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Parasites and Wildlifejournal homepage: www.elsevier.com/locate/ijppawA new coccidian, *Isospora rheae* sp. nov. (Apicomplexa, Eimeriidae),
from *Rhea americana* (Aves, Rheidae) from South AmericaSamira S.M. Gallo ^{a,*}, Nicole B. Ederli ^b, Bruno P. Berto ^c, Francisco C.R. de Oliveira ^a^a Laboratório de Sanidade Animal, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ 28013-600, Brazil^b Universidade Federal do Pará, Campus Universitário do Marajó-Breves, Av. Anajás, s/n, Bandeirantes, Breves, PA 68800-000, Brazil^c Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465 – Km 7, Seropédica, RJ 23851-970, Brazil

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ABSTRACT

A new species of coccidian (Protozoa: Apicomplexa: Eimeriidae) obtained from rheas, *Rhea americana*, is reported in Brazil. Oocysts of *Isospora rheae* sp. nov. are spherical to subspheroidal, measuring $22.6 \times 21.0 \mu\text{m}$, and have a double and smooth wall that is approximately $1.7 \mu\text{m}$ thick. The micropyle, oocyst residuum and polar granule are absent. Sporocysts are slightly ovoid, measuring $13.9 \times 9.6 \mu\text{m}$. The Stieda body is flattened, the substieda body is pointed, irregular and wavy and the sporocyst residuum is composed of scattered granules of varying sizes. Sporozoites have an oblong refractile body and one nucleus. This is the first description of an isosporid coccidian infecting birds of the family Rheidae.

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1. Introduction

Rheas are native birds from South America that live in fields and cerrado biomes, which are very common on this continent. Taxonomically, these birds are classified as the order Struthioniformes Latham, 1790, family Rheidae (Bonaparte, 1849) and genus *Rhea* (Brisson, 1760) (Dunning and Belton, 2004). The rhea is a long-legged, large bird, the largest bird from Brazil, and it belongs to the ratite group (flightless birds). As part of the Brazilian native wildlife, it is controlled and protected by the government, which defines and regulates the standards for its breeding and bans their hunting in national territory (Ludwig and Marques, 2008). However, unlike Darwin's rhea, *Rhea pennata* (Orbigny, 1834), which is considered an endangered bird and is present in the highlands (3500–4500 masl) and semi-arid regions from southern Peru and the forests and steppes of Argentina, the species *R. americana* is described by Tully and Shane (1996) as distributed worldwide in commercial flocks, including Brazil (Giannoni, 2004). With an increasing number of ratites being bred in captivity throughout Australia, Asia, Africa, Europe and North America (Tully and Shane, 1996), as well as in South America (Giannoni, 2004), the spread of infectious and parasitic diseases associated with these birds has also increased (Tully and Shane, 1996).

Infections with species of *Isospora* eventually kill host cells during protozoa development. As in other coccidiosis, there is a balance between the number of cells killed and their replacement. Animals do not show clinical signs when such a balance is favorable. However, coccidiosis can develop in some birds when a large amount of oocysts is ingested in a short time period (Atkinson et al., 2008).

Faust and Pappas (1977) recovered isosporid oocysts from the feces of an *R. americana* from the Columbus Zoo in Ohio. Reissig et al. (2001) found *Eimeria* spp. oocysts in 97 stool samples from *R. pennata* from Argentina. Ponce Gordo et al. (2002) identified coccidian oocysts in the feces of *R. americana* in Spain; however, the oocysts were not morphologically described, and no specific identification was given. The present study describes a species of isosporid coccidian infecting captive rheas *R. americana* in Brazil.

2. Materials and methods

Fresh fecal samples were collected before, during and after the reproductive period of six birds from a scientific breeding facility at the Universidade Estadual do Norte Fluminense Darcy Ribeiro, which is located in the city of Campos dos Goytacazes, Rio de Janeiro, Brazil. Samples were collected in individual plastic bags, which were identified, packed in ice boxes ($8\text{--}10^\circ\text{C}$) and immediately transported to laboratory located in the same university. The feces were mixed with a 2.5% potassium dichromate solution ($\text{K}_2\text{Cr}_2\text{O}_7$), filtered with double gauze and aerated with the aid of an aquarium pump coupled to plastic tubes to facilitate sporulation. Oocysts were

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recovered using the Sheather flotation method with sucrose solution and examined microscopically using the method described by Duszynski and Wilber (1997). For image capture, a digital camera (Canon PowerShot A640, USA) coupled to a binocular microscope (Carl Zeiss, Germany) was used. For oocyst measurements, the Zeiss Axion Vision Sample Images Software was used, with measurements given in micrometers (μm). Drawings were made by overlapping images in different focus with the aid of the software Corel Photo Paint version 11. Size ranges are given in parentheses, followed by the mean, standard deviation and morphometric ratio (length/width).

3. Results

Of the six examined rheas, five (four females and one male) shed oocysts in their feces during the reproductive period. Oocyst sporulation started at the tenth day after aeration, and at fifteenth day, 70% of the oocysts had sporulated. Higher sporulation rates were not observed by 20 days, which was the maximum period of observation. All observed oocysts were characteristic of *Isoospora* (Fig. 1), which is described and named as follows:

***Isoospora rhea* sp. nov.** (Figs. 1a–e, 2a,b).

Type host: *Rhea americana* (Aves, Struthioniformes, Rheidae).

Type specimens: Phototypes and line drawings of sporulated oocysts are deposited and available (<http://r1.ufrj.br/lcc>) in the Parasitology Collection of the Laboratório de Coccídios e Coccidioses, at UFRJ, located in Seropédica, Rio de Janeiro, Brazil. Photographs of the type-host specimens (sybiontypes) are deposited in the same collection. The repository number is P-56/2014.

Type locality: Scientific breeding of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, State of Rio de Janeiro, Brazil (21°45'16"S; 41°19'28"W).

Prevalence: 5/6 (83%).

Sporulation: 10–15 days.

Site of infection: Unknown.

Etymology: The species epithet is derived from the host specific name.

Description of sporulated oocysts: Oocyst shape ($n = 27$): spherical to subspheroidal; double-layered wall, $\sim 1.7 \pm 0.2$ (1.2–2.0) thick; smooth outer wall representing 2/3 of the total thickness; $L \times W$:

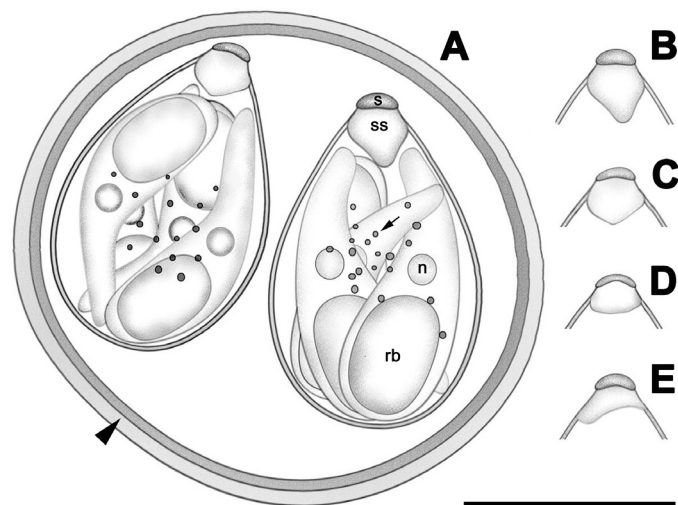


Fig. 1. Schematic drawing of *Isoospora rhea* sp. nov. (A) Sporulated oocyst containing two sporocysts each with four sporozoites; (B–E) variations in Stieda and substieda body. Arrowhead indicates double-layered wall; (S) Stieda body; (SS) substieda body; (n) nucleus; (rb) refractile body and arrow indicates residual granules of sporocyst. Bar: 10 μm .

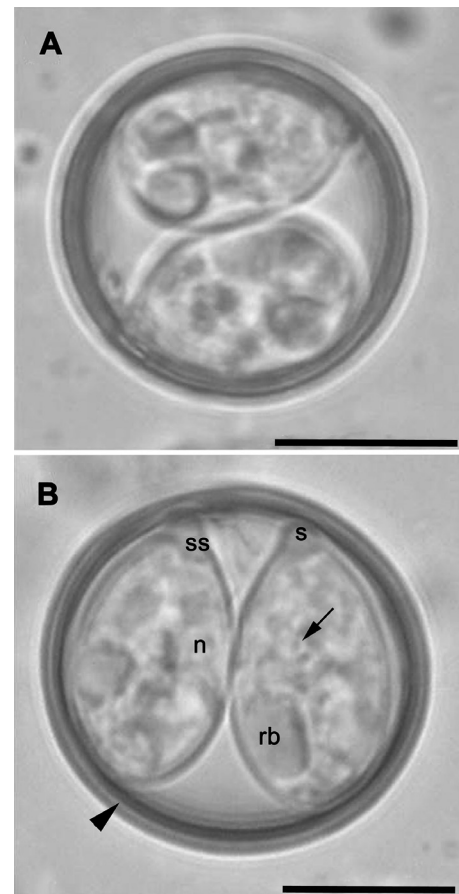


Fig. 2. Optical micrograph of *Isoospora rhea* sp. nov. sporulated oocyst (A and B). Arrowhead indicates double-layered wall; (S) Stieda body; (SS) substieda body; (n) nucleus; (rb) refractile body and arrow indicate residual granules of sporocyst. Bar: 10 μm .

22.6 ± 3.0 (16.6–27.3) \times 21.0 ± 2.7 (15.9–26.7), L/W : 1.0 ± 0.05 (1.0–1.2); micropyle, oocyst residuum and polar granule absent (Figs. 1 and 2).

Description of sporocyst and sporozoites: Sporocyst shape ($n = 18$): slightly ovoid; $L \times W$: 13.9 ± 1.8 (10.8–18.9) \times 9.6 ± 0.9 (7.6–11.4); L/W : 1.4 ± 0.1 (1.3–1.7); Stieda body present, flattened, $L \times W$: 0.8 ± 0.2 (0.5–1.1) \times 1.3 ± 0.2 (0.9–1.6), substieda body present, pointed, irregular and wavy, $L \times W$: 1.2 ± 0.2 (0.9–1.5) \times 2.1 ± 0.3 (1.3–2.6); parastieda body absent; sporocyst residuum formed by scattered granules of different sizes; sporozoites claviform, with an oblong refractile body and one nucleus (Fig. 2).

4. Discussion

Due to the lack of descriptions of coccidian parasites in birds from the family Rheidae, *I. rhea* sp. nov. was compared with the single species of *Isoospora* described from ratites: *I. struthionis* from ostriches, *Struthio camelus* Linnaeus, 1758 (Struthioniformes, Struthionidae), as suggested by Duszynski and Wilber (1997) for description of a new species. The highest prevalence of oocysts in females can be explained by the low immune system during the stance being more susceptible to diseases.

Oocysts of *I. rhea* sp. nov. obtained from rhea feces in the present study had a spherical to subspheroidal shape with double wall, whereas the oocysts collected from ostriches by Yakimoff (1940) and named *I. struthionis* are described as spheroidal with a double oocyst wall. *Isoospora* from rheas have no polar granule, unlike those oocysts

from ostriches that have double polar granule. Sporocysts from rhea coccidian are described as having an ovoid shape, with a flattened Stieda body, a prominent or wavy pointed substieda body and a residuum body with scattered granules, and as varying in size, whereas the sporocysts from the ostrich *Isospora* had a lemon shape, a pointed Stieda body and lacked a substieda body and residual granules. Sporozoites from the rhea *Isospora* present an oblong refractile body and one nucleus, whereas *I. struthionis* sporozoites were not characterized.

Faust and Pappas (1977) found oocysts of *Isospora* sp. in a female *R. americana* from the Columbus Zoo, Ohio, United States, which sporulated at about 2 days at room temperature. This differs from the time of sporulation observed in our study.

The mean diameters of oocysts and sporocysts measured from *I. rhae* sp. nov. in the present study are smaller than the diameter of *I. struthionis* from ostriches cited by Yakimoff (1940) (oocysts 30.6 µm, sporocysts 18 × 10.8 µm). However, the coccidian oocysts shed in the feces of rheas observed in this study are larger than the measurements made by Ponce Gordo et al. (2002) (12–15 µm) from rheas in Europe. These researchers did not cite the measured species, most likely because the oocysts were measured when nonsporulated.

Based on the differences in the morphological and morphometric characteristics mentioned in this study, *I. rhae* is considered a new species. Moreover, it is the first isosporan reported in the rhea *R. americana*.

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