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Differential diagnosis between endogenous stages of *Cyniclomyces guttulatus* (Robin) Van Der Walt and Scott, 1971 and *Eimeria caviae* Sheather, 1924 from Guinea pig *Cavia porcellus* Linnaeus

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Abstract Flausino G, Furtado TT, McIntosh D, Teixeira Filho WL. 2013. **Differential diagnosis between endogenous stages of *Cyniclomyces guttulatus* (Robin) Van Der Walt and Scott, 1971 and *Eimeria caviae* Sheather, 1924 from Guinea pig *Cavia porcellus* Linnaeus** [Diagnóstico diferencial entre estágios endógenos de *Cyniclomyces guttulatus* (Robin) Van Der Walt and Scott, 1971 and *Eimeria caviae* Sheather, 1924 from Guinea pig *Cavia porcellus* Linnaeus]. *Coccidia* 1, 21-24. Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro. BR-465 km 7, 23897-970 Seropédica, RJ, Brasil. E-mail: gilflausino@ufrrj.br

In the intestinal microbiota of Guinea pigs *Cavia porcellus* Linnaeus there exists a wide variety of species that may be pathogenic or non-pathogenic; including the ascomycete, *Cyniclomyces guttulatus* (Robin) Van Der Walt and Scott, 1971 and the coccidium, *Eimeria caviae* Sheather, 1924, both observed in the digestive tract of *C. porcellus*. This study aimed to compare the developmental stages of gastrointestinal *C. guttulatus* with *E. caviae* present in guinea pigs derived from rustic breeders, to allow their differential diagnosis between them. Three Guinea pigs naturally infected with *C. guttulatus* were infected with 10^6 oocysts of *E. caviae*. Mucosal swabs stomach, small intestine and large intestine were stained by Giemsa and Ziehl-Neelsen staining, respectively, for the identification of endogenous forms of *C. guttulatus* and *E. caviae*, respectively. Assessment of the differences between endogenous stages of *E. caviae* and *C. guttulatus* was markedly more difficult than the straight forward comparison of merozoites of the coccidia with vegetative cells of the ascomycete. Polymorphism was observed in *C. guttulatus* which complicated comparison to the first and second generations of merozoites of *E. caviae* present in smears of the large intestine of Guinea pigs. Based on the results obtained it was concluded that the endogenous forms of *C. guttulatus,* characterized as small vacuolated cells, and merozoites of *E. caviae* should be examined carefully to avoid mistakes in diagnosis.

Keywords oocysts, morphology, diagnosis, Ascomycota, Apicomplexa

Resumo Na microbiota intestinal de cobaios *Cavia porcellus* Linnaeus existe uma grande variedade de espécies que podem ser patogê nicas ou não, como o ascomiceto, *Cyniclomyces guttulatus* (Robin) Van Der Walt and

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Scott, 1971 e o coccídio, *Eimeria caviae* Sheather, 1924, ambos observados com certa frequência no sistema digestório de *C. porcellus*. O presente trabalho teve como objetivo comparar as fases de desenvolvimento gastrintestinais de *C. guttulatus* com as de *E. caviae* procedentes de criações rústicas de cobaios, traçando um diagnóstico diferencial entre as mesmas. Três cobaios naturalmente infectados com *C. guttulatus* foram infectados com 106 oocistos esporulados de *E. caviae*. Esfregaços com mucosas estomacal, de intestino delgado e de intestino grosso foram coradas pelos métodos de Giemsa e Ziehl-Neelsen, respectivamente, para a identificação das formas endógenas de *C. guttulatus* e *E. caviae*. A dificuldade em avaliar as diferenças entre as formas endógenas de *E. caviae* e as de *C. guttulatus* foi mais acentuada quando comparou-se os merozoítas deste coccídio com as células vegetativas do ascomiceto. Acentuado polimorfismo pode ser observado em *C. guttulatus* quando comparados aos merozoítos de *E. caviae* em esfregaços de intestino grosso. Com base nos resultados obtidos concluiu-se que as formas endógenas de *C. guttulatus* caracterizadas como células vacuoladas pequenas e merozoítos de *E. caviae* são as que devem ser analisadas criteriosamente para que não haja equívoco no diagnóstico diferencial.

Palavras-chave oocistos, morfologia, diagnóstico, Ascomycota, Apicomplexa

Introduction

In the intestinal microbiota of Guinea pigs *Cavia porcellus* Linnaeus there exists a wide variety of species, both pathogenic or non-pathogenic, including some which, although classified in different Kingdoms, share developmental stages with similar morphologies. Among them, an ascomycete, *Cyniclomyces guttulatus* (Robin) Van Der Walt and Scott, 1971 and the coccidium, *Eimeria caviae* Sheather, 1924, have both been observed in the digestive tract of *C. porcellus*.

Oftentimes, the differential diagnosis between one or more etiological agents is essential to determine the responsible for the disease process, be it infectious, nutritional, or a combination of both. The association of nutritional factors to infectious agents has been pointed out by Scrimshaw et al. (1959), who affirmed the existence of this interaction. Moreover, the differential diagnosis of microorganisms is essential to determine their role in the disease process. Costa et al. (2001) observed morphological similarities between *C. guttulatus* cells and zoytes of coccidian, in Geisma stained mucosal scrapings of rabbits Subsequently, Van Praag (2009) commented on the difficulty of separating different forms of *C. guttulatus* from coccidial development stages, in rabbits with coccidiosis. However, these two studies did not address the issue of how to differentiate between the numerous developmental stages of these organisms that may result in confusing during evaluation of outbreaks of coccidiosis. It should also be considered that *C. guttulatus* has been reporeted as being pathogenic in dogs (Flausino et al. 2012, Furtado et al. 2013), and therefore it is important to correctly determine the morphological forms of all developmental stages, to ensure effective differential diagnosis. Thus, this study aimed to compare the developmental stages observed in the gastrointestinal mucosa of guinea pigs derived from rustic breeding facilities.

Materials and methods

Three Guinea pigs derived from a rustic breeding facility located in the Municipality of Seropédica in the State of Rio de Janeiro, Brazil were used to obtain *C. guttulatus* and *E. caviae* were used three Guinea pigs coming from a rustic breeding located in the Municipality of Seropédica in the State of Rio de Janeiro, Brazil.

The Guinea pigs were transported to the Federal Rural University of Rio de Janeiro (UFRRJ), and were reared and fed in a single cage without the admisitration of anticoccidial medication. Feed and water were administered *ad libitum*.

Animals infection

The Guinea pigs from the rustic breeding were positive by microscopy for *C. guttulatus* but they were negative for *E. caviae*. Thus, an inoculum for experimental infection was produced by recovering and isolating oocysts

from fecal samples of positive Guinea pigs by flotation in Sheather's sugar solution (S.G. 1.20) according to the protocol of Duszynski & Wilber (1997). The oocysts were preserved in a 2.5% (w/v) solution of $K_2Cr_2O_7$ to induce sporulation and maintained in a refrigerator (2–5 °C) until use. Three Guinea pigs were inoculated, using inocula, quantified using a Neubauer chamber, containing approximately 100 sporulated oocysts of *E. caviae*.

Necropsy of the animals

After confirming that the three animals eliminated oocysts of *E. caviae* in their feces, a single animal was randomly selected to be euthanized in CO_2 chamber, as recommended by Cobea (2013). Upon confirmation of death, the animal was placed in the supine position and incised in the mento-pubic direction to expose the thoracic-abdominal cavity and for removal of the digestive tract. Thereafter, the tract was divided into stomach, small intestine and large intestine. Mucosal scrapings from each section were made with the aid of a glass slide for preparation of smears. Smears were air dried and dehydrated in methanol for 5 minutes. In parallel to this activity, fragments of these segments were adhered to a piece of porous paper by the serosal side, and placed in a flask containing 10% formalin for histological fixation.

Laboratorial analysis

Mucosal scrapies

Smears originating from each segment were stained with Giemsa or Ziehl-Nielsen (Behmer et al. 1976). The identification of endogenous forms of *C. guttulatus* and *E. caviae* was conducted using a binocular microscope (Carl Zeiss; Germany) at 400X and 1000X magnification.

Photographs

Photomicrographs were produced using a binocular microscope model star Primo, Zeiss (Germany) coupled to a digital camera model Panasonic Lumix F2 ® (Japan).

Statistical analysis

The Student's *t* test was used to compare measurements of the length, width and shape-index. The software Microsoft ® Excel 2007 was used to calculate the mean, variance, degree of freedom and p value (Sampaio 2002).

Results and discussion

The difficulty in assessing the differences between the endogenous forms of *E. caviae* and *C. guttulatus* becomes more pronounced when coccidian merozoites are compared with vegetative cells of the ascomycete (Figure 1, Table 1).

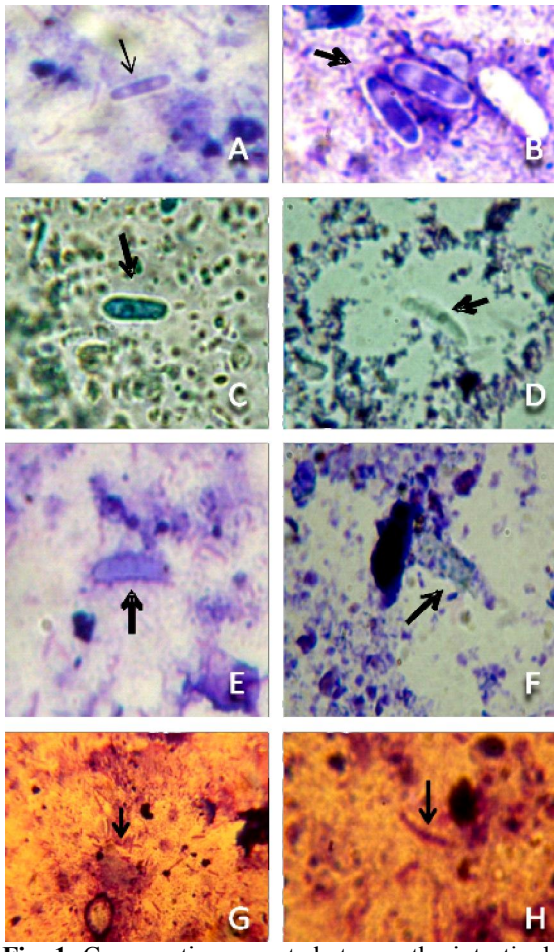

Fig. 1. Comparative aspects between the intestinal stages of *Cyniclomyces guttulatus* and *Eimeria caviae* (arrows) from a Guinea pig *Cavia porcellus*. Small (A, C) and large (B, D) vegetative cells of *C. guttulatus* stained with Giemsa (A, B) and Ziel-Nielsen (C, D). Macromerozoites (E, G) and micromerozoites (F, H) of *Eimeria caviae* stained with Giemsa (E, F) and Ziel-Nielsen (G, H). Magnification of 1000X (A-F, H) and 400x (G).

Strong polymorphism was observed in *C. guttulatus* which complicated differention

Table 1. Comparative aspects of free living forms in the large intestine of a Guinea pig *Cavia porcellus*

Species (n= 10)	Forms[a]	Diameters (μm)	
		length	width
Cyniclomyces guttulatus (Robin) Van Der Walt and Scott, 1971	Large vegetative cells	15.91[k] (18.7-13.86)	4.22[y] (7.92-5.50)
	Small vegetative cells	10.89[w] (14.08-9.24)	3.19[z] (4.18-2.42)
Eimeria caviae Sheather, 1924	Macromerozoites	12.85[k] (14.96-10.12)	3.10[y] (3.96-2.42)
	Micromerozoites	8.58[w] (9.46-6.38)	2.51[z] (2.86-1.98)

[a]Giemsa stain
[k]Same letters in each column significant at $p < 0.001$ by Student's t test

from first and second generation merozoites from smears of the large intestine. The basis for these difficulties was revealed by morphometric analyisis of the different morphological forms (Table 1). Specifically, it was noted that once the mean measurements were equivalent between small vegetative cells and micromerozoites; and between large vegetative cells and macromerozoites.

Observations similar to those encountered in the current work, were reported by da Costa et al. (2001), who highlighted the marked similarity between vegetative cells of *C. guttulatus* and zoytes of coccidia (*Cystoisospora felis,)* found in the Peyer's patches of experimentally infected rabbits. Furthermore, Van Praag (2009), emphasized the difficulties encountered for distinction between vegetative cells of *C. guttulatus* containing vesicles and oocysts of Eimeria in rabbits with coccidiosis.

Based on our data it can be concluded that the endogenous forms of *C. guttulatus* and *E. caviae*, in particular the developmental stages of small vacuolated cells and merozoites respectively, should be examined carefully to avoid possible mistakes in diagnosis.

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