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Cryptosporidium spp. in calves: a description of an outbreak. *Cryptosporidium* spp. em bezerros: descrição de um surto.

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Abstract

The aim of this study was to characterize an outbreak of cryptosporidiosis in calves reared on a beef cattle farm. Nelore bovines (n=65) of up to 45 days old presented intense diarrhea and 18 died. Faecal samples (n=40) were analysed using the centrifugal-sedimentation technique in formol-ether with modified Ziehl-Neelsen staining, and the immunoassay MERIFLUOR® test. Oocysts of *Cryptosporidium* spp. were observed in 35% and 45% of the samples by the modified Ziehl-Neelsen technique and immunoassay, respectively. The diagnosis of *Cryptosporidium* spp. in calves was described for the first time in this study in the state of Pernambuco. Preventive measures should be taken to reduce the economic impact caused.

Keywords: Protozoan infection. Ruminants. Zoonosis. MERIFLUOR® test.

Resumo

O objetivo deste estudo foi caracterizar um surto de criptosporidiose em bezerros criados em uma fazenda de gado de corte. Bezerros Nelore (n=65) com até 45 dias de idade apresentaram diarreia intensa, sendo que 18 foram a óbito. Amostras fecais (n=40) foram analisadas usando a técnica centrífugo-sedimentação em formol-éter com coloração Ziehl-Neelsen modificada e o teste de imunoensaio MERIFLUOR®. Oocistos de *Cryptosporidium* spp. foram observados em 35% das amostras pela técnica de Ziehl-Neelsen modificada e 45% pelo imunoensaio. O diagnóstico de *Cryptosporidium* spp. em bezerros foi descrito pela primeira vez neste estudo no estado de Pernambuco. Medidas preventivas devem ser tomadas para reduzir o impacto econômico causado.

Palavras-chaves: Infecção por protozoário. Ruminantes. Zoonose. Teste MERIFLUOR®.

Introduction

Cryptosporidium spp. are zoonotic protozoa belonging to the Phylum Apicomplexa which parasitizes intestinal cells of vertebrate hosts and occasionally cells of the respiratory and excretory systems (CAVALIER-SMITH, 2014; RYAN; FAYER; XIAO, 2014; THOMSON et al., 2017). Although this parasite may infect a wide range of hosts, in cattle considerable damages have been observed (FAYER; MORGAN; UPTON, 2000; RYAN et al., 2015). Currently, this parasite has been considered a re-emerging pathogen affecting humans, being one of the most important causes of diarrheal disease in immunosuppressed patients (MADRID; BASTOS; JAYME, 2015; STRIEPEN, 2013). It is known that during development, *Cryptosporidium* spp. cause atrophy of intestinal cells, reducing the absorption of nutrients and, consequently, leading to weight loss, which characterizes cryptosporidiosis (ORTOLANI; SOARES, 2003; ZAMBRISKI et al., 2013). From a clinical point of view, cryptosporidiosis is characterized especially by diarrhea and dehydration, which may lead to death in young animals (OLSON et al., 2004; ZAMBRISKI et al., 2013).

Presently, more than 30 species of *Cryptosporidium* have been identified (THOMSON et al., 2017), and at least 14 have been detected in bovines (COUTO; BOMFIM, 2012; RODRIGUES et al., 2016; SANTIN; TROUT; FAYER, 2008). Amongst the species that infect these animals, *C. parvum* has been extensively studied due to high pathogenicity and zoonotic aspects (GONG et al., 2017). In general, the mortality is low, but when affecting young animals co-infected with other pathogens (e.g., *Eimeria* spp., *Salmonella* spp., coronaviruses and rotaviruses) high mortality may occur (DELAFOSSE et al., 2015; THOMSON et al., 2017).

The prevalence of *Cryptosporidium* spp. in cattle can vary. In Brazil, it has been reported in different areas, with the majority of cases registered in the South, Southeast, and Midwest. However, in the North-eastern region data are limited (ABREU et al., 2019), therefore the aim of this study was to characterize an outbreak of cryptosporidiosis in calves raised on a beef cattle farm.

Material and methods

Animals and ethical aspects

On November 2018, calves (Nelore breed) reared on a beef cattle farm located in the municipality of São Benedito do Sul (08º48'30" South and 35º57'06" West), state of Pernambuco, North-eastern region of Brazil, presented clinical signs suggestive of cryptosporidiosis. These animals were from a herd composed of 100 calves up to 45 days old, raised in a semi-intensive system, where they maintained contact with adult animals from other herds. The diet was based on Tifton grass and water *ad libitum*. Sixty-five calves presented the symptoms mentioned and of these 18 died approximately 20 days after disease onset. The sick animals were treated with Enrofloxacin (2,5mg/kg) and Doramectin (200 mcg/kg), but no improvement in their clinical status was observed.

At the visit, a humid environment with the presence of organic matter and mud were recorded. During the outbreak (November 2018) an atypical situation with intense rainfall was observed in the area.

All live calves (n = 47) were physically examined and clinical data reported in individual clinical charts. Subsequently, faecal samples (n = 40) were collected from the rectal ampoule of

calves aged 30 (n = 20) and 45 (n = 20) days, stored in plastic vials and maintained in isothermal boxes (8 °C) until laboratory processing. Seven animals did not present enough faeces at the moment of sampling.

Laboratorial procedures

Parasitological analysis

The identification of *Cryptosporidium* spp. was performed through the centrifugal-sedimentation technique in formol-ether (DAVID; FREBAULT; THOREL, 1989; RITCHIE, 1948) modified and stained by the Ziehl-Neelsen technique (HENRIKSEN; POHLENZ, 1981). Slides were observed under an optical microscope at different magnifications (40X and 100X). All parasitic structures detected were measured using an optical microscope equipped with a camera using AxioVision software (release 4.8).

Immunoassay analysis

The immunoassay evaluation was performed using the commercial kit MERIFLUOR® *Cryptosporidium*/*Giardia* (Bioscience) following the manufacturer's instructions.

Data analysis

Absolute and relative frequencies were obtained through the descriptive analysis.

Results

A total of 47.5% (19/40) of faeces were diarrheic and the presence of blood was observed in 17.5% (7/40) of samples. Oocysts of *Cryptosporidium* spp. were detected in 35% (14/40) of animals. In particular 42.9% (6/14) of positive animals were 30 days old and 57.1% (8/14) 45 days old. Oocysts presented a mean length of 3.86 ± 0.71 and width of 4.07 ±0.66 μm.

The immunological test scored positive in 47.5% (19/40) of samples, being 30% (12/40) reagent for *Cryptosporidium* spp., 2.5% (1/40) for *Giardia* spp., and 15% (6/40) co-infected by both parasites. Positive samples in the parasitological examination were confirmed by the immunological assay.

All positive animals presented diarrhea, weight loss, dehydration, and apathy, clinical signs suggestive of cryptosporidiosis.

Discussion

This study characterizes for the first time an outbreak of cryptosporidiosis in calves reared on a beef production farm in the state of Pernambuco, North-eastern Brazil.

Although protozoan infection is a common finding in cattle herds, the positivity may vary, ranging from 10.2% to 63.54% (ABREU et al., 2019; HECKLER et al., 2015; RODRIGUES et al., 2016; TOLEDO et al., 2017). These differences can be attributed to the different methods employed

for this kind of diagnosis. For instance, in the present study positivity values of 35% and 45% were found using the modified Ziehl-Neelsen technique and immunological assay, respectively.

The clinical signs herein reported were nonspecific and may be confused with other gastrointestinal disorders; therefore, the laboratorial confirmation is pivotal (FAYER; MORGAN; UPTON, 2000; MUNIZ NETA et al., 2010; OLSON et al., 2004). In the current study, it is believed that the hygiene conditions of the farm could facilitate the maintenance and dissemination of protozoan. It is known that excessive humidity and accumulation of organic matter (e.g., faeces and mud), as well as the sharing of the same environment between young and adult animals may be considered risk factors for this infection (GONG et al., 2017; OLSON et al., 2004; SILVA JUNIOR et al., 2011). From an epidemiological perspective, adult animals are very important as although they are asymptomatic, they are able to eliminate oocysts, contaminating the environment (DELAFOSSE et al., 2015; GONG et al., 2017; XIAO; FAYER, 2008;).

The atypical rainfall observed at the study area during the outbreak, associated with the humid environment in which the calves were maintained, most likely favoured the spread of the oocysts and environmental contamination, increasing the risk of animal infection, as previously observed in other studies (ALMEIDA; OLIVEIRA; TEIXEIRA, 2008; BOUZID et al., 2013; FEITOSA et al., 2004).

Diarrhea in infected animals may be an absent finding. In fact, this clinical sign is dependent on the species involved, and the immunological status of the animal (FEITOSA et al., 2008; RYAN et al., 2015). Both parasites (i.e., *Cryptosporidium* spp., *Giardia* spp.) reported herein in some animals cause damage to the host intestinal epithelium, leading to reduction in nutrient absorption, weight loss, and important economic impact (SILVA JUNIOR et al., 2011; ZAMBRISKI et al., 2013).

In general, the presence of *Cryptosporidium* spp. has been considered an opportunistic infection (MOORE; ZEMAN, 1991; VARGAS JUNIOR et al., 2014), however findings from this study suggest that this parasite could be the primary cause of the disease, since some animals with intense diarrhea were parasitized exclusively by *Cryptosporidium* spp.

Conclusions

This is the first report of an outbreak of cryptosporidiosis in calves reared on a beef cattle farm in the area of study. Therefore, considering that this disease may be facilitated by interaction between improper management and exposure of susceptible animals, preventive measures should be adopted on beef production farms in order to reduce the losses and economic impact caused by this neglected protozoan.

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