**Eimeria spp. from Japanese quails (Coturnix japonica): new characteristic features and diagnostic tools**


**ABSTRACT.** Berto B.P., Borba H.R., Lima V.M., Flausino W., Teixeira-Filho W.L. & Lopes C.W.G. 2013. *Eimeria spp. from Japanese quails (Coturnix japonica): new characteristic features and diagnostic tools*. Pesquisa Veterinária Brasileira 33(12):1441-1447. Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23897-970, Brazil. E-mail: bertobp@ufrrj.br

The Japanese quail *Coturnix japonica* originated from North Africa, Europe and Asia, is used worldwide as an experimental animal and model for aviculture. The current paper characterizes *Eimeria bateri*, *Eimeria tsunodai* and *Eimeria uzura* recovered from *C. japonica*. Based on the fact that quails have a global distribution, as are their coccidia, the findings of this study should provide the means for diagnosis of those *Eimeria* spp. in other regions and continents. *Eimeria bateri* showed the greatest intensity of infection and shed oocysts from the fourth day after infection; in contrast, *E. tsunodai* and *E. uzura* shed oocysts from the fifth day after infection. The three species shared a high degree of similarity and were all polymorphic. Yet, the application of line regressions, histograms and ANOVA provided means for the identification of these species. Finally, the algorithm was very efficient since verified that resultant values were not superimposed.

**INDEX TERMS:** Coccidiosis, diagnostic, *Eimeria bateri*, *Eimeria tsunodai*, *Eimeria uzura*.

**INTRODUCTION**

The breeding of Japanese quails *Coturnix japonica* has excelled in aviculture, due to increased consumption of exotic meats and eggs, and represents an alternative to chicken production. In the USA, Japanese quails were introduced in the 1950s, and at that time the breeding of Japanese quails was already widespread in European countries. In Brazil, Japanese quails were introduced in the 1950s, but large scale production began in the mid 1960’s (Howes 1964, Murakami & Arik 1998, IUCN 2013, Jatoi et al. 2013).

In addition to its importance for aviculture, the Japanese quail has become, in recent decades, an important experimental animal for scientific research. It is used extensively in studies examining genetics, nutrition, toxicology, em-

In common with the situation encountered in breeding of other poultry species, parasites represent a limiting factor in high-production commercial rearing farms and even in rustic breeding systems, where economic losses can be significant. Among the most pathogenic parasites, coccidiosis caused by species of *Eimeria* is distinguished by severe enteropathy, which promotes anorexia, decreased reproduction and posture in adults, and is responsible for high levels of mortality in young animals (Mazurkiewicz et al. 1967, Norton & Pierce 1971, Tsunoda & Muraki 1971, Ruff et al. 1984, Teixeira et al. 2004).

The present study characterizes three *Eimeria* spp. commonly encountered on farms breeding Japanese quails *C. japonica*, with focus on the dynamics of oocyst shedding and the provision of detailed morphological and morphometrical data. In addition, an algorithm designed to enable reliable identification of individual species of *Eimeria* during routine diagnosis of Japanese quail pathogens is proposed.

**MATERIALS AND METHODS**

**Japanese quails and facilities**

Eight one-day-old Japanese quails *Coturnix japonica* were obtained from a commercial rearing farm located in the Municipality of Seropédica in the State of Rio de Janeiro, Brazil. The chicks were transported to the Federal Rural University of Rio de Janeiro (UFRRJ), and were reared and fed in a single cage without anticoccidial additives. Feed and water were administered ad libitum. Sample processing and data analysis, as described below, were conducted at the Laboratório de Coccídios e Coccidiose in the Departamento de Biologia Animal (Biologica area), both located at UFRRJ. This study was approved by UFRRJ Ethics Committee under protocol No. 333/2013.

**Eimeria spp.**

The adult Japanese quails from the commercial rearing farm used to provide the chicks were naturally parasitized by three species of *Eimeria*: *Eimeria bateri* (Bhatia et al., 1965), *Eimeria tsunodai* (Tsutsumi, 1972) and *Eimeria uzura* (Tsunoda et Muraki, 1971). An inoculum for experimental infection was produced by recovering and isolating oocysts from fecal samples of positive Japanese quails 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 K2Cr2O7 to induce sporulation and maintained in a refrigerator (2-5°C) until use. All eight *Coturnix japonica* chicks were inoculated at the age of 7 days, using inocula, quantified using a Neubauer chamber, containing approximately 6.5 × 104 sporulated oocysts per chick, at the approximate proportion of: 50% *E. bateri*; 25% *E. tsunodai*; and 25% *E. uzura*.

**Study of oocysts**

The number of oocysts shed in the feces was determined on days 2, 3, 4, 5, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28 after infection (DAI). Enumeration of oocysts per gram of feces (OoPG) was performed according to Menezes & Lopes (1995), as modified by Cardozo et al. (2010).

Oocysts were examined microscopically using the technique described by Duszynski & Wilber (1997). Morphological observations and measurements, given in micrometres, were made using a Carl Zeiss binocular microscope 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. Photomicrographs were taken using a digital camera (Sony CD Mavica MVC-CD250). Size ranges are shown in parentheses followed by average and shape index (L/W ratio). Abbreviations: micropyle [M], oocyst residuum [OR], polar granule [PG], Stieda body [SB], substieda body [SSB], parasitied body [PSB], sporocyst residuum [SR], sporozoite [SZ], refractile body [SRB], nucleus [N], length [L] and width [W].

**Statistical evaluation**

Three statistical methods were employed: 1) Histograms were prepared to plot the values of the length, width and shape-index of the oocysts, as well as their relative frequencies, according to Sampaio (2002) and Berto et al. (2008, 2011); 2) Analysis of variance (ANOVA) was used to compare measurements of the length, width and shape-index of the oocysts and sporocysts of individual *Eimeria* spp. The software Microsoft® Excel 2007 was used to calculate the mean, variance, degree of freedom and p value (Gomez et al. 1982, Sampaio 2002, Berto et al. 2011); 3) Linear regression to determine the distribution of *Eimeria* spp. sporulated oocysts using methods proposed by Norton & Joyner (1981) and subsequently modified by Sampaio (2002) and Berto et al. (2011). The graphs and coefficient of regression line were obtained using the software Microsoft Excel 2007®.

**Algorithm for identification**

Morphological and morphometrical characteristics from a total of 150 oocysts were analyzed to create an identification algorithm. The size of SSB and shape of the oocyst wall ends were estimated and transformed to numerical values (SSB appearance: 0 = prominent, 0.5 = barely discernible; Oocyst wall ends: 0 = rounded ends, 0.7 = one of the ends flattened). Thereafter, the following equation was developed to differentiate *E. bateri*, *E. tsunodai* and *E. uzura*:

\[
A = \left(\frac{W_0}{L}\right) - \left[\frac{nPG}{x^{1/3}}\right] + OWe + SSBa
\]

where A is the algorithm value, W0 = width of oocyst, LS = length of sporocysts, nPG = number of PG, OWe = oocyst wall ends, and SSBa = SSB appearance.

**RESULTS**

**Dynamic of oocysts shedding**

*Eimeria bateri* oocysts were observed commencing on the fourth day after infection, whereas, oocysts of *E. tsunodai* and *E. uzura* were shed from the fifth day after infection (Fig. 1). *Eimeria bateri* demonstrated a greater intensity of infection shedding a total of 849,425 (71%) oocysts, followed by *E. tsunodai* with 268,465 (22%) and *E. uzura* with 77,718 (7%) oocysts. It was observed that each *Eimeria* spp. showed four peaks of oocyst shedding until the 28th DAI, with some shedding periods being of extended duration and at different periods when the three species were compared (Fig.1).
Eimeria spp. oocysts characterization

All the Japanese quails experimentally infected shed oocysts in feces. Initially, the oocysts were non-sporulated, by day three, 70% of them were sporulated.

Eimeria bateri (Bhatia et al. 1965). Oocysts (n= 50) (Figs. 2a,d-f, 3a-b) were sub-spheroidal to ellipsoidal. OW bi-layered and smooth, ~1.0 thick. M and OR absent, but one or two PG present. Sporocysts ovoidal. SB nipplelike, ~1.0 high × 1.5 wide. SSB rounded, prominent, ~1.0 high × 2.0 wide. PSB absent. SR composed of granules of different sizes. SZ vermiform with single posterior SRB and centrally located N. The morphometry of the sporulated oocysts of E. bateri can be visualized in Table 1. The histograms and linear regressions can be visualized in Figures 4a,d and 5 respectively.

Eimeria tsunodai (Tsutsumi, 1972). Oocysts (n=50) (Figs. 2b,g-i, 3c-d) were sub-spheroidal to ellipsoidal. OW bi-layered and smooth, ~1.0 thick. M and OR absent, but one or two PG present. Sporocysts ovoidal. SB knob-like to half-moon-shaped, ~0.5 high × 1.5 wide. SSB rounded, prominent, ~1.0 high × 2.0 wide. PSB absent. SR composed of many granules. SZ vermiform with single posterior SRB and centrally located N. The morphometry of the sporulated oocysts of E. tsunodai can be visualized in Table 1. The histograms and linear regressions can be visualized in Figures 4b,e and 5 respectively.

Eimeria uzura (Tsunoda et Muraki, 1971). Oocysts (n=50) (Figs. 2c,j-l, 3e-f) were ovoidal. OW bi-layered and smooth, ~1.0 thick. M and OR absent, but five or more PG present. Sporocysts elongate. SB knob-like to half-moon-shaped, ~0.5 high × 1.5 wide. SSB rounded, prominent, ~1.0 high × 2.0 wide. PSB absent. SR composed of many granules. SZ vermiform with single posterior SRB and centrally located N. In Table 1, the morphometry of the sporulated oocysts of E. uzura can be visualized. The histograms and linear regressions can be visualized in Figures 4c,f and 5 respectively.

Algorithm for identification

The values obtained using the identification algorithm as applied to 150 eimerian oocysts identified in fecal samples of experimentally infected quails are presented Table 1. It was observed that the range of values obtained from the three Eimeria spp. were not superimposed, and in addition they showed significant differences between means.

DISCUSSION

Japanese quail are not a Brazilian species, having originating from North Africa, Europe and Asia, therefore the coccidia characterized in this work are not regional, but instead should represent those which parasitized the first lots of quails that were imported into Brazil in the 1950s (Howes 1964, Murakami & Ariki 1998, IUCN 2013, Jatoi et al. 2013).
Table 1. Comparative morphology of *Eimeria* spp. recovered from *Corturnix* spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>References</th>
<th>Oocysts</th>
<th>Sporocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Shape</td>
<td>Length (µm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eimeria dispersa</em></td>
<td><em>Coturnix</em></td>
<td>Tyzzer (1929)</td>
<td>ovoidal</td>
<td>17.2</td>
</tr>
<tr>
<td><em>Eimeria coturnicis</em></td>
<td><em>C. coturnix</em></td>
<td>Chakravarty &amp; Kar (1947)</td>
<td>ovoidal</td>
<td>26.4</td>
</tr>
<tr>
<td><em>Eimeria taldykyrogaica</em></td>
<td><em>C. coturnix</em></td>
<td>Svambaev &amp; Utebaeva (1973)</td>
<td>ovoidal</td>
<td>21.9</td>
</tr>
<tr>
<td><em>Eimeria tahanensis</em></td>
<td><em>Coturnix</em></td>
<td>Amoudi (1987)</td>
<td>ellipsoidal</td>
<td>36.5</td>
</tr>
<tr>
<td><em>Eimeria bateri japonica</em></td>
<td><em>C. coturnix</em></td>
<td>Berto et al. (2008)</td>
<td>subspherical</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current work</td>
<td>subspherical</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>to ellipsoidal</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eimeria tsunodai japonica</em></td>
<td><em>C. japonica</em></td>
<td>Berto et al. (2008)</td>
<td>current work</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eimeria fluminensis</em></td>
<td><em>C. japonica</em></td>
<td>Teixeira et al. (2004)</td>
<td>subspherical</td>
<td>17.3</td>
</tr>
<tr>
<td><em>Eimeria sp.</em></td>
<td><em>C. japonica</em></td>
<td>Bashtar et al. (2010)</td>
<td>ovoidal to ellipsoidal</td>
<td>17.7</td>
</tr>
<tr>
<td><em>Eimeria sp.</em></td>
<td></td>
<td></td>
<td>spherical</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Algorithm equation applied for the differentiation of *E. bateri*, *E. tsunodai* and *E. uzura*. a, b, c Means with the same letters within columns are not significantly different (P < 0.05).

The prepatent periods recorded for the three Eimeria spp. from experimentally infected quails in this study (4 days for E. bateri, and 5 days for E. tsunodai and E. uzura) were similar to those described by Bhatia et al. (1965), Tsunoda & Muraki (1971) and Bashtar et al. (2010). With regards to transmission, E. bateri appeared to be the most successful in mixed infections, based on the observation that it shed a greater quantity of oocysts during the experimental period (Fig.1).

Bhatia et al. (1965), Tsunoda & Muraki (1971) and Tsutsumi (1972) described the three species characterized in this study, but did they lacked the current optical resources that allowed improved visualization and detailed descriptions of sporulated oocysts. Teixeira & Lopes (2002) and Teixeira et al. (2004) described morphologic details of oocysts of these species, but some data essential for differentiation were not provided, such as flattening of one of the ends of the oocysts of E. tsunodai and the presence of SSB in all three species. Furthermore, in those articles the description of the SB as piriform in E. uzura and the SB as triangular in E. tsunodai was in fact making reference to the SB and SSB complex. More recently, Berto et al. (2008) reported oocysts of E. bateri similar to those described in the present study, but they did not characterize the species. Table 1 compares the morphology and morphometry of all Eimeria spp. recovered from Coturnix japonica.

Morphometric data from the current study served to characterize the polymorphic nature of the oocysts of each of the three Eimeria spp., which was more pronounced for E. uzura and less so for E. bateri. This polymorphism could be verified using linear regression analysis (Fig.5), which demonstrated multiple variations for values of width on length, as evidenced by the low value of R². Oocyst polymorphism has been described previously by several authors for a variety of coccidia and it is caused by different factors associated with the environment and host parasite relationship (Duszynski 1971, Catchpole et al. 1975, Fayer 1980, Joyner 1982, Gomez et al. 1982, Parker & Duszynski 1986, Gardner & Duszynki 1990, Berto et al. 2011). Importantly, the proximity of the regression lines and superimposed points demonstrated the inability to distinguish the three Eimeria spp. based solely on the length and width data (Fig.5), although ANOVA resulted in significant differences between length and width means (Table 1). In addition, some morphometric characteristics were evidenced in linear regression. The regression line was more inclined for E. bateri, which indicated a tendency for the oocysts to adopt an ellipsoidal shape; E. bateri has larger oocysts which are separated from those of E. tsunodai and E. uzura in the graph (Fig.5).
The histograms based on length and width data (Fig. 4a-c) showed a regular distribution, besides revealing tendencies in the dimensions: 24.7-27.2 × 18.0-20.0 μm for *E. bateri*; 18.6-19.9 × 15.7-16.6 μm for *E. tsunodai*; and 23.1-24.7 × 17.5-18.3 μm for *E. uzura*. The histograms of shape-index (Fig. 4d-f) revealed higher frequencies in the range of values from 1.2 to 1.5 for the three *Eimeria* spp. This observation indicated a tendency for the oocysts to adopt an ellipsoidal or ovoidal shape. In *E. uzura* specifically, it can be affirmed that there is no subspherical oocyst, since the values begin at 1.1 (Table 1, Fig. 4f). Berto et al. (2011) observed similar aspects for *Isospora* spp. recovered from passerines birds on Marambaia Island in the State of Rio de Janeiro, Brazil.

The ANOVA applied for sporocysts also allows some morphometric comparisons (Table 1): *Eimeria tsunodai* and *E. bateri* have equivalent means of length and significant differences of width, readily justified by the greater width of *E. bateri* sporocysts; the equivalent means between the values of shape-indexes are also justified because the sporocysts are larger or smaller in the three *Eimeria* spp., however, the measures increased and decreased at the...
same rate; and the significant differences of length among *E. uzura* and *E. tsunodai* and *E. bateri* conditioned part of the preparation of the algorithm through association with the oocyst width, which is not proportional in the three *Eimeria* spp. The algorithm was completely efficient for the identification of oocysts, since it verified that species specific values did not superimpose following the application of the equation. Thus, values between 1.1 to 1.6 provided confident identification of *E. bateri*, whereas values lower than, or equal to, 1.0 supported the identification of *E. uzura* and values greater than or equal to 1.7 provided the identification of *E. tsunodai*. Algorithms for the differentiation of porcine *Eimeria* spp. dividing seven species into groups were implemented by Daugschies et al. (1999), and Hassum et al. (2007) created algorithms for the differentiation of *Eimeria* spp. from sheep. In the current research, the lower number of species (n=3), allowed the creation of a single algorithm which was sufficiently robust for differentiation. Based on the successful application of this tool, we would encourage the development of more algorithms for identification of oocysts in other hosts.

**Acknowledgements**- The study was supported by grants from the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) to B.P. Berto (E-26/110.987/2013). We also thank the commercial rearing farm of Japanese quails at the Municipality of Seropédica in the State of Rio de Janeiro, Brazil, for permitting and assisting in the collection of material.

**REFERENCES**


