10 days (larger paddocks) or three to four days (smaller paddocks).

The brood farms performed a sanitary protocol consisting of the application of 1 mL of doramectin at birth, 1 mL for every 50 kg of animal live weight of 3.15% ivermectin at four months of age, and this same dose of 1% ivermectin right at weaning. The criterion for choosing the drugs was by convenience, according to the farmer’s demand. When they arrived at the breeding and fattening property, the animals received a polyvalent vaccine against diseases caused by clostridia and a vaccine against foot-and-mouth disease. Sometimes weaned calves remained on the brood farms for three to four months, but were not dewormed again.

Clinical and epidemiological information, BCS assessment, fecal samples and necropsy were performed as described in Outbreak I. Blood samples from six animals were obtained by jugular venipuncture in tubes containing EDTA, to perform a complete blood count. Morbidity and lethality coefficients were calculated using the following formulas:

\[
\text{Morbidity coefficient} \left( \% \right) = \frac{\text{number of cattle that showed clinical signs of hemoncosis}}{\text{total cattle at risk}} \times 100
\]

\[
\text{Lethality coefficient} \left( \% \right) = \frac{\text{number of cattle that died of hemoncosis}}{\text{number of animals that showed clinical signs of hemoncosis}} \times 100
\]

Of the necropsied cattle in Outbreak II, an aliquot of the abomasal content was used for the generic identification of helminths, which was performed under a stereoscopic microscope (Optika SZR-10), and the specific identification (Ueno & Gonçalves 1998) under a light microscope (Nikon Eclipse E100).

In one of the calf farms responsible for the production of calves that were reared and finished on the farm involved in Outbreak II, an efficacy test of anthelmintic formulations was carried out. The principles were evaluated: 3.15% ivermectin, 2.25% ivermectin + 1.25% abamectin, and 4% ivermectin. To carry out the efficacy test, 120 animals with egg counts per gram of feces greater than or equal to 25 (OPG≥25) were selected. The animals were divided into four groups (n=30), including an untreated control group, using a randomized block design.

For each of the four groups formed, a treatment (control or anthelmintics) was randomly assigned. In addition to the pre-treatment OPG, performed seven days before treatment (D-7), there was a new collection at 14 days post-treatment (D14). For the calculation of efficacy, D14 OPG values, control versus treatment, were used. The efficacy percentages and confidence intervals were calculated using ‘eggCounts-2.3’ on the R version 3.6.1 program (Wang & Paul 2018).

After diagnosis, in both outbreaks, treatments were instituted based on technical criteria.

RESULTS

Outbreak I (trichostrongylosis)

From May to July 2016, there were five frosts in the region, sharply reducing the supply and quality of pasture on the property. Fifteen cows (pregnant and empty) died during this period, in two different paddocks, 10-20 days after presenting diarrhea and weight loss and remaining in recumbency, though some had already presented clinical signs before the frost. One of them was received by the LAP-UFMS and underwent necropsy, which observed, in addition to a poor body condition (score 1) and a uterus containing a fetus of approximately three months, abomasum with extensive reddish areas in the mucosa and edema of the folds, especially those located in the proximal half of the organ. Distributed throughout the mucosa were numerous multifocal to irregular coalescent areas, whitish, wrinkled, and slightly elevated, as well as small multifocal ulcers (Fig. 1-4).

Histopathology showed transverse and longitudinal sections of larvae morphologically compatible with nematodes of the superfamility Trichostrongyloidea, which measured between 100 and 300 µm in length and 30 and 60 µm in width and were inside glands of the abomasal mucosa, in the middle of the mucosa of the duodenum and in the lumen of both organs. The larvae were characterized by having a platymerian musculature and cuticle with a thickness of 8 to 10 µm. Nematodes observed in the duodenum had cuticular ridges and were surrounded by few macrophages and a moderate amount of lymphocytes (Fig. 1-4).

The OPG count performed using the feces collected during the necropsy revealed 125 strongyloide-like eggs. The adult helminths collected from the abomasum and duodenum were identified as belonging to the genera Cooperia, Trichostrongylus, and Haemonchus. Among Trichostrongylus species, T. axei was present in a greater quantity.

On a visit to the property, it was noticed that most of the cows were thin (score 1 to 2) and with the perineum dirty with feces. In the coproparasitological evaluation of 10 cows, OPG counts resulted in values between 25 and 175. Treatment with Valbazen® (albendazole) 5 mg/kg was established and deaths ceased.

Outbreak II (hemoncosis)

The cases began in October and worsened in December 2018, a period in which the measured rainfall index was 188 mm in October, 58 mm in November, and 51 mm in December. Only cattle aged between eight and 18 months became ill. On the first visit to the property, the clinical signs identified were emaciation, weakness, dehydration, edema in the submandibular region, and nasty stools adhered to the perineum.

The OPG counts in the feces of six symptomatic animals ranged from 175 to 10,000, and in two of them, the mean cell volume (MCV) were 38.6 fL (reference = 40-60 fL) indicating microcytosis without the occurrence of anemia (hemoglobin concentration (Hb) 11.5 and 12.1 g/dL; reference = 8-15g/dL) was verified on the hemogram. All clinically affected cattle were treated with oral levamisole (Ripercol®), Ferrodex®, and Aminofort at the dosages indicated by the manufacturer. Approximately one month after the first treatment, the farm was visited again and 86 calves received moxidectin (Cydectin®) at the dose indicated by the manufacturer, as they were still weak. Of the total of 4,000 cattle, 673 got sick and 117 died, so the morbidity and lethality coefficients were 16.82% and 17.38%, respectively.
The two necropsied cattle had poor body scores, pale ocular and oral mucosa, moderate hydropericardium, abomasum containing abundant amounts of *Haemonchus* spp. adhered to the wall or amidst its contents, edema of the folds, multiple ulcers measuring between 0.1 and 0.3 cm in diameter (Fig. 5-6), and diffusely reddened bone marrow. Histopathological examination showed increased medullary erythropoiesis and extramedullary erythropoiesis in the liver. Nematodes collected during necropsy were classified as *Haemonchus placei*.

The efficacies (EF%) calculated for the different anthelmintics in one of the breeding farms were: ivermectin 3.15%, EF% = 21.2% (95%CI = 1.32% to 50.9%); ivermectin 2.25% + abamectin 1.25%, EF% = 32.6% (95%CI = 3.29% to 61.3%); and ivermectin 4%, EF% = 20% (95%CI = 1.15% to 58.8%).

**DISCUSSION**

In the two outbreaks described, the diagnosis was based on the clinical picture, post-treatment clinical evolution, necropsy, and histopathology findings, as well as the identification of etiological agents recovered from the mucosa and abomasum content of necropsied cattle. The methodology used for the diagnosis is in accordance with the protocols recommended for this purpose and allows the agents involved to be safely identified (Ueno & Gonçalves 1998).

According to the data presented, *Trichostrongylus axei* and *Haemochus placei* can cause serious and fatal outbreaks in both young and adult cattle. Deaths in cattle from hemonchosis and mixed infections were described in a retrospective study carried out in Mato Grosso do Sul (Pupin et al. 2019) and in other states in Brazil (Lucena et al. 2010, Oliveira et al. 2017, Rondelli et al. 2017) but they occurred sporadically and rarely bring morbidity and lethality coefficients.

This is one of the rare cases of mortality in adult cattle due to *T. axei* infection described in Brazil. During the literature review, the only report found of trichostrongylosis in the country was in Mato Grosso do Sul and it affected cows (Bianchin et al. 1979). *T. axei* is a species with a prevalence close to 70% (Santos et al. 2010); however, it has low parasitic
intensity (Bianchin et al. 1993, Borges et al. 2001). Thus, to cause the death of cattle, there is a need for environmental or management conditions favoring their survival in pastures and inside the abomasum, resulting in massive infections (Bianchin et al. 1979). On the other hand, H. placei, in addition to being prevalent, has high parasitic intensity and high pathogenicity (Bianchin et al. 1993, Borges et al. 2001). In addition, larvae have a good ability to survive in the environment (Heckler & Borges 2016), which makes it essential to adopt devermination protocols to avoid the occurrence of outbreaks (Carmo et al. 2011, Hossa et al. 2013).

The clinical signs presented in the two outbreaks were nonspecific and can be observed in other diseases that affect cattle, such as food deprivation, mineral deficiencies such as cobalt, phosphorus, and copper (Tokarnia et al. 2010), and ostertagiosis (Uzal et al. 2016). In this context, the importance of performing the differential diagnosis is highlighted, according to criteria involving the epidemiological approach, clinical examination, and the performance of necropsies and complementary laboratory tests (Maxie & Miller 2016).

The macroscopic and microscopic changes observed in cattle necropsied in both outbreaks are compatible with the diagnosed parasitic diseases (Bianchin et al. 1979, Carmo et al. 2011, Hossa et al. 2013, Uzal et al. 2016). Cattle with severe T. axei infections have poor body condition, dehydration, edema of the abomasal folds, reddening and erosion and/or ulceration of the mucosa, in addition to irregular or rounded white plaques that cover the affected mucosa together with a layer of mucus (Bianchin et al. 1979, Uzal et al. 2016). In mild infections, there may be only reddening of the mucosa (Uzal et al. 2016). Histologically, there are changes restricted mainly to the abomasal mucosa, such as hyperplasia or metaplasia, lymphocytic or eosinophilic inflammation, and a decrease in the number of parietal glands associated with the presence of morphologically compatible larvae with trichostrongylid nematodes (Uzal et al. 2016). It is noteworthy that the morphological characteristics of T. axei observed in histological sections are identical to those of other trichostrongylids, including Ostertagia spp., which causes clinical signs and microscopic changes similar to those observed in trichostrongylosis (Gardiner & Poynton 1999, Uzal et al. 2016). Thus, the recovery and identification of larvae present in the abomasum are essential for a conclusive diagnosis (Uzal et al. 2016).

In Outbreak II, although the histopathological examination was performed as a complement to the necropsy, as in all cases received by the LAP, the macroscopic changes are sufficient to conclude the diagnosis of hemonchosis, as it is possible to see specimens of Haemonchus spp. in the content of the abomasum or adhered to the mucosa of this organ (Carmo et al. 2011, Hossa et al. 2013). In addition to the macroscopic changes observed in cattle in this outbreak, pale and friable liver, edema of the subcutaneous tissue in the ventral and mesenteric region, hydrothorax, hydropericardium, and ascites are changes that may occur and reflect anemia and hypoproteinemia caused by the helminth (Carmo et al. 2011, Hossa et al. 2013, Uzal et al. 2016). In an attempt to increase the production of erythrocytes, the bone marrow of adult cattle becomes active and, consequently, redden, as observed in the present case. Extramedullary erythropoiesis found in the liver indicates a chronic anemia secondary to parasitism by Haemonchus spp. (Uzal et al. 2016).

The morbidity and lethality coefficients obtained in this report were high, resulting in 16.82% and 17.38%, respectively. Morbidity and lethality coefficients ranging between 8% and 22.8%, and between 16.1% and 45% in H. placei infections, have previously been described (Carmo et al. 2011, Hossa et al. 2013, Oliveira et al. 2017). Despite these lethality rates being higher than those obtained in Outbreak II, it is noteworthy that the number of cattle at risk, sick, and dead in the reports of the first two authors is lower than that of the present outbreak, which ends up increasing this coefficient.

In both outbreaks, failures were identified in the sanitary programs, more specifically in the worm control protocols, but in Outbreak I, the occurrence of successive frosts resulted in a low food supply, which may have triggered immunological stress and contributed to deaths.

The anthelmintics ivermectin, abamectin, and albendazole were used in the Outbreak I property for many years without knowledge of the effectiveness of these drugs in the control of helminths. The first failure identified was the choice of antiparasitic, performed in the absence of a previous reduction

Fig. 5-6. Macroscopic findings of hemonchosis in cattle, Outbreak II. (5) Conjunctival mucosa markedly pale indicating anemia. (6) Abomasum. There is edema of the folds and numerous specimens of Haemonchus placei over the mucosa.
test. Although the control protocol involves two annual dosages (May and November) and rotation between active ingredients, as the choice of these principles did not meet technical criteria, favorable conditions were created for the occurrence of the outbreak. The fact that cows get sick even after deworming carried out in May can be explained by the choice of albendazole, which, although it is an anthelmintic recommended for the treatment of trichostrongylosis and is efficient in the control of other helminths in most of the properties of Mato Grosso do Sul (Feliz 2011), has short action, thereby allowing for rapid reinfections. The frosts that occurred during the outbreak may have contributed to the reduction in food availability and, consequently, immunological stress due to food deprivation, as well as to the increase in the population of \textit{T. axei}. Some authors report the increase in the parasite load of adult cattle (indicated by OPG) as a result of immunosuppressive factors, such as food deprivation, peripartum, transport, long walks, the use of anthelmintics that cause temporary immunosuppression, and the administration of steroids (Bianchin et al. 1987, Bianchin et al. 1993, Sajid et al. 2006). In addition, cold weather may favor the increased occurrence and survival of pre-infective and infective stages of \textit{T. axei} (Roberts et al. 1952, Lee et al. 1960), favoring greater contamination and reinfections.

Two flaws in the parasite control protocol were decisive for the occurrence of Outbreak II, as immunosuppressive conditions favoring the severity of infections were not identified. One of them was the absence of a calendar for the dosing of cattle in the rearing and finishing property; the other was the resistance of \textit{H. placei} to ivermectin, proven through an efficacy test carried out on one of the brood properties. Outbreaks of haemonchosis related to the absence of parasite control in cattle have already been reported, demonstrating that this is a part of the sanitary protocol that should not be neglected (Carmo et al. 2011, Hossa et al. 2013). In Brazil, the recommendation is that strategic parasite control in cattle from weaning to 18 or 24 months of age is carried out in May, July, and September (Bianchin et al. 1993) or May, August, and November (Heckler et al. 2016), and not just twice a year (May and November). Despite the higher cost, the protocol with three annual dosages resulted in a greater economic advantage (Conde et al. 2019). The low efficacy of ivermectin in the control of \textit{H. placei} in cattle herds was demonstrated by studies carried out in the Southeast, Midwest, and South regions of Brazil, where different populations of this nematode were resistant to different concentrations of ivermectin, even the highest ones (Souza et al. 2008, Felippelli et al. 2014, Borges et al. 2015). In general, albendazole sulfoxide, levamisole, moxidectin, and doramectin are molecules that are still effective against the genus \textit{Haemonchus} in cattle (Soutello et al. 2007, Souza et al. 2008, Ramos et al. 2016).

The treatments instituted in both outbreaks showed good results, with the recovery of affected animals and the ending of deaths. There are no hemonchosis treatment protocols for cattle, except for the indication of the use of an effective anthelmintic, which many times may not be sufficient for the recovery of animals in serious condition. Two of the hemograms performed showed microcytic erythrocytes, a characteristic alteration of iron deficiency. Lambs infected with \textit{H. contortus} and supplemented with iron had less severe anemia than those not supplemented (Casanova et al. 2018). This data may indicate that the association of supplements containing iron, minerals, amino acids, and vitamins is interesting with respect to increasing the speed of recovery of sick cattle.

**CONCLUSION**

Infections by \textit{Trichostrongylus axei} and \textit{Haemonchus placei} can cause serious and fatal outbreaks in cattle, especially when they are kept under an inadequate parasite control protocol.

**Acknowledgements.** One of the authors (R.A.A.L.) has a research fellowship from the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq). This study was partially funded by the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES), Brazil - Finance Code 001 and by “Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul”. This study was carried out with the support of the “Universidade Federal de Mato Grosso do Sul” (UFMS/MEC), Brazil.

**Conflict of interest statement.** The authors have no conflicts of interest to declare.

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