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Parasitological and Molecular Detection of Cryptosporidium parvum in Rheas (Rhea americana)

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ABSTRACT

Background: The rhea (Rhea americana) is a Brazilian wild bird that produce meat, leather and feathers of excellent quality. Rhea production has been increasing every day in Brazil due to many favorable conditions for breeding and there are also large native populations in various regions of the country. The incidence of parasites is a limiting factor when raising many animals, and rheas are not an exception. The occurrence of Cryptosporidium spp in captive rheas in a Brazilian zoo and Spain was described. However, little is known about cryptosporidiosis in rhea, which makes the need for further studies. Thus, this study aimed to detect Cryptosporidium parvum in rheas from the South of Brazil.

Case: This study evaluated two properties located in Southern Brazil. Property A was located in Rio Rufino, Santa Catarina State, Brazil and it had 40 rheas for commercial purposes. Property B was located in Santa Maria, a small town in Rio Grande do Sul State, Brazil and it had 10 rheas. Fresh fecal samples were collected and kept refrigerated from adult birds (n = 4) and chicks (n = 2) from property A, and chicks (n = 3) of three months of age from property B. Samples were analyzed by the method of direct examination, followed by centrifugal flotation with zinc sulfate. Only the centrifugal flotation technique allowed the observation of Cryptosporidium spp. oocysts in three adults and one chick. Fecal samples were stored in ethanol and analyzed by PCR for C. parvum, all being positive for this protozoan. Feces contaminated by C. parvum oocysts from one young rhea was used to inoculate two mice orally (BALB/c), previously confirmed protozoan free by faecal examination and PCR. Feces from inoculated mice were collected on days 1, 3 and 5 post-inoculation for analysis by the centrifugal flotation technique. After five days of inoculation all mice presented diarrhea and high numbers of oocysts of protozoan in their feces.

Discussion: Cryptosporidiosis can evolve into severe diarrhea, followed by abdominal cramps, anorexia, vomiting, dehydration, nausea and fever in different animals. However the incubation period of this disease in rheas is unknown because this is only the third report of cryptosporidiosis in this wild bird. The Cryptosporidium spp. is an obligate parasite of vertebrates, and its colonization occurs at the periphery of the intestinal cells of the host and it may cause atrophy of these structures and enteritis, but these findings have not been described in rheas yet. The species of Cryptosporidium that are mainly reported in birds are Cryptosporidium meleagridis, Cryptosporidium baileyi and Cryptosporidium galli. In our study, the molecular analysis was performed in order to identify the protozoan, being detected the C. parvum, a zoonotic agent reported in several mammals. The rhea is wild birds with habits similar to ostriches. Already, in studies have reported the occurrence of Cryptosporidium in ostriches; however the species identified were different from that described in this study. Based on these results, we conclude that rhea may be parasitized by C. parvum, an important zoonotic parasite. Prevalence studies should be conducted in this area to estimate the role and impact of rhea as reservoirs and disseminators of this zoonotic parasite.

Keywords: protozoan, ratites, cryptosporidiosis, rhea, Cryptosporidium parvum, Rhea americana.
INTRODUCTION

The rhea (*Rhea americana*) is a Brazilian wild bird that produce meat, leather and feathers of excellent quality, being able to contribute economically to many farms. Rhea production has been increasing every day in Brazil due to many favorable conditions for breeding and there are also large native populations in various regions of the country [4]. Therefore, further studies are needed regarding health issues related to these animals, especially about the incidence of parasites, an area of scarce research and large economic losses.

Researchers report occurrence of *Cryptosporidium* spp. in captive rheas in a Brazilian zoo [5] and Spain [9]. *Cryptosporidium* spp. is an obligate parasite of vertebrates, and its colonization occurs at the periphery of the intestinal cells of the host or at its edge grooved, but not within, wrapped by microvilli, originating from a vacuole which causes atrophy of these structures and enteritis. Moderate hyperthermia, diarrhea and malabsorption are signs of the infection by this protozoan [3,6]. The oocyst of this protozoa is released in the feces and may infect its host by the oral or nasal route, usually by contaminated water or food [10]. Being zoonotic, *Cryptosporidium parvum* can be transmitted to humans causing gastrointestinal problems [3,6,10].

The species of *Cryptosporidium* that are mainly reported in birds are *C. meleagridis*, *C. baileyi* and *C. galli* [14]. Clinical signs of cryptosporidiosis in birds include gastrointestinal problems such as diarrhea, lethargy, weight loss, and respiratory problems [1]. Therefore, this paper is aimed to report the parasitological and molecular detection of *Cryptosporidium parvum* in rheas in Southern Brazil.

CASE

This study evaluated two properties located in Southern Brazil. Property A was located in Rio Rufino, Santa Catarina State, Brazil and had 40 rheas for commercial purposes, being 21 adults of 3 to 7 years of age, and 19 young animals aged between 4 and 6 months. Property B was located in Santa Maria, a small town in Rio Grande do Sul State, Brazil. Three adult females and seven young animals with three months of age were evaluated from property B. In both farms, rheas were kept in paddocks of natural pasture, and fed commercial feed.

In the property A, four fresh fecal samples of adults and two from young animals were collected, stored in plastic bags, and kept under refrigeration until analysis (24 h). Property B had fresh fecal samples collected from three young animals, and their samples were analyzed immediately. Samples were processed by the method of direct examination and by centrifugal flotation with supersaturated solution of zinc sulfate to search for eggs, parasite cysts and oocysts under optical microscopy. The centrifugal flotation technique allowed the detection of *Cryptosporidium* spp. oocysts in some fecal samples as described below.

PCR was performed in order to confirm the microscopic diagnosis, and to identify which *Cryptosporidium* species was infecting the rheas. Oocysts were isolated from feces using sucrose flotation methods, and subsequently preserved in ethanol. DNA extraction was performed as previously described by Nichols and Smith [7]. Purified DNA samples were used as templates for PCR amplification of a spiked leader gene sequence using specific primers (SB012 F: 5’-CTCCGTTCGATGATGCAGATG-3’; SB012 R: 5’-CGGCCCCCTGTAGAAATAAGTCA-3’) under reaction conditions previously described [13]. DNA from *C. parvum* was used as positive control. *C. baileyi* and *C. meleagridis* were also included as controls in both PCR assays. Amplified DNA fragments were resolved in 2% agarose gel, stained with ethidium bromide and visualized under UV light.

In both properties all rheas were apparently healthy. However, fecal analysis detected *Cryptosporidium* spp. in three adult birds from property A and one young animal from property B (Figure 1). This diagnosis was confirmed by molecular testing since samples were positive by PCR for *Cryptosporidium parvum*. Molecular tests also showed that the samples were negative for *C. baileyi* and *C. meleagridis*, the main species that usually infect birds.

Once confirmed the presence of *Cryptosporidium* spp oocysts in rhea feces from property B, we decided to conduct an inoculation of these contaminated feces into mice. For this, two mice with negative parasitological examination for parasites received orally 0.2 mL of rhea stool diluted in physiological solution. Feces from inoculated mice were collected on days 1, 3 e 5 post-inoculation for analysis by the centrifugal flotation technique.
The inoculated mice exhibited negative parasitological examination on days 1 and 3 post-infection, and had feces with normal appearance (bolus). However, on day 5, inoculated mice presented diarrhea and large numbers of oocysts of *C. parvum* in their feces. After nine days of infection, one mouse died (showing diarrhea, chills and weight loss) and another was apparently healthy.

**DISCUSSION**

Although the animals were apparently healthy, tests confirmed the presence of the parasite, *C. parvum*. In different mammals, cryptosporidiosis can evolve into severe diarrhea, followed by abdominal cramps, anorexia, vomiting, dehydration, nausea and fever [1]. However the incubation period of this disease in rheas is unknown because this is only the third report of cryptosporidiosis in this wild bird. Infection by *C. parvum* can cause major economic losses and can be transmitted to humans. Being a zoonosis, is paramount efficient sanitary control measures against this protozoan since the transmission can be through ingestion of contaminated feces of infected animals, as proved in this study when mice infected orally developed the disease. Therefore, rheas are potential reservoirs and disseminators of this protozoosis, and consequently of great epidemiological importance to the disease.

*Cryptosporidium* spp. occurrence in rheas was reported in only two previous studies [5,9]. However, molecular analysis was not performed in order to identify the protozoan, differently from our study where the presence of *C. parvum*, a zoonotic agent reported in several mammals was detected. Based on the literature, there are only a few descriptions of cryptosporidiosis in rheas, unlike of what happens in other ratites, as well as in ostriches. The incidence of cloacal prolapse in ostrich chicks was investigated, however the cause was not known, but the authors called attention to the presence of a *Cryptosporidium* in affected birds [2]. Studies have reported the occurrence of *Cryptosporidium* in ostriches confirmed by the molecular characterization of the parasite [8,11,12]. Recently, researchers evaluated ostriches in a farm in Central Vietnam, and confirmed a prevalence of 23.7% (110/464 positives) for *Cryptosporidium* spp. [8] and a prevalence of 11.7% in China (53/452) [12], being found only *Cryptosporidium baileyi*. Like ostriches, the rheas also can be parasitized by different species of *Cryptosporidium*. In this study, we could not perform the molecular characterization of the parasite, which must be done in a future research.

In the stool samples of adult rheas it was observed the presence of eggs of *Capillaria* spp., helminth from *Trichostrongylidae* family and oocysts of *Eimeria* spp. These and others parasites have been reported in a large study conducted in Europe parasitizing ostriches and rheas [9]. Another study conducted in Brazil reported the occurrence of seven species of nematodes, including a species of *Capillaria* [15], a parasite also found in the current study. Based on these results it is possible to conclude that *Rhea americana* may be parasitized by *Cryptosporidium parvum*. *Cryptosporidium* isolated from rheas was able to infect and replicate in mice, causing clinical signs of the disease and mortality. Prevalence studies should be conducted in this area to estimate the role and impact of rhea as reservoirs and disseminators of this zoonotic parasite.

**SOURCES AND MANUFACTURERS**

1. Agarose gel - Sigma-Aldrich, St. Louis, MO, USA.
2. Ethidium bromide - Sigma-Aldrich, St. Louis, MO, USA.
**Ethical approval.** The procedure was approved by the Animal Welfare Committee of Universidade do Estado de Santa Catarina (UDESC) [number 1.50.12].

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**REFERENCES**