Endoparasites and ectoparasites of rheas (*Rhea americana*) from South America

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**Abstract.** The aim of the present study was to identify the endoparasites and ectoparasites found in *Rhea americana* in captivity in Brazil. Faecal samples of seven adult rheas were collected and evaluated using the Sheather technique and samples positive for oocysts were submitted to sporulation. Molecular analysis was also performed for diagnosis of the genera *Cryptosporidium*, *Giardia* and *Entamoeba*. Feathers and skin of rheas were analysed for ectoparasites. Eggs of *Capillaria* sp., *Procyrnea* sp. and *Procyrnea* sp.-type and other nematode eggs of the Strongylida order as well as cysts of *Entamoeba* sp. and oocysts of *Isospora rheae* and *Eimeria* sp. were found in the faeces. Six faecal samples (85.7%) were diagnosed as positive for *Entamoeba* by PCR, and no positive samples for *Cryptosporidium* or *Giardia* were detected. Specific malophagous lice classified as *Struthiolipeurus nandu* were found distributed throughout the animals’ bodies. It was concluded that rheas of the present study were infected enzootically by nematode and protozoan species in addition to being infested with lice.

**INTRODUCTION**

Rheas are birds native to South America that live in fields and Brazilian savanna vegetation (cerrado), which are very common on this continent. Taxonomically, these birds are classified as belonging to the order Struthioniformes Latham, 1790, family Rheidae Bonaparte, 1849 and genus *Rhea* Brisson, 1760 (Dunning & Belton, 1993). The rhea is a long-legged, large bird, the largest bird in 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 & Marques, 2008). Knowledge about parasitism in these animals is desirable for their management under natural conditions and reproduction in captivity. Additionally, descriptions of the parasites and parasitic diseases in free-living and captive animals may help in the evaluation of the importance of host-parasite relationships in each environment (Zettermann et al., 2005).

The first and necessary step before implementing appropriate control measures is knowing which parasites can be found in ratites. In general, little is known about parasites in these birds, not only in their original distribution areas but also in importing countries (Ponce Gordo et al., 2002). Few works about endoparasites of greater rheas have been published in the last 20 years (Martínez-Díaz et al., 2013), and little is known about diseases that can be caused by ectoparasites in ratites, which in part explains the small number of studies related to this topic. Together with feather mites (Acari: Acaridida), Malophaga are the ectoparasites most frequently found in wild
birds, and few studies have emphasized these ratite parasites (Freitas et al., 2002; Silva et al., 2004). The aim of this study was to contribute to the knowledge of ratite parasites by describing endoparasites and ectoparasites found among rheas (*Rhea americana*) in captivity in Brazil.

**MATERIAL AND METHODS**

The analysed biological material consisted of faecal samples and feathers of seven adult rheas (*R. americana*) (four females and three males) belonging to a scientific breeding program at the Universidade Estadual do Norte Fluminense (UENF), located in Campos dos Goytacazes city, Rio de Janeiro state, Brazil. Fresh faeces were collected five times with a two-day interval between collections, stored in labelled plastic bags, placed in isothermal boxes (8-10°C) and immediately transported to the Núcleo de Pesquisas Avançadas em Parasitologia (NUPAP) located at the same university. Special care was taken when sampling to avoid faecal contamination by the soil. For each sample, a flotation technique was used in a sucrose-saturated solution (Sheather, 1923), and the slides were analysed under an optical microscope (objective 40× and 100×). Samples positive for oocysts were mixed with a 2.5% potassium dichromate solution (K$_2$Cr$_2$O$_7$), passed through double gauze and aerated with an aquarium pump coupled to hoses to facilitate sporulation, and they were subsequently examined microscopically. For image capture, a digital camera (Canon PowerShot A640, USA) coupled to a binocular microscope (Carl Zeiss, Germany) was used, and for cyst, oocyst and egg measurements, the Zeiss AxioVision Sample Images Software was used, with measurements given in micrometres (µm).

For the molecular analysis, a pool of faeces from each animal was made and the samples were processed by centrifugation with sucrose to concentrate and purify cysts according to Fiuza et al. (2008). For DNA extraction, a DNeasy kit (Qiagen, Valencia, California) was used with reagents provided by the manufacturer. Modifications of the protocol included overnight incubation with proteinase K and the elution step was made with the volume recommended by the manufacturer. For *Cryptosporidium* spp. and *Giardia* spp., the nested PCR technique was used with two-step amplification of an 18S rRNA gene fragment, and for *Entamoeba* spp., only one amplification of the same fragment was conducted. The primer sequences used are shown in Table 1. The PCR products were analysed using electrophoresis in 1% agarose gel stained with GelRed and immersed in 1X TAE buffer in a horizontal chamber. Bands were visualized using the Gel Logic 6000 PRO imaging system (Carestream®, USA) after the electrophoretic run, and the fragment sizes were compared with Low DNA Mass Ladder marker (Invitrogen®) and with positive and negative controls already existing in the laboratory. One positive amplicon of an *Entamoeba* PCR product was selected for sequencing based on the PCR product visualization. That amplicon was purified using a QIAquick PCR purification kit (QIagen, UK) according to the manufacturer’s instructions and then subjected to sequencing using the same primers from the PCR. The obtained nucleotide sequence was deposited in GenBank® databased and submitted to Basic Local Alignment Search Tool (BLAST) analysis to identify similarities with sequences in GenBank® (Altschul et al., 1997).

After the birds were contained, their feathers and skin were analysed, and specimens were placed in bottles with 70% alcohol for later observation at the NUPAP. For optical microscopy, the lice were placed in 10% KOH, dehydrated in a series of alcohol solutions, diaphanized in Faya’s creosote, mounted on permanent slides with Damar gum (Palma, 1978) and analysed using the same microscope as previously described. Images were captured using a digital camera (Canon PowerShot A640, USA) coupled to a stereoscope microscope (TIM-2T OPTON, Brazil).
Table 1. Sequences of specific primers for protozoa of pathogenic and zoonotic potential and their amplified fragment sizes

<table>
<thead>
<tr>
<th>Protozoa</th>
<th>Primers</th>
<th>Sequences</th>
<th>Fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium spp.</td>
<td>1st PCR</td>
<td>CRYPTOF 5'-TTCTAGAGCTAATAACATGCG-3'1</td>
<td>~1300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRYPTOR 5'-CCCATTTCCTCTACAAACAGGA-3'1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd PCR</td>
<td>AL3032 5'-GGAAGGGTTGTATTTAGATAAAAG-3'1</td>
<td>~800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AL1598 5'-AAGGAGTAAGGAAACCTTCCA-3'1</td>
<td></td>
</tr>
<tr>
<td>Giardia spp.</td>
<td>1st PCR</td>
<td>GiaF 5'-AAGTGTGGTGCAGACGGACTC-3'2</td>
<td>~500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GiaR 5'-CTGCTGCCGTCCTTGATGT-3'3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd PCR</td>
<td>RH11 5'-CATCCGGTCGATCCTGCC-3'3</td>
<td>~300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RH4 5'-AGTCGAAACCTCATTCTCCGACCC-3'3</td>
<td></td>
</tr>
<tr>
<td>Entamoeba spp.</td>
<td>PCR</td>
<td>ENTAM1 5'-GTTGATCCTGCCAGTATTATG-3'4</td>
<td>~550</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ENTAM2 5'-CAGCTTGGAGCTGAAATTAC-3'4</td>
<td></td>
</tr>
</tbody>
</table>

Xiao et al., 1999; 2Appelbee et al., 2003; 3Hopkins et al., 1997; 4Sukprasert et al., 2008.

RESULTS

The analysis of rhea faeces revealed the presence of the cysts and oocysts of parasitic protozoa and nematode eggs. The parasite most frequently found in the bird faeces was Entamoeba sp. (Fig. 1a), with cysts observed in all the samples collected, which averaged 41.0 µm and 37.7 µm for the largest and smallest diameter, respectively (Table 2). Of the seven birds with mononuclear cystic forms, the presence of Entamoeba was confirmed in six using PCR, and the sequencing from one of the samples was deposited at GenBank under accession number KY286575. Among the seven fecal samples examined using nested PCR from the rheas in our study, none were positive for Cryptosporidium and Giardia.

Oocysts of Isospora rheae (Fig. 1b; 1c), with the larger and smaller diameters measuring 23.6 µm and 22.3 µm, respectively (Table 3), were observed (Fig. 1b; 1c) during the reproductive period in the faeces of five rheas, four females and one male. No oocysts were found in the faeces of two other males in all samplings. Oocysts of Eimeria sp. (Fig. 1d) were also observed in one of the breeding animals in all samplings, and for both Eimeria and Isospora oocysts, the sporulation period lasted for 10 to 15 days.

Helminth eggs identified as Strongylida-type (Fig. 1e), Procyrnea sp. (Fig. 1f), Procyrnea sp.-type (Fig. 1g) and Capillaria sp. (Fig. 1h) were also found in the faeces of rheas, and mean, standard deviation, minimum, maximum, and morphometric index values for the length and width of eggs are provided in Table 2.

The lice found in the analysed rheas (Fig. 2) are ratite-specific malophagous lice, and based on the identification key provided by Mey (1998), specimens were identified as belonging to the species Struthiolipeurus nandu Eichler, 1950, a chewing louse of the Ischnocera suborder and Philopteridae family, whose dark ventral spots from the second to the seventh abdominal segment of the female and male genital organs were observed as taxonomic characteristics of the species. Adults (Fig. 2a, b, c, d) and nymphs (Fig. 2e, f, g, h) of both sexes were found distributed throughout the host’s body, with a greater concentration in the wings.

DISCUSSION

Low number of parasitic forms was observed in the rhea faeces, which can be related with a low parasite load due to the good hygienic conditions of the installations. However, the
Figure 1. Cyst and oocysts of protozoa and nematode eggs found in faecal samples of rheas, *Rhea americana*. (a) *Entamoeba suis* cyst with a well visible nucleus (Bar: 10 µm, 1000x), (b) Non sporulated oocyst (Bar: 15 µm, 1000x), (c) Sporulated oocyst of *Isospora rheae* (Bar 10 µm, 1000x), (d) Similar forms to *Eimeria* sp. with four sporocysts visible inside the oocyst (Bar: 10 µm, 400x), (e) Strongyliida-type egg (Bar: 20 µm, 1000x), (f) *Procyrnea* sp. egg (Bar: 20 µm, 1000x), (g) Non-larval egg similar to *Procyrnea* sp. (Bar: 10 µm, 1000x), (h) *Capillaria* sp. egg (Bar: 20 µm, 400x).
Table 2. Morphometry in micrometres of *Entamoeba* cysts and nematodes eggs isolated in faeces of rhea, *Rhea americana*

<table>
<thead>
<tr>
<th>Cysts and Eggs</th>
<th>n¹</th>
<th>Larger diameter²</th>
<th>Minor diameter²</th>
<th>Morphometric Index²</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Entamoeba</em> sp.</td>
<td>13</td>
<td>41.0±7.8 (30.5-57.3)</td>
<td>37.7±5.5 (29.4-46.8)</td>
<td>0.9±0.06 (0.8-1.0)</td>
</tr>
<tr>
<td>Strongylida</td>
<td>12</td>
<td>119.6±11.2 (96.0-140.7)</td>
<td>69.6±5.8 (55.5-77.9)</td>
<td>0.6±0.03 (0.5-0.6)</td>
</tr>
<tr>
<td>Capillaria sp.</td>
<td>29</td>
<td>30.6±8.7 (16.4-48.2)</td>
<td>16.8±4.2 (9.0-24.0)</td>
<td>0.6±0.06 (0.5-0.7)</td>
</tr>
<tr>
<td>Procyrene sp.</td>
<td>17</td>
<td>44.1±2.9 (38.2-49.5)</td>
<td>26.1±2.7 (22.6-33.0)</td>
<td>0.6±0.06 (0.5-0.7)</td>
</tr>
</tbody>
</table>

¹Number of cysts and eggs measured.
²Media; standard deviation; larger and minor observed measures.

Table 3. Morphometry in micrometres of *Isospora rheae* oocysts isolated in faeces of rhea *Rhea americana*

<table>
<thead>
<tr>
<th>Bird</th>
<th>n¹</th>
<th>Wall²</th>
<th>DIAMETER²</th>
<th>Morphometric Index²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Larger</td>
<td>Minor</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1.7±0.2 (1.2-2.1)</td>
<td>27.3±2.1 (23.0-34.8)</td>
<td>25.8±2.5 (20.8-34.6)</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>1.6±0.1 (1.3-2.1)</td>
<td>24.5±1.1 (18.9-29.4)</td>
<td>22.5±0.9 (18.0-27.2)</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>1.5±0.02 (1.1-1.9)</td>
<td>23.5±0.4 (18.7-29.7)</td>
<td>22.5±0.4 (17.3-28.8)</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>1.6±0.04 (1.2-2.1)</td>
<td>22.7±0.5 (16.4-34.5)</td>
<td>21.5±0.5 (15.5-31.0)</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>1.6±0.08 (1.5-1.9)</td>
<td>25.3±1.0 (21.5-27.6)</td>
<td>23.0±1.5 (18.7-26.3)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>1.6±0.02 (1.1-2.1)</td>
<td>23.6±0.3 (16.4-34.8)</td>
<td>22.3±0.3 (15.5-34.7)</td>
</tr>
</tbody>
</table>

¹Number of oocysts measured.
²Media; standard deviation; larger and minor observed measures.

diversity of species present in their faeces can be explained by the fact that rheas have a habit of feeding on almost everything that is available because of their poorly developed palate and habit of directly swallowing food (Giannoni, 2004). This study also indicates that it is a common habit of this bird to feed its own faeces (coprophagia) and sometimes the faeces of other animals. Although parasitic infection of ratites mostly occurs directly, through ingestion of cysts, oocysts, eggs and infecting larvae, that may be present in intermediate hosts, usually insects, that are also ingested directly or are present in foliage, faeces or any other matter with which birds come into contact, causing a greater risk of infection by parasites. Thus, the management type and breeding system adopted have a direct influence on the levels of parasitic infections of the gastrointestinal tract (Giannoni, 2004), which may explain the low number of parasite forms observed. Some studies have shown that ratites are very vulnerable to several types of parasites, especially those infecting the gastrointestinal tract (Oliveira *et al.*, 2009), since other organs do not seem to exhibit significant rates of parasitism (Ponce Gordo *et al.*, 2002; Foreyt, 2005). These authors also report that even in large numbers, the presence of parasites in these birds may not be accompanied by characteristic clinical signs, which makes the analysis of faecal samples even more interesting and important.

*Entamoeba* cysts with a mean and standard deviation of 13.5±2 µm were observed in ostriches from Spain (Martínez-Díaz *et al.*, 2000). Sotiraki *et al.* (2001) and Pennycott & Patterson (2001) also observed cysts with one nucleus that were 10-15 µm diameter in ostriches from Europe. Subsequently, Ponce Gordo *et al.* (2004) also observed uninucleated cysts in ratites, that measured 8 to 20 µm in diameter, proposing...
a new species that was designated *E. struthionis* (Ponce Gordo et al., 2004). The values found in the present study were higher than those found for the *E. bovis* group described by the previously cited authors, and this may be considered a peculiar morphological differential characteristic. Martínez-Díaz et al. (2013) observed mononuclear cystic forms in faecal samples from *R. americana* chicks on farms in Argentina with eight nuclei in adult rheas (*Rhea pennata*), which were characterized as *E. bovis*-like and *Entamoeba coli*-like, respectively. In our study, only mononuclear forms (Fig. 1a) of *Entamoeba* sp. were observed; however, the identification of species of the genus *Entamoeba* or even species of other genera, such as *Iodamoeba* and *Endolimax*, cannot be reliably conducted using only morphological characters (Ravdin, 1994; Haque et al., 1997; Verweij et al., 2003). The sequencing of the PCR products from one of the samples showed that the *Entamoeba* species (KY286575) found in the rheas in the present study had 99% identity with *Entamoeba suis* (FR686456) found in non-human primate (*Gorilla gorilla*) faeces from a zoo in the United Kingdom (Stensvold et al., 2011) and 98% identity with *E. suis* found in Vietnamese pigs (Clark et al., 2006). Therefore, it can be stated that, in addition to the *E. struthionis* species isolated from ostrich faeces from six different breeding sites in Spain (Ponce Gordo et al., 2004), ratites may harbour another uninucleate *Entamoeba* species. This shows that *E. suis* is not restricted to swine and gorillas, as it can also infect birds, with this being the

Figure 2. *Struthiolipeurus nandu* lice. In a and b is observed dorsal and ventral surface of adult female and in c and d, dorsal and ventral surface of adult male. Bars: 1000 µm. In e and f, dorsal and ventral surface of female nymph and in g and h, dorsal and ventral surface of male nymph. Bars: 500 µm.
first report of *E. suis*-like in rheas (*R. americana*). Several protozoa have been described in ratite faeces, including *Cryptosporidium* spp., oocysts compatible with *Isospora* spp. and *Eimeria* spp., *Balantidium struthionis*, *Entamoeba* spp., *Endolimax* sp., *Iodamoeba* sp., *Histomonas meleagridis*, *Monocercomonas* sp., *Tetra-trichomonas gallinarum*, *Trichomonas gallinae*, *Giardia* spp., *Spironucleus meleagridis*, *Chilomastix gallinarum*, *Retortamonas* sp., *Pleuromonas jaculans*, *Euglenoidea* and *Blastocystis* sp. (Ponce Gordo et al., 2002). However, almost none of these was molecularly confirmed in the observed species.

There are reports in the literature regarding the presence of *Cryptosporidium* in rhea and ostrich faeces (Ponce Gordo et al., 2002; Penrith & Burger, 1993; Meireles et al., 2006). *Cryptosporidium* was implicated as the cause of phallic and cloacal prolapse (Bezuidenhout et al., 1993; Penrith & Burger, 1993; Penrith et al., 1994), enteritis (Huchzemeyer, 1999), and pancreatic necrosis (Jardine & Verwoerd, 1997). Although negative results were obtained for *Cryptosporidium* by both the microscopic and molecular analyses, one of the rheas in the study underwent cloacal prolapse, suggesting that this parasite is not related to cloacal prolapse in this particular case. *Giardia* was found by Ponce Gordo et al. (2002) in rheas born and raised in Europe.

From economic and sanitary points of view, the coccidia group is the most important among protozoa. The higher occurrence of oocysts in females may be explained by their low immune system during the reproductive period, which makes them more susceptible to disease. The oocysts of *Eimeria* sp. found in the present study were not measured because of the large amount of faecal debris. Oocysts of *Eimeria* sp. observed in rheas (*R. pennatana*) in Argentina had a mean larger diameter of 26 µm and a mean minor diameter of 23.7 µm (Reissig et al., 2001); however, these researchers did not report whether the measured oocysts had sporulated and did not morphologically describe the observed coccidia, which makes it impossible to compare, since oocysts cannot be diagnosed when they have not sporulated.

There are a few reports regarding the presence of *Isospora* and *Eimeria* genera in ratites, and the specimens are designated *Isospora* sp. (Jansson & Christensson, 2000; Reissig et al., 2001) or *Eimeria* sp. (Sotiraki et al., 2001; Reissig et al., 2001) in most of studies. In 1940, the first *Isospora* species in ratites was described, *Isospora struthionis*, which was found in an ostrich from a Russian zoo (Yakimoff, 1940). In 2014, we described *Isospora rheae*, a new and unique species found in rheas (*R. americana*) (Gallo et al., 2014), and in the same year, *Isospora dromaii*, isolated from emus (*Dromaius novaehollandiae*), was described, with this being the first report of an *Isospora* species in emus (Teixeira et al., 2014). The scarcity of reports on the morphology of these coccidia can be justified by the delay in the sporulation of isolated oocysts, which makes their identification difficult.

Coccidia oocysts were found in the faeces of practically all birds analysed, and all animals were apparently healthy, since no clinical disease or pathology was observed during the collection period except the cloacal prolapse observed in a bird that was free of coccidian parasites which shows that the pathogenicity of coccidia found in adult rheas requires experimental investigations for evaluation of the possible damage caused to this bird. In general, coccidia show variation in pathogenicity in most hosts (Soulsby, 1986), and failure to observe clinical signs may have been because low values were obtained in the oocyst counts, since clinical signs are generally observed in association with high oocyst counts of mainly pathogenic strains (Reissig et al., 2001). Another hypothesis is attributed to the nonpathogenic character of the species observed. According to Mushi et al. (1998), young ostriches have a higher proportion of apparent infection and, similarly, the oocyst count was higher in young birds, with more characteristic signs of disease being observed in this age group. In agreement with these authors, the adult rheas in our study may have developed
resistance to the identified coccidia in addition to eliminating a higher quantity of oocysts in the first infection.

The Strongylida-type eggs in the faeces of rheas (Fig. 1e) had a mean diameter (119.6 µm) that was smaller than those described for *Deletrourecephalus dimidiatus* (150-160 µm) (Vaz, 1936; Taylor et al., 2000) and *Deletrourecephalus cesarpintoi* (157 µm) (Avelar et al., 2014) found in *R. americana* from Minas Gerais state and considerably lower than the larger diameter of Strongylida-like eggs found in *R. americana* from Argentina (Martínez-Díaz, 2013) and the eggs of *Paradiselurocephalus minor* (190-200 µm) described by Acomolli et al. (2006). In addition, it was observed that the mean of the minor diameter of the eggs (69.6 µm) was slightly smaller than that of *D. cesarpintoi* eggs (81 µm) (Avelar et al., 2014) and similar to the minor diameter of *D. dimidiatus* eggs (70 µm) (Vaz, 1936; Taylor et al., 2000), Strongylida-type eggs observed by Martínez-Díaz (2013) in *R. americana* (65 µm) and eggs of *P. minor* (60-70 µm) (Acomolli et al., 2006). According to these data, mainly based on the larger diameter of the Strongylida-type eggs in rheas (Table 2), it can be inferred that these are from *Deletrourecephalus* sp. The most frequent intestinal nematode in *R. americana* is *D. dimidiatus* (Zettermann et al., 2005), which is considered to be of high importance because, at high levels of infection, it is responsible for the appearance of anemia in rheas due to its habit of feeding on host blood (Craig and Diamond, 1996). However, the eggs of *D. dimidiatus* and *D. cesarpintoi* cannot be differentiated based only on morphological and morphometric measurements.

Eggs similar to those described by Ederli (2012) are small eggs, with a thick and smooth wall, and containing a larva with a small operculum on both sides were observed in the faeces of the investigated birds. This study observed that these eggs inside *Procyrnea uncinipenis* females had parasitized the gizzard of a necropsied rhea. In addition to the morphological similarity, the mean measurements of the largest and smallest diameter (Table 2) were very similar to those of the eggs measured by Ederli (2012), which had a larger diameter of 44.6±3.2 (35.5-50.7) µm and a minor diameter of 25.6±1.9 (20.8-31.6) µm, thus confirming the diagnosis of the presence of *P. uncinipenis* eggs in the faeces of rheas. Similar measurements were also cited by other authors for eggs similar to those of *Procyrnea* sp. (Vaz, 1936; Freitas & Lent, 1947, Avelar et al., 2014).

Non-larval eggs of the same size and shape were found in the faeces of rheas and characterized as similar to those of *Procyrnea* sp. (Fig. 1g), which were likely expelled unfertilized, as the formation of larvae still occurs inside nematode uterus in this species, as described by Ederli (2012). This highlights the hypothesis of infertile egg elimination with the low parasitic forms observed in the performed examinations, which may be influencing the ability of males to fertilize the large number of eggs produced by females, as observed by Ederli (2012).

Eggs of helminths identified as *Capillaria* sp. (Fig. 1h), with a mean larger diameter of 30.6 µm and a mean minor diameter of 16.8 µm, were observed in the faeces of rheas. In ratites, the species *Capillaria parvumspinosa* has been described in rheas from Europe (Yamagüti, 1961) and the species *Capillaria venteli* was found in free-living *Rhea americana* from Brazil (Zettermann et al., 2005). Eggs identified as *Capillaria* sp. were found in *R. pennata* by Reissig et al. (2001), in *R. americana* by Uhart et al. (2006), in samples from *D. novaehollandiae* by Jansson and Christensson (2000) and in the faeces of *Struthio camelus* by Ponce Gordo et al. (2002). However, species of the *Capillaria* genus cannot be identified by egg morphology (Yabsley, 2008); therefore, it is preferential to identify this egg as belonging to a nematode of the subfamily Capillarioidea until a diagnosis based on the morphology of the infecting larvae of this parasite or a molecular analysis can be conducted.

Chewing lice and mites are widely found in wild birds (Valim et al., 2005), but few papers have been published on this subject, especially in regard to rheas. These
arthropods are common in rheas and their transmission among birds occurs through direct contact between animals. According to some authors (Hoover et al., 1988; Huchzermeyer, 1999), although these ectoparasites are generally harmless, they can cause the loss of feathers and intense itching, driving the rheas to stop feeding. In addition, lice can compromise weight gain in young birds and lead to death when over infested. In the rheas in the present study, it was observed that the feathers were brittle and lacklustre, which highlights the importance of the control of these ectoparasites, since feathers are responsible for the retention of temperature, acting in protection and thermal insulation in addition to their economic potential.

CONCLUSION

Rheas, *R. americana*, of South America were enzootically parasitized by nematode species of the *Capillaria* and *Procyrnea* genera and others of the Strongylida order in addition to coccidia, such as *Isospora rheae* and members of the *Eimeria* genus. In addition, rheas can be considered a new host for *E. suis* -like, being this the first report of these protozoans parasitizing these birds. In addition to the endoparasites, we highlight the potential pathogenic effect of *S. nandu* lice, which can parasitize these birds, damaging their growth and generating economic losses in commercial breeding. More detailed analyses are needed to determine not only the host’s specific conditions but also the risk of infection for other domestic and wild animals as well as humans.

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