Gastrointestinal parasites of a population of emus (Dromaius novaehollandiae) in Brazil

S. S. M. Gallo a, C. S. Teixeira a, N. B. Ederli b and F. C. R. Oliveira a*

a Laboratório de Sanidade Animal – LSA, Centro de Ciências e Tecnologias Agropecuárias – CCTA, Universidade Estadual do Norte Fluminense – UENF, CEP 28035-302, Campos dos Goytacazes, RJ, Brasil

b Instituto do Noroeste Fluminense de Educação Superior – INFES, Universidade Federal Fluminense – UFF, 28470-000, Santo Antônio de Pádua, RJ, Brasil

*e-mail: oliveirafcr@gmail.com

Received: January 9, 2018 – Accepted: June 19, 2018 – Distributed: February 28, 2020

(With 2 figures)

Abstract
Emus are large flightless birds in the ratite group and are native to Australia. Since the mid-1980s, there has been increased interest in the captive breeding of emus for the production of leather, meat and oil. The aim of this study was to identify gastrointestinal parasites in the feces of emus Dromaius novaehollandiae from a South American scientific breeding. Fecal samples collected from 13 birds were examined by direct smears, both with and without centrifugation, as well as by the fecal flotation technique using Sheather’s sugar solution. Trophozoites, cysts and oocysts of protozoa and nematode eggs were morphologically and morphometrically evaluated. Molecular analysis using PCR assays with specific primers for the genera Entamoeba, Giardia and Cryptosporidium were performed. Trophozoites and cysts of Entamoeba spp. and Giardia spp., oocysts of Eimeria spp. and Isospora dromaii, as well as eggs belonging to the Ascaridida order were found in the feces. Three animals were diagnosed with Giardia spp., and three were positive for Entamoeba spp. based on PCR techniques. After analyzing the data, we concluded that emus were infected enzootically by nematode and protozoan species.

Keywords: coccidia, helminth, protozoan, ratite.

1. Introduction
The emu Dromaius novaehollandiae is the largest bird native to Australia and the only living member of the genus Dromaius. It is the second-largest bird in the world by height, after its ratite relative, the ostrich Struthio camelus. The emu inhabits most areas of mainland Australia, with the exceptions of heavily populated areas, dense forests and arid areas (Davies, 1976).

In birds, protozoa can be found in the digestive tract (including the oropharynx, intestines and cloaca), where they are an important cause of disease, as well as in the...
bloodstream and internal organs (Silvanose et al., 1998). Protozoa commonly found in birds include *Eimeria* spp., *Isospora* spp., *Sarcocystis* spp., *Cryptosporidium* spp., *Giardia* spp., *Trichomonas* spp., *Histomonas* spp., *Hexamita* spp. and *Toxoplasma gondii* (Greiner and Ritchie, 1994; Martínez-Diaz et al., 2013; Gallo et al., 2014).

There is a dearth of studies on the parasites of ratites. In many cases, parasites are implicated as the cause of illness, but there are no studies that have demonstrated the presence of these parasites as a cause of pathology in emus. Often, disease is related to low levels of immunity, mixed infections, stress and malnutrition, but these animals commonly remain asymptomatic (Craig and Diamond, 1996).

The goal of the present study was to identify gastrointestinal parasites present in captive emus (*Dromaius novaehollandiae*) in Campos dos Goytacazes municipality, Brazil.

### 2. Material and Methods

Fresh feces were collected from 13 emus *Dromaius novaehollandiae* of both sexes belonging to a scientific breeding program of the Universidade Estadual do Norte Fluminense, Campos dos Goytacazes city, Rio de Janeiro state, Brazil. Samples were collected four times from each animal with intervals of three days between each collection. All animals were numbered to guarantee samples from all birds. The samples were identified, placed in isothermal containers on ice and later processed at the Núcleo de Pesquisas Avançadas em Parasitologia of the Veterinary Hospital of the Universidade Estadual do Norte Fluminense. For the morphological identification of trophozoites, cysts, oocysts and protozoal eggs in the feces of emus, centrifugation fecal flotation in Sheather’s sugar solution as well as in direct smears, in both stained and unstained samples. Using centrifugation fecal flotation, sporulated and non-sporulated oocysts of coccidia of the *Eimeria* genus (Figure 2C) and *Isospora dromaii* (Figure 2D) were observed and identified, as were eggs of the Ascaridida order (Figure 2E-F).

### 3. Results

Trophozoites and cysts of *Entamoeba* spp. (Figure 1A-F) and *Giardia* spp. (Figure 2A-B) were observed in the fecal samples analyzed with centrifugation with Sheather’s sugar solution as well as in direct smears, in both stained and unstained samples. Using centrifugation fecal flotation, sporulated and non-sporulated oocysts of coccidia of the *Eimeria* genus (Figure 2C) and *Isospora dromaii* (Figure 2D) were observed and identified, as were eggs of the *Ascaridida* order (Figure 2E-F).

The trophozoites of *Entamoeba* sp. presented on average 44.8±6.3 μm and 29.4±7.6 μm (n=3) of larger and smaller diameter. Uninucleate and multinucleate cysts (with at least six nuclei) measured on average 27.7±9.6 and 25.6±8.9 μm (n=11) of larger and smaller diameter. Two cysts of *Giardia* spp. were observed in the feces of emus by the centrifugation fecal flotation technique measuring 11.7 × 9.8 μm and 10.8 × 10.1 μm, in addition to a trophozoite with a diameter of 14.6 × 12.2 μm. The mean values of the largest and smallest diameters of the *Eimeria* spp. oocysts observed were 16.5 ± 2.4 μm and 16.0 ± 2.6 μm (n = 3), respectively. Oocysts of *Isospora dromaii*, averaging 21.6 ± 2.9 μm for the larger diameter and 19.8 ± 2.7 μm (n = 62) for the smaller diameter, were observed in the feces of the emus. In addition, several sporulated oocysts of *Eimeria* spp., characterized as ovoid to ellipsoid and double walled with rounded sporocysts, were observed, although other details, such as the sporozoite shape and absence or presence of Stieda and Substieda...
Endoparasites in *Dromaius novaehollandiae*

Figure 1. Photomicroscopy of *Entamoeba* sp. observed in emu feces, *Dromaius novaehollandiae*. In A and B, characteristic trophozoites with hyaline projections of the ectoplasm and very visible nucleus. In C cyst with a very visible nucleus and in D multinucleated cyst. E and F shows uninucleated and multinucleated cysts respectively stained by 2% Lugol. Bars: 20 μm.

bodies, could not be ascertained. The sporulation time of both *Isospora* and *Eimeria* oocysts was 15-25 days at room temperature with forced aeration.

Fertile and infertile Ascaridia-type eggs found in the feces of the emus had larger and smaller mean diameter of 89.2±34.7 μm and 79.0±35.1 μm (n=9). The PCR technique did not demonstrate the presence of *Cryptosporidium* spp. in the analyzed feces of the emus, but three animals were diagnosed with *Giardia* spp., and three were positive for *Entamoeba* spp.
4. Discussion

The prevalence of gastrointestinal parasites can be high in places where living conditions and basic sanitation are minimal or nonexistent. Many parasites, such as protozoa of the genera *Entamoeba* and *Giardia*, helminths of the genus *Ascaris* and *Trichuris*, and many species of cestodes are transmitted through contaminated water or food, while other helminths are transmitted by larvae that are present in the soil (Neves, 2005).

In a study from Greece (Sotiraki et al., 2001), small diameter (10-15 μm) uninucleate cysts of *Entamoeba* spp. were identified in the feces of ostriches; these cysts were much smaller than the uninucleate and multinucleate cysts observed in the emu feces of the present study. The cysts of *E. gallinarum*, described in bird feces, each have eight nuclei but are also smaller (12-15 μm) than the cysts of *Entamoeba* spp. found in the emu feces, whereas *E. coli* cysts were measured at 10-33 μm (Tyzzer, 1920). In 2000 Martinez-Diaz et al. (2000) described *Entamoeba* sp.

![Figure 2](image_url). Trophozoite, cyst, oocysts of protozoa and nematode eggs found in fecal samples of emu, *Dromaius novaehollandiae*. Trophozoite (A) and cyst (B) of *Giardia* sp. Bars: 10 μm. Oocysts of *Eimeria* sp (C) and *Isospora dromaii* (D). Bars: 5 μm. Fertile (E) and infertile (F) Ascaridia-type eggs. Bars: 50 μm.
cysts with one nucleus in ostrich feces from Spain. Nearly 90% of the samples contained single-nucleus cysts with a mean diameter of 13.5 μm (8-20 μm), whereas the trophozoites had a mean diameter of 19.9 μm (8-35 μm). The mean diameters of the cysts and trophozoites of the present study were greater than those found by the previously cited author. Later in 2004, Ponce-Gordo et al. (2004) analyzed this parasite genetically and after finding differences in the sequence of RNA, they concluded be that a new species, naming it Entamoeba struthionis. In 2002, Ponce-Gordo et al. described cysts of eight nuclei in rhesus Rhea americana of Europe that had a morphometry of 20 μm on average, being smaller than those found in emus of the present study. The authors also suggested that this new species may infect hosts other than ratites. In rhesus in Argentina, cysts of Entamoeba spp. containing one nucleus and others containing eight nuclei were found and identified as Entamoeba bovis-like and Entamoeba coli-like, respectively. The cysts found in the Argentine rhesus had a range in diameter of 14-22 μm (Martínez-Díaz et al., 2013), again confirming that the diameters found in our study are greater than those described in other studies of ratites.

The authors Craig and Diamond (1996) cited the presence of Entamoeba sp. in ostriches, but in emus no reports were found. Once the presence of Entamoeba sp. in the emus feces was confirmed by the PCR technique and the cysts and trophozoites were characterized as larger than those found in other ratites we can hypothesically infer that Entamoeba sp. observed in the emus are different from the species already described in other birds including ratites. However, a molecular diagnosis with sequencing of the PCR product would be required before this hypothesis could be confirmed. Trophozoite size can vary depending on environmental conditions, but the same is not true for cysts (Neal, 1966). Further studies will need to be conducted, including experimental infection, cross-transmission and genetic analysis, to clarify the Entamoeba species for which emus are hosts.

In a study done on feces of the budgerigar Melopsittacus undulatus, Giardia trophozoites measuring 14 μm in length and 6 μm in width (Erlandsen and Bemrick, 1987) were morphologically distinct from previously described species, leading to the naming of a new species, G. psittaci. Trophozoites of G. duodenalis are approximately 10-12 × 5-7 μm. These organisms have been isolated from humans, dogs, cats, guinea pigs, beavers, gerbils, birds, reptiles, sheep and cattle, and some of these animals are thought to act as reservoirs for human infection (Faubert, 1988). This trait has also been described in ostriches and emus (Craig and Diamond, 1996). Only two species of Giardia have been described in birds: G. ardea, with trophozoites measuring 6.5 × 10 μm in diameter, and G. psittaci (Thompson, 2002). Further studies by these researchers describe the trophozoite sizes of the five species of this genus, in which the diameters range from 4 to 30 μm. None of those species have diameters that are comparable to those found in our study.

There are many reports of Cryptosporidium spp. infection in ostriches (Penrith et al., 1994; Penrith and Burger, 1993) and a few in rhesus (Ponce-Gordo et al., 2002). Cryptosporidium is a genus commonly found in ostrich feces (Huchzermeyer, 2002), but the same has not been demonstrated to occur in emus. In our study, no oocysts of this coccidian were found through any of the techniques performed.

In a study in Europe, in which 500 ostriches and a number of rhesus were analyzed for the presence of ectoparasites and endoparasites, 29 species of parasites were identified, most of which were protozoans. Non-sporulated oocysts compatible with Isospora and Eimeria species were observed (Ponce-Gordo et al., 2002). At an ostrich breeding facility in Espírito Santo state, the presence of oocysts of the Isospora genus were identified (Batista et al., 2008). According to the author, the birds were in good health, with appropriate hygiene and husbandry, as were the emus in our study. Although coccidiosis has been described in ostriches and emus, infection with coccidia has not been confirmed to cause clinically important disease in ratites (Jensen, 1993), but has been reported to be primarily a disease of emu chicks (Jurajda, 2002).

Studies on the morphology of Isospora spp. and Eimeria spp. oocysts in ratites are scarce. Non-sporulated oocysts found in the feces of rhesus and ostriches have been reported to measure 12-15 μm (Ponce-Gordo et al., 2002), but it is not possible to definitively identify the genus by oocyst size alone. As previously discussed, the number of Eimeria spp. oocysts found in this study was small, thereby preventing us from being able to fully describe these oocysts. In a study from Spain, feces were collected from ostriches in a zoo, and Eimeria spp. oocysts were identified (Pérez Cordón et al., 2008), however, the authors did not provide a morphological or morphometric description of the sporulated oocysts, preventing a comparison between our results and theirs. An analysis of feces from two emus in a zoo in Italy demonstrated the presence of coccidia, but the genus was not identified (Papini et al., 2012).

A study of ostrich feces described oocysts with shapes ranging from ellipsoid (28 × 22 μm) to spherical (21 × 21 μm) (Mushi et al., 1998). The researchers of this study were unable to induce sporulation, and thus the genus of this coccidian is unknown. The author reported that oocyst size in their study was similar to that of Eimeria tropicalis (Soulsby, 1987), which is spherical and subspherical, with dimensions of 19-24 μm × 18-23 μm; this species has been previously described in the ostrich. In another study, oocysts of Eimeria spp. with a mean size of 19 × 23 μm were found in 42% of the ostrich feces examined on Greek farms (Sotiraki et al., 2001). Oocysts of Eimeria spp. with a mean diameter of 26 μm × 23.7 μm (24-28 μm × 20-28 μm) were observed in fecal samples from rhesus (Chang Reissig et al., 2001) which is larger than Isospora dromaii demonstrated in emus in the present study and described in a 2014 study (Santos Teixeira et al., 2014). In the same year, Isospora rheae was first described in the feces of rhesus Rhea americana (Gallo et al., 2014). Oocysts of Isospora spp., which were identified in the feces of an ostrich from a Russian zoo and described as spherical.
with a diameter of 30.6 μm and containing Stieda bodies, were designated *Isospora struthionis* (Yakimoff, 1940).

*Baylisascaris* spp. is a nematode of great clinical importance in ostriches. This parasite can be transmitted by eggs present in the feces of opossums and raccoons, causing serious damage to the central nervous system and great economic losses to the ostrich industry, since these eggs can remain viable in the soil for years (Nemejc and Lukesova, 2012). In Rio Grande do Sul state, where the feces of 452 ostriches were analyzed, 30% of those animals were found to be parasitized by eggs of *Heterakis* spp., *Ascaridia* spp., *Capillaria* spp., *Hymenolepis* spp., *Codiostomum* spp., *Isospora* spp. and *Balantidium* spp. (Mattos et al., 2011). Feces of 50 ostriches from Formiga, Minas Gerais state, were analyzed, and 10% were positive for Ascaridoidea eggs. Similar results were found in another study (Rosa, 2003) that reported that endoparasite eggs of the Ascaridoidea and Strongiloidea superfamilies were the most commonly observed parasites in rataite fecal flotation tests. Following the necropsy of an emu that presented with problems of locomotion and bilateral paralysis, an Ascaridoidea was identified in the cerebellum of the animal (Winterfield and Thacker 1978). In our study, eggs similar to *Baylisascaris* spp. were found in the feces of the emus. Previously, only a single study, in 1982, demonstrated the presence of *Baylisascaris* spp. in the feces of emus (Kazacos et al., 1982). *Ascaris* spp.-type eggs are prevalent throughout the world and are frequently demonstrated in the feces of domestic poultry as well as other animals (Rey, 2001). Infection by this nematode greatly impacts weight gain in affected animals, retarding growth by competing for nutrients with the host. *Ascaris* eggs remain viable in the environment for long periods of time, resulting in the infection and reinfection of animals. The presence primarily of protozoan parasites in the feces of the emus can be explained by the fact that these birds are prone to coprophagia, which contributes to infection with these parasites. Another reason for the development and maintenance of protozoan parasites is the local topography, which is flat and creates favorable sites for standing water (Dias et al., 2008). The emus studied here do not seem to be vulnerable to developing disease as a result of these infections, and no clinical signs were observed in any of the animals, which supports our assertion that some of these parasites may have no clinical significance.

Emus *Dromaius novaehollandiae* were enzootically parasitized by helminths characteristic of the Ascaridida order as well as by protozoa of the genera *Entamoeba*, *Giardia*, *Isospora* and *imeria*. Further analyses are needed to determine the implications of these infections for emus and the risk of infection in domestic animals, wildlife and humans.

**Acknowledgements**

The authors thank the research funding agency CAPES (Coordenação de perfeiçoamento de Pessoal de Nível Superior) for financial support.

**References**


6/7
Endoparasites in Dromaius novaehollandiae


