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Phenotypic and Genotypic Characterization of *Eimeria caviae* from Guinea Pigs (*Cavia porcellus*)

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Abstract. Coccidiosis in Guinea pigs (*Cavia porcellus*) has been frequently associated with the presence of *Eimeria caviae*; however, this coccidium has never been characterized in detail. This study aimed to present the phenotypic and genotypic characterization of *E. caviae* from guinea pigs reared under rustic breeding conditions in Brazil. *Eimeria caviae* oocysts are polymorphic, being sub-spherical, ovoidal or ellipsoidal, 20.9 × 17.7 µm, with a smooth or slightly rough and bi-layered wall, ~ 1.0 µm. Micropyle and oocyst residuum are absent, but one polar granule is present. Sporocysts are ellipsoidal, 10.8 × 6.4 µm. Stieda and parastieda bodies are present, sporocyst residuum is present and sporozoites posses a refractile body and a nucleus. Linear regressions and histograms were performed and confirmed the polymorphism of the oocysts. The internal transcribed spacer 1 of the ribosomal RNA gene (ITS-1 rRNA) of the isolates was sequenced and showed no significant similarity to the orthologous region of other *Eimeria* species.

Key words: oocysts, coccidiosis, diagnostic, morphology, morphometry, ITS-1, Rio de Janeiro, Brazil.

INTRODUCTION

Guinea pigs (*Cavia porcellus*) are small rodents raised in the South American Andes Mountains primarily as a food source. However, the breeding of Guinea pigs has long been established worldwide, as a source of pets and laboratory animals (Sachser 1998).

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Guinea pigs can be affected by various non-infectious and infectious (bacterial, viral, fungal and protozoan) diseases. Among the protozoa, *Balantidium* sp., *Cyathodinium* sp., *Giardia muris*, *Leishmania enrietti* and coccidia are the most frequently reported (Rigby 1976, Andrade *et al*. 2002). Coccidiosis in Guinea pigs has frequently been associated with the presence of *Eimeria caviae*, although other coccidian species, namely *Cryptosporidium* spp., *Klossiella* spp., *Toxoplasma gondii* and *Sarcocystis caviae* have also been observed as parasites of these animals.

Eimeria cavie was first described by Sheather (1924), where oocysts and endogenous stages were observed in the epithelium of the colon. Subsequently, Henry (1932) and Lapage (1940) provided morphological data in relation to gametocytes and oocysts of this coccidian. Interestingly, Zwart and Strik (1961) considered *E. caviae* and *E. dolichotis* as representing a single species; however, the latter did not develop in experimentally infected Guinea pigs. Moore (1976) performed experimental infections in Guinea pigs in conjunction with a cytological study on the sporozoites of *E. caviae*. More recently, the studies of Hankinson *et al*. (1982), Muto *et al*. (1985), Fujino *et al*. (1991) and Matsui *et al*. (1996) served to improve our knowledge of the life cycle of the parasite. Nevertheless, it is somewhat surprising that despite having been the subject of numerous studies, the phenotypic characterization of this species has not been fully developed. Moreover, relative to other *Eimeria* species, there exists a dearth of genotypic data available for this parasite relative to other *Eimeria* species.

The present study aimed to produce a detailed phenotypic description of this parasite, isolated from Guinea pigs reared under rustic breeding conditions in Brazil, and to provide preliminary genotypic characterization using nucleotide sequences of the internal transcribed spacer region of the ribosomal RNA (ITS-1 rRNA).

MATERIALS AND METHODS

Sample collection and processing

Fecal samples were collected from 30 Guinea pigs derived from a single rustic breeding facility located in the Municipality of Seropédica (22°44′29″S and 43°42′19″W), in the State of Rio de Janeiro, Brazil. Samples were collected immediately after defecation and placed into plastic vials with potassium dichromate 2.5% solution ($K_2Cr_2O_7$), at a ratio of 1:6 (v/v). Samples were transported to the Laboratório de Coccídios e Coccidioses located at Universidade Federal Rural do Rio de Janeiro (UFRRJ). They were placed in a thin layer (~ 5 mm) $K_2Cr_2O_7$ 2.5% solution in Petri plates, and incubated at 23–28°C for 10 days or until 70% of oocysts were sporulated. Oocysts were recovered by flotation in Sheather's sugar solution (sp. g. 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997).

Observation, measurement and illustration of oocysts

Morphological observations and measurements, provided in micrometres, were made using a Carl Zeiss binocular microscope fitted with an apochromatic oil immersion objective lens and an ocular micrometer (K-15X PZO, Poland). Line drawings were prepared using a Wild M-20 binocular microscope with a drawing tube. Size ranges are shown in parenthesis followed by the average and shape index (L/W ratio).

Statistical evaluation

Two statistical methods were employed: (1) Histograms were prepared to plot the values of length, width and the shape-index of the oocysts, as well as their relative frequencies, according to the methods of Sampaio (2002) and Berto *et al*. (2008a, b, c, 2011); (2) Linear regression determined the distribution of *E. cavie* oocysts, using methods proposed by Norton and Joyner (1981) and applied by Sampaio (2002) and Berto *et al*. (2008a, b, c, 2011). The graphic and coefficient of regression line was obtained using the software Microsoft Excel 2007®.

DNA extraction

Concentrated oocysts with the number of 10^6 per sample were suspended in duplicate in 300 µl of ice cold phosphate buffered saline (pH 7.2), in screw capped 1.5 mL microcentrifuge tubes containing 50 mg of glass beads (Sigma-Aldrich; product # G8772). Tubes were placed in a mini-beadbeater-16 apparatus (Biospec; Bartlesville, OK, USA), and the cells were disrupted using a single cycle of agitation (60 seconds). Cell lysis was completed by the addition of 300 µl of cell disruption solution (20 mM Tris-HCl, 20 mM EDTA, 400 mM NaCl, 1% sodium dodecyl sulphate), with lysates incubated overnight at 56°C. DNA was extracted by single rounds of phenol and phenol chloroform treatment, followed by precipitation with an equal volume of isopropanol for 20 minutes at room temperature. Precipitated (16,000 × g for 15 min) DNA pellets were desalted twice with 70% ethanol and re-suspended overnight at 4°C in 50 µl of molecular biology grade water (Sigma-Aldrich), with subsequent storage at –20°C. DNA concentration was determined spectrophotometrically using a Nanodrop 2000 apparatus (Thermo-Scientific).

Polymerase chain reaction (PCR) and DNA sequencing

We used genus-specific primers, reagents and cycling conditions as described by Kawahara *et al*. (2010) to amplify ITS-1 rDNA targets. An aliquot (5 µl) of each PCR reaction was examined by agarose gel electrophoresis to confirm the presence of a single amplicon. Remaining PCR products were treated with Exo-Sap-IT (USB) according to the manufacturer's protocol and sequenced in both directions, using the amplification primers by use of the BigDye Ready Reaction mix (ABI Corp); reaction products were analyzed on a Prism 3700 automated DNA analyser (ABI Corp). Sequence alignments were performed using Sequencher (Version 5.0, Genecodes Corporation). In order to find possible orthologs, we submitted the sequences to similarity searches using the BLAST program (Altschul *et al*. 1990) against the non-redundant (nr) database.

RESULTS

In total fecal samples from 30 Guinea pigs were examined and 3 were positive for *Eimeria caviae*. Oocysts isolated were not sporulated but approximately 70%

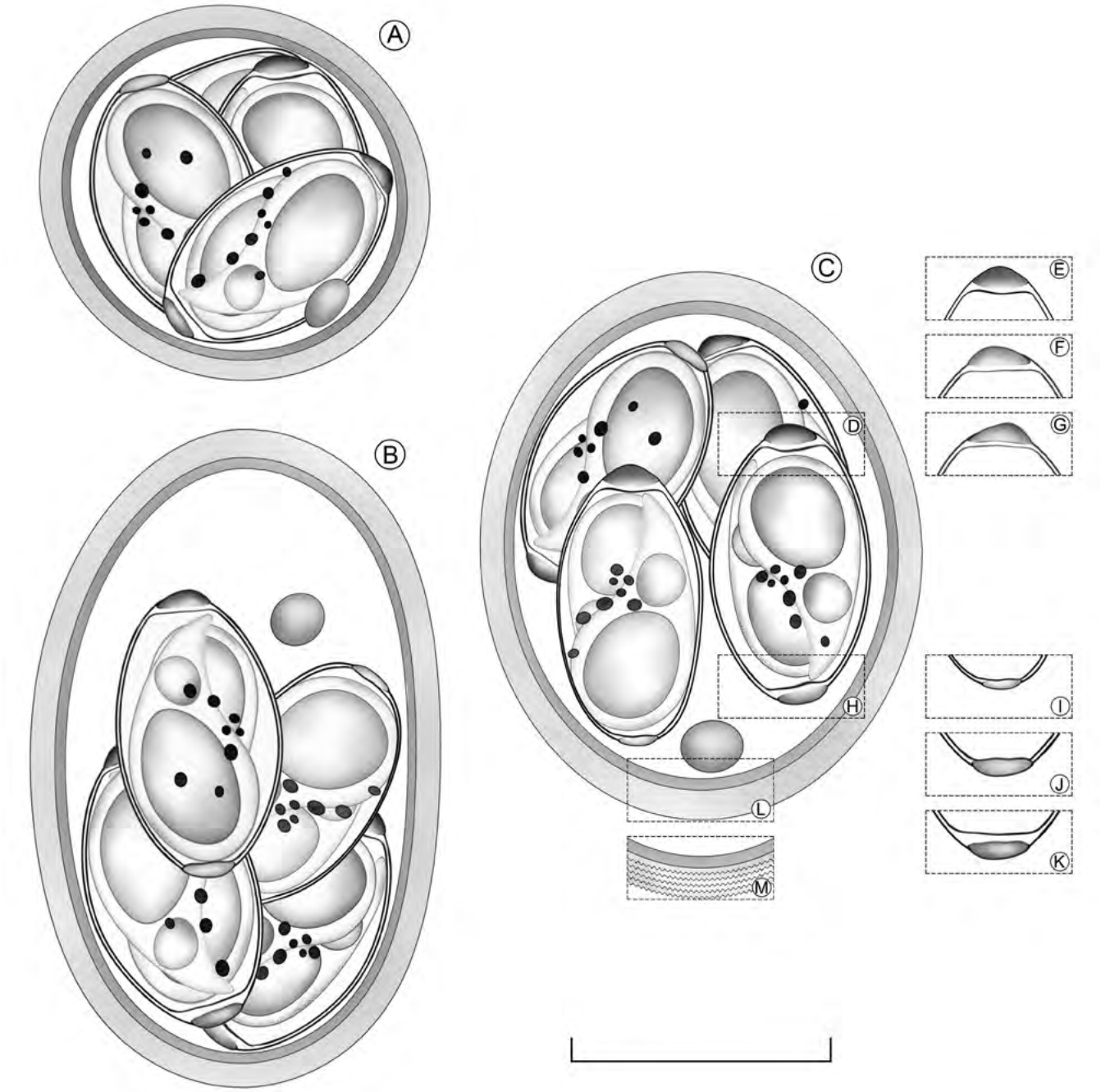

Fig. 1. Line drawings of sporulated oocysts of *Eimeria caviae*, a coccidium species recovered from Guinea pigs *Cavia porcellus*: (A) sub-spherical, (B) ellipsoidal, and (C) ovoidal oocysts; (D–G) variations of the Stieda bodies; (H–K) variations of the parastieda bodies; (L–M) variations of roughness of the oocyst wall. Scale bar: 10 μm.

sporulated 4–7 days after being placed in 2.5% potassium dichromate and incubated at 23–28°C.

***Eimeria caviae* Sheather, 1924** (Figs 1, 2)

Species description: Sporulated oocysts are polymorphic, being sub-spherical, ovoidal or ellipsoidal, measure 20.9 (14–25) × 17.7 (14–21) μm (n = 150) and present shape-index 1.2 (1.0–1.5). They are smooth or slightly rough, with a 1.0 (0.9–1.2) μm thick bi-layered wall. Micropyle and oocyst residuum are absent, but one polar granule is present. Sporocysts are ellipsoidal and measure 10.8 (7–13) × 6.4 (4–9) μm (n = 150).

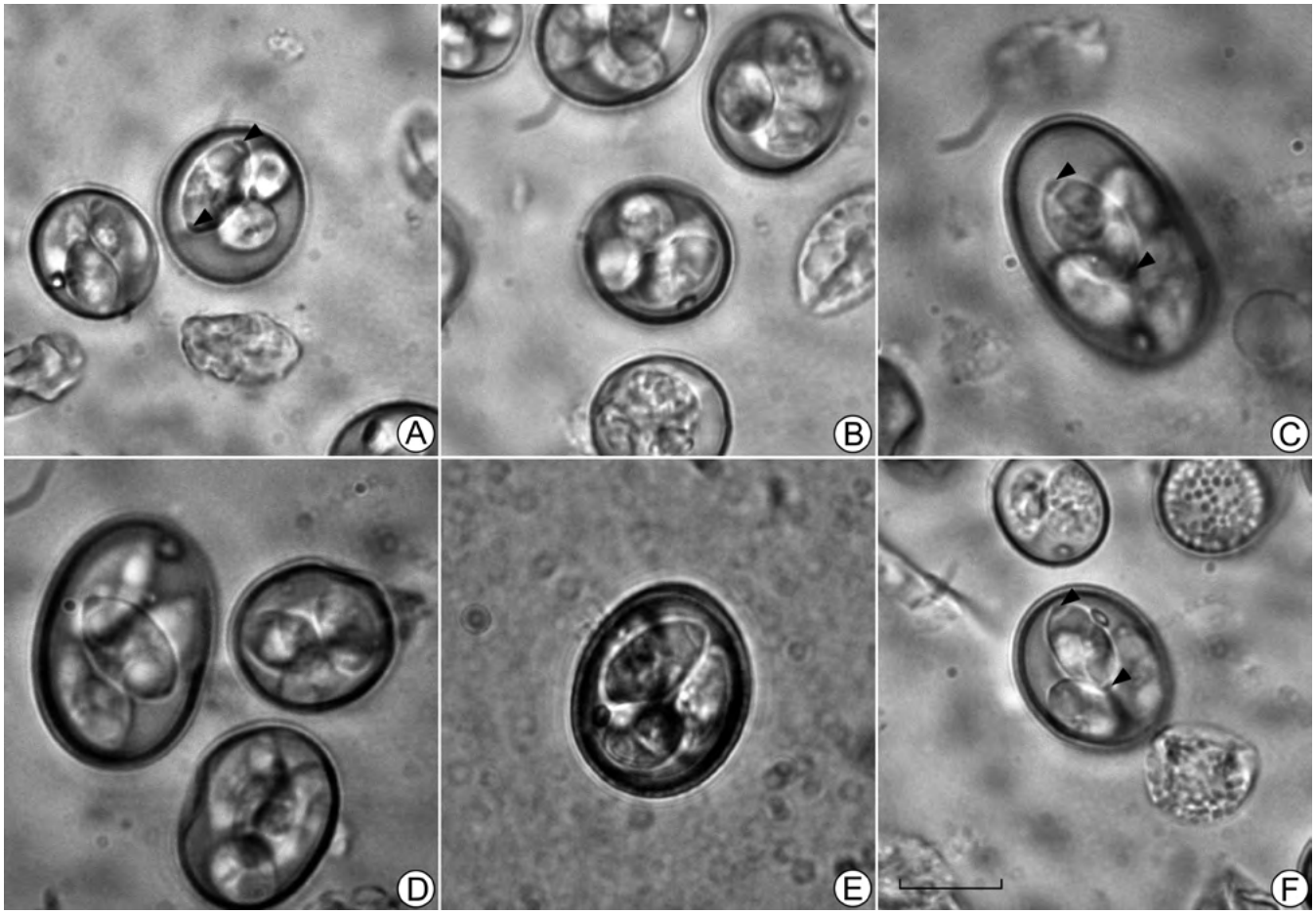

Fig. 2. Photomicrographs of sporulated oocysts of *Eimeria caviae*, a coccidium species recovered from Guinea pigs *Cavia porcellus*: (A, B, D) sub-spherical, (C, D) ellipsoidal, and (D, E, F) ovoidal oocysts. In (D) three shapes can be observed in the same field. The arrowheads point the Stieda and parastieda bodies. Sheather's sugar solution. Scale bar: 10 µm.

They present shape-index 1.7 (1.2–2.3), are smooth, thin, with single-layered wall. Stieda body is evident and triangular, ~ 1.0 µm high × 2.0 µm wide. The parastieda body is flattened, ~ 0.5 µm high × 2.0 µm wide. Sporocyst residuum is composed of few scattered granules. The sporozoites have a robust posterior refractile body and a centrally located nucleus.

Host: The Guinea pig, *Cavia porcellus* Linnaeus, 1758 (Rodentia: Caviidae).

Locality: Municipality of Seropédica (22°44′29″S and 43°42′19″W) in the State of Rio de Janeiro, Brazil.

Material deposited: Phototypes and line drawings are deposited and available (http://r1.ufrrj.br/lcc) in the Parasitology Collection of the Laboratório de Coccídios e Coccidioses, UFRRJ, located in Seropédica, Rio de Janeiro State, Brazil. The repository number is 54/2014.

Morphometric characterization: The histograms and linear regression of *E. caviae* sporulated oocysts can be visualized in Figs 3 and 4, respectively. In the histograms of length, width and shape-index it was observed that the frequencies of the different classes increased and declined gradually; in other words, the oocyst measurements are in smaller quantities in the limits of values and in greater quantity in median values. In relation to linear regression, the R^2 value was lower than 0.4 and the points were distributed distant from the regression line on the graph, which served to demonstrate the polymorphism of the oocysts.

Genotypic characterization: Amplifications using 100fg of DNA as template, generated single amplicons with an approximate size of 350 base pairs (bp). Two individual amplicons were sequenced in both direc-

tions and aligned using Sequencher 5.0. To determine the size of the ITS-1 component of the *E. caviae* amplicon, sequences corresponding to the genes encoding the 18S ribosomal RNA (rRNA) and 5.8S rRNA of *Eimeria tenella* (GenBank accession number AF026388) were aligned with the *E. caviae* sequence. This analysis demonstrated that the ITS-1 region was 232 nucleotides in length. The sequence did not demonstrate significant nucleotide similarity with any other sequence deposited in the GenBank, database. The sequence of the entire amplicon (352 bp), was deposited in the GenBank with the accession number (KC484340).

DISCUSSION

The morphological data reported in the present study showed a high degree of similarity to the descriptions made by Henry (1932), Lapage (1940), Zwart and Strik (1961), Moore (1976) and Muto *et al*. (1985) (Table 1). However, the current study provided insight into morphological characteristics not previously noted for oocysts of *E. caviae*, including the possibility of a slightly rough wall and the presence of a parastieda body. It is noteworthy that the latter feature has been described infrequently in Eimeriidae species (Duszynski and Wilber 1997). Those authors characterized this structure as representing a substieda body located at the opposite end of the Stieda body; however, in this study we identified the parastieda body as a structure resembling another Stieda body, located at the opposite end, which served to complicate the localization of the anterior and posterior ends. Furthermore, we observed bands below the Stieda and parastieda bodies, with similarity to substieda bodies (Fig. 2). Owing to the lack of opacity of this structure is not possible to conclusively describe it as a substieda body; however, this observation has been depicted in the line drawing (Fig. 1).

Morphometric data from the current study served to characterize the polymorphic nature of the oocysts, which can be sub-spherical, ovoidal or ellipsoidal. This polymorphism can be observed in the line drawings (Fig. 1A–C) and photomicrographs (Fig. 2) and was verified in the linear regression (Fig. 3C), which stated a low proportionality for the values of width on length.

Oocyst polymorphism has been described previously by several authors for a variety of coccidia including, *Isospora lacazei* from the house sparrow *Passer domesticus*, in the Province of Cordoba, Spain (Gomez

Table 1. Comparative morphology of *Eimeria caviae* recovered from the Guinea pigs, *Cavia porcellus*.

References	Oocysts				Sporocysts				
	Shape	Measurements (µm)	Shape index	Polar granule	Shape	Measurements (µm)	Stieda body	Parastieda body	Residuum
Sheather (1924)	–	(15.9–24.6 × 12.2–17.4)			–	–	present	–	–
Ellis and Wright (1961)	–	18 × 15			–	–	present	–	–
Lapage (1940)	–	19 × 16 (17–25 × 13–18)	–	–	–	–	present	–	–
Zwart and Strik (1961)	–	(17.6–27.0 × 17.0–21.0)	1.24	–	–	(8–13 × 5–7)	–	–	–
Moore (1976)	ellipsoidal	22.6 × 20.8 (19.9–25.9 × 17.2–24.3)	1.1	present	–	13.1 × 7.2	present	–	–
Muto *et al.* (1985)	sub-spheroidal to ellipsoidal	20 x 17	–	–	–	–	–	–	–
Present study	sub-spherical, ovoidal or ellipsoidal	20.9 × 17.7 (14–25) × (14–21)	1.2 (1.0–1.5)	present	ellipsoidal	10.8 × 6.4 (7–13) × (4–9)	evident and triangular, ~ 1 × 2	flattened, ~0.5 × 2	diffuse

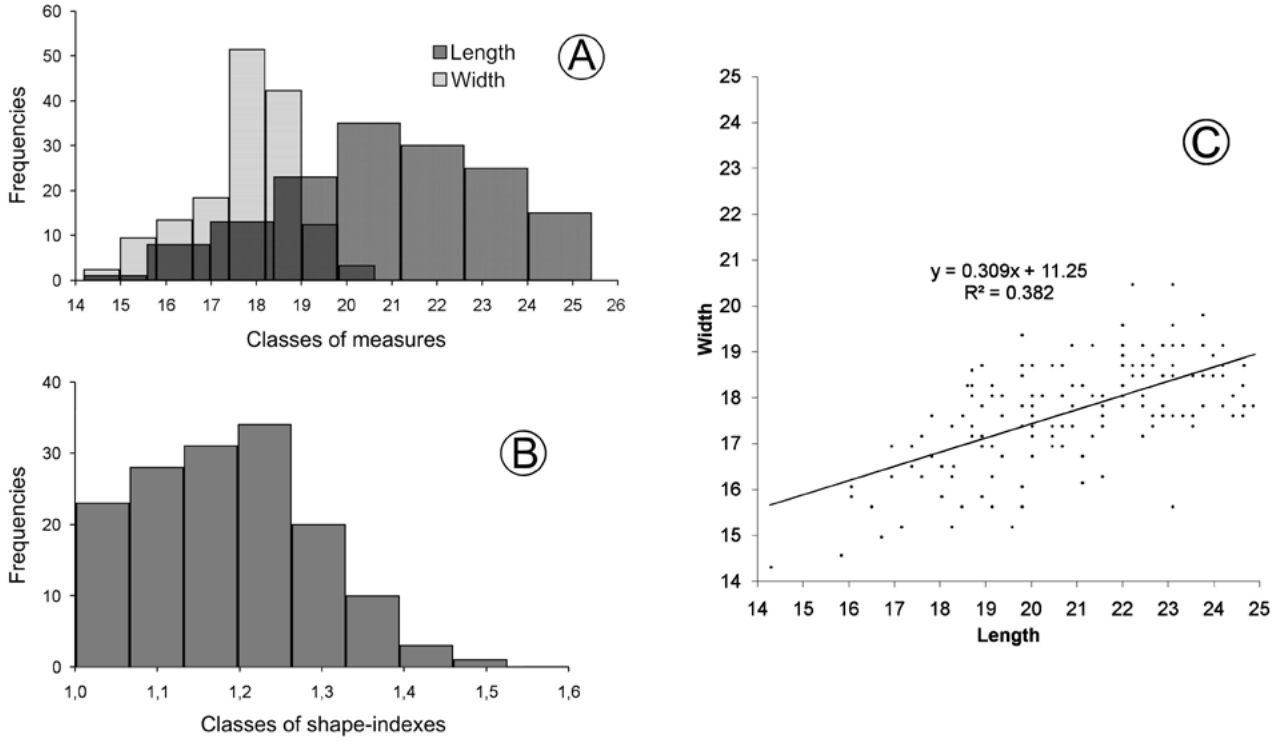

Fig. 3. Histograms of (A) length, width and (B) shape-index, and (C) linear regression of the oocysts of *Eimeria caviae*, a coccidium species recovered from Guinea pigs *Cavia porcellus*.

et al. 1982), *E. opimi*, from tuco-tucos *Ctenomys* spp., in Bolivia (Gardner and Duszynki 1990), *Tyzzeria parvula* from greylag geese *Anser anser* (Berto *et al*. 2008b), *Eimeria bareillyi* of domestic water buffalos *Bubalus bubalis* (Ramirez *et al*. 2009b) and *Isospora sepetibensis* from thraupid passerines in Marambaia Island, Rio de Janeiro, Brazil (Berto *et al*. 2011). Several factors may have a role in the existence of the observed polymorphism: (1) stress, (2) nutrition, (3) immunity of the host, (4) the infecting dose (Fayer 1980, Joyner 1982), (5) the time of oocysts discharge during the patent period (Duszynski 1971, Catchpole *et al*. 1975, Joyner 1982), and (6) phenotypic plasticity, when a coccidium activates different phenotypes in response to its environment (Parker and Duszynski 1986, Gardner and Duszynski 1990).

The histograms based on length and width data (Fig. 3A) showed a regular distribution. However, the histogram of shape-index (Fig. 3B) revealed a higher frequency in the range of values from 1.0 to 1.3. This observation indicates a tendency for the oocysts to adopt a sub-spherical to ovoidal shape. It is of interest that similar results were recorded by Berto *et al*. (2008a, c, 2011) for *Isospora hemidactyli* recovered from the house gecko, *Hemidactylus mabouia*; *Eimeria bateri* from the Japanese quail, *Coturnix japonica*; and *Isospora* spp. from thraupid passerines at the Marambaia Island, Rio de Janeiro, Brazil.

Transcription of the ITS-1 region of the nuclear genome does not result in a functional product, which means that these sequences can tolerate an elevated degree of mutation relative to the genes encoding the different subunits of the ribosomal RNA (Oliviera *et al*. 2010). As such, this marker may be indicated for population studies making use of the intra-specific sequence variations as discriminatory characters (Schnitzler *et al*. 1998). The taxonomy of the coccidia has historically been morphologically based. However, the ITS-1 region of the nuclear genome has been used as a molecular marker, and as the basis for discriminatory detection, in numerous studies of coccidian parasites including seven species of *Eimeria* from domestic

chickens (Schnitzler *et al*. 1998, 1999), six species of *Eimeria* which infect bovines (Kawahara *et al*. 2010), and eleven known species of *Eimeria* associated with the domestic rabbit (Oliveira *et al*. 2011). More recently it has been applied, in conjunction with ITS-2, to examine how *Eimeria callospermophili* isolated from ground dwelling squirrel hosts (Rodentia: Sciuridae) are related to eimerian species from other sciurid hosts (Motriuk-Smith *et al*. 2011).

The ITS-1 sequence recorded for *E. caviae* showed no significant similarity the ITS-1 region of any other *Eimeria* species. Clearly, sequencing of additional isolates will be necessary to confirm the utility of this region as a genotypic marker for this species. The DNA used for PCR was derived from cultures which contained the three morphotypes of the parasite and was amplified using primers which were designed to anneal to conserved regions of the 18S and 5.8S rDNA of the genus *Eimeria* (Kawahara *et al*. 2010). Without exception the sequencing chromatograms were composed of clearly defined peaks, with no evidence for secondary peaks which would indicate the presence of more than one amplicon in the PCR product. As such, the genotyping data provide support to the conclusion that the three types of oocyst observed in the phenotypic analysis represented polymorphic variants of the same species rather than a mixture of different species.

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REFERENCES

Altschul S. F., Gish W., Miller W., Myers E. W., Lipman D. J. (1990) Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410

Andrade A., Pinto S., Oliveira R. (2002) Animais de Laboratório: Criação e experimentação. Fiocruz, Rio de Janeiro

Berto B. P, Lopes B. do B., Flausino W., Teixeira-Filho W. L., Lopes C. W. G. (2008a) Contribution on the study of *Isospora hemidactyli* Carini, 1936 and a report of an adeleid pseudoparasite of the house gecko *Hemidactylus mabouia*, from the Rio de Janeiro Metropolitan Region, Brazil. *Rev. Bras. Parasitol. Vet.* **17:** 150–154

Berto B. P., Flausino W., Almeida C. R. R., Lopes C. W. G. (2008b) Polymorphism of *Tyzzeria parvula* (Kotlán, 1933) Klimes, 1963 (Apicomplexa: Eimeriidae) oocysts from the greylag geese *Anser anser* L., 1758 conditioned in two distinct sites. *Rev. Bras. Med. Vet.* **30:** 215–219

Berto B. P., Cardozo S. V., Teixeira-Filho W. L., Ferreira A. M. R., Lopes C. W. G. (2008c) Aflatoxin effect on the oocysts morphometry and contribution on the morphology of *Eimeria bateri* Bhatia, Pandey and Pande, 1965 of the Japanese quail *Coturnix japonica*, in Brazil. *Rev. Bras. Parasitol. Vet.* **17:** 235–238

Berto B. P., Luz H., Flausino W., Teixeira-Filho W. L., Ferreira I., Lopes C. W. G. (2011) Isosporoid Coccidia (Apicomplexa: Eimeriidae) parasites of tanagers (Passeriformes: Thraupidae) from the Marambaia Island, Brazil. *Pesq. Vet. Bras.* **31:** 798–805

Catchpole J., Norton C., Joyner L. (1975) The occurrence of *Eimeria weybridgensis* and other species of coccidia in lambs in England and Wales. *Br. Vet. J.* **131:** 392–401

Duszynski D. (1971) Increase in size of *Eimeria separata* oocysts during patency. *J. Parasitol.* **57:** 948–952

Duszynski D., Wilber P. (1997) A guideline for the preparation of species descriptions in the Eimeridae. *J. Parasitol.* **83:** 333–336

Fayer R. (1980) Epidemiology of protozooan infection: the Coccidia. *Vet. Parasitol*. **6:** 75–103

Fujino T., Matsui T., Morii T. (1991) Endogenous stages and the other biological characteristics of *Eimeria caviae* in Guinea pigs. *Kisechugaku Zasshi.* **40:** 368–373

Gardner S., Duszynski D. (1990) Polymorphism of eimerian oocysts can be a problem in naturally infected hosts: an example from subterranean rodents in Bolivia. *J. Parasitol*. **76:** 805–811

Gomez F., Navarrete I., Rodriguez R. (1982) Influencia de los factores ambientales sobre diferentes poblaciones de *Isospora lacazei* Labbé, 1983 (Protozoa: Apicomplexa). *Rev. Iber. Parasitol*. **42:** 185–196

Hankinson G., Murphy J., Fox J. (1982) Diagnostic exercise. *Eimeria caviae* infection with concurrent *Balantidium coli* infection. *Lab. Anim. Sci*. **32:** 35–36

Henry D. (1932) Coccidiosis of the guinea pigs. *Univ. Calif. Publ. Zool*. **37:** 211–268

Joyner L. (1982) Host and Site specificity. In: Long P., The biology of the Coccidia. University Park Press, Baltimore, 35–62

Kawahara F., Zhang G., Mingala C. N., Tamura Y., Koiwa M., Onuma M., Nunoya T. (2010) Genetic analysis and development of species-specific PCR assays based on ITS-1 region of rRNA in bovine *Eimeria* parasites. *Vet. Parasitol*. **174:** 49–57

Lapage G. (1940) The study of coccidiosis in the Guinea pig. *Vet. J.* **96:** 144–154, 190–202, 242–254, 280–295

Matsui T., Fujino T., Kobayashi F., Tsutsumi Y., Tsuji M. (1996) Attenuation of *Eimeria caviae* by selection for precocious development. *Int. J. Parasitol.* **26:** 1243–1248

Moore C. (1976) A cytological study of the sporozoites of *Eimeria caviae*, a coccidian parasite of the domestic Guinea pig, *Cavia porcellus*. Thesis. Drake University, School of Graduate Studies. United States

Motriuk-Smith D., Seville R. S., Quealy L. L., Clinton E., Oliver C. E. (2011) Comparison of the ITS1 and ITS2 rDNA in *Emeria callospermophili* (Apicomplexa: Eimeriidae) from sciurid rodents. *J. Parasitol.* **97:** 305–310

Muto T., Sugisaki M., Yusa T., Noguchi Y. (1985) Studies on coccidiosis in guinea pigs. Clinico-pathological observations. *Experim. Anim.* **34:** 23–29

Norton C. C., Joyner L. P. (1981) *Eimeria acervulina* and *E. mivati*: oocysts, life-cycle and ability to develop in the chiken embryo. *Parasitology*, **83:** 269–279

Oliveira U. C., Fraga J. S., Licois D., Pakandl M., Gruber A. (2011) Development of molecular assays for the identification of the 11 *Eimeria* species of the domestic rabbit (*Oryctolagus cuniculus*). *Vet. Parasitol*. **176:** 275–280

Parker B., Duszynski D. (1986) Polymorphism of eimerian oocysts: a dilemma posed by working with some naturally infected hosts. *J. Parasitol.* **72:** 602–604

Ramirez L., Berto B. P., Teixeira-Filho W., Flausino W., Meireles G., Rodrigues J., Almeida C., Lopes C. W. G. (2009b) *Eimeria bareillyi* from the domestic water buffalo, *Bubalus bubalis*, in the State of Rio de Janeiro, Brazil. *Rev. Bras. Med. Vet.* **31:** 261–264

Rigby C. (1976) Natural infections of Guinea pigs. *Lab. Anim.* **10:** 119–142

Sachser N. (1998) Of domestic and wild Guinea pigs: Studies in sociophysiology, domestication, and social evolution. *Naturwissen* **85:** 307–317

Sampaio I. B. M. (2002) Estatística aplicada à experimentação animal. FEP MVZ Editora, Belo Horizonte

Schnitzler B. E., Thebo P. L., Mattsson J. G., Tomley F. M., Shirley M. W. (1998) Development of a diagnostic PCR assay for the detection and discrimination of four pathogenic *Eimeria* species of the chicken. *Avian Pathol.* **27:** 490–497

Schnitzler B. E., Thebo P. L., Tomley F. M., Uggla A., Shirley M. W. (1999) PCR identification of chicken *Eimeria*: A simplified read-out. *Avian Pathol.* **28:** 89–93

Sheather A. (1924) Coccidiosis in the Guinea Pig. *J. Comp. Path.* **37:** 243–246

Zwart P., Strik W. (1961) Further observations on *Eimeria dolichotis*, a coccidium of the Patagonian cavy (*Dolichotis patagonica*). *J. Protozool*. **8:** 58–59

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