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

Gilberto FLAUSINO¹, Bruno P. BERTO², Douglas MCINTOSH³, Tassia T. FURTADO¹, Walter L. TEIXEIRA-FILHO³ and Carlos Wilson G. LOPES³

¹Curso de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Brasil; ²Departamento de Biologia Animal, Instituto de Biologia, UFRRJ, Seropédica, Brasil; ³Departamento de Parasitologia Animal, Instituto de Veterinária, UFRRJ, Seropédica, Brasil

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).

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. (1996) served to improve our knowledge of the life cycle of the parasite. Nevertheless, it is something 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 (K₂Cr₂O₇), at a ratio of 1:6 (v/v). Samples were transported to the Laboratório de Coccídios e Coccidiose located at Universidade Federal Rural do Rio de Janeiro (UFRJR). They were placed in a thin layer (~ 5 mm) K₂Cr₂O₇ 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 Sheather 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 10⁶ 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, Gene Codes 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%
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).

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**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.
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 Coccidiose, 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-
Eimeria Caviae from Guinea Pigs

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1.24 present 13.1 × 7.2 present – –

Muto et al. (1985) sub-spheroidal to ellipsoidal 20 x 17 ellipsoidal ellipsoid 0.9 × 2.0 1.2 present

Present study sub-spheroidal, ovoidal or ellipsoidal 20.9 × 17.7 ellipsoid 10.8 × 6.4 ellipsoid, – , 1.2 present

Ellis and Wright (1961) 18 × 15 – – – –

Lapage (1940) 19 × 16 (17–25 × 13–18) present – –

Zwart and Strik (1961) 19 × 16 (17.6–27.0 × 17.0–21.0) 1.24 present (8–13 × 5–7)

Moore (1976) ellipsoidal 22.6 × 20.8 ellipsoid 1.1 present 13.1 × 7.2

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

<table>
<thead>
<tr>
<th>References</th>
<th>Shape</th>
<th>Measuremets (µm)</th>
<th>Polar granule</th>
<th>Shape index</th>
<th>Stieda body</th>
<th>Parastieda body</th>
<th>Residuum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheather (1924)</td>
<td>–</td>
<td>(159–246 × 12.2–17.4)</td>
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<td>–</td>
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<tr>
<td>Ellis and Wright (1961)</td>
<td>–</td>
<td>(17–25 × 13–18)</td>
<td>–</td>
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<tr>
<td>Lapage (1940)</td>
<td>19 × 16</td>
<td>(17.6–27.0 × 17.0–21.0)</td>
<td>1.24</td>
<td>present</td>
<td>(8–13 × 5–7)</td>
<td>–</td>
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<tr>
<td>Zwart and Strik (1961)</td>
<td>–</td>
<td>(17.6–27.0 × 17.0–21.0)</td>
<td>1.24</td>
<td>present</td>
<td>(8–13 × 5–7)</td>
<td>–</td>
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<tr>
<td>Moore (1976)</td>
<td>ellipsoidal</td>
<td>22.6 × 20.8</td>
<td>ellipsoid</td>
<td>1.1</td>
<td>present</td>
<td>13.1 × 7.2</td>
<td>–</td>
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<tr>
<td>Muto et al. (1985)</td>
<td>sub-spheroidal</td>
<td>20.9 × 17.7</td>
<td>ellipsoid</td>
<td>1.2</td>
<td>present</td>
<td>13.1 × 7.2</td>
<td>–</td>
</tr>
<tr>
<td>Present study</td>
<td>–</td>
<td>20.9 × 17.7</td>
<td>ellipsoid</td>
<td>1.2</td>
<td>present</td>
<td>13.1 × 7.2</td>
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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
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*.

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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