

Two new species of coccidia, *Eimeria pavota* and *Eimeria egyptica* parasitic in white peacocks, *Pavo cristatus* (Galliformes: Phasianidae) in Egypt

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Abstract. Two *Eimeria* species were identified in faeces collected from a white peacock (*Pavo cristatus*) housed at the El-Gharbia city park in Egypt, and each is described as a new species. Sporulated oocysts of one species (*Eimeria pavota*) are ellipsoidal, measuring 23.9 x 16.5 (19.5–25 x 14.5–20) μm . The oocysts have a smooth wall made up of two layers and approximately 1.0 μm in thickness. An oocyst residuum, micropyle and polar granule are present. Sporocysts have a rounded end opposite knob-like Stieda body and are 14.3 x 7.3 (12–16.5 x 5.5–8) μm in size. The sporozoites contain one refractile body and a diffuse granular residuum; the entire content of each sporocyst is enclosed by a thin membrane. Sporulated oocysts of the second eimerian (*Eimeria egyptica*) are subspherical, measuring 14 x 20 (12–17 x 18–22) μm . The oocysts are without a micropyle but with a two-layered wall, which is approximately 1.3 μm thick, with the outer layer having a smooth surface texture. The oocyst residuum and polar granule are present. Sporocysts have a rounded end opposite the nipple-like Stieda body atop a prominent sub-Stieda body and are 14.4 x 8.0 (12–15.5 x 6.5–9) μm . Sporocysts contain a diffuse granular residuum, and each sporozoite has two different-sized refractile bodies.

INTRODUCTION

Pavo cristatus, Linnaeus (Galliformes: Phasianidae) is of Indian origin where it is designated as a national bird and it still exists as a wild bird. Currently peacocks are kept as ornamental birds, worldwide. They usually suffer from parasitic infections, which are mostly subclinical but may also cause mortality (Freitas *et al.*, 2002). Amongst parasitic diseases, protozoan diseases especially coccidiosis tops the list of parasites affecting birds worldwide.

Although parasites usually cause little or no distress to healthy individuals in the wild, parasitic infections are among the most common sanitary problems affecting captive birds, especially in high-density populations (Barnes, 1986).

Avian coccidiosis, an intestinal disease caused by protozoan parasites of the genus *Eimeria*, occurs worldwide. It is considered to be one of the most economically important diseases characterized by morbidity, mortality and reduced production efficiency of affected poultry and other livestock (Dolnik, 2006).

To date, eight species of eimerian parasites have been described from Indian peacocks worldwide, of which five species of *Eimeria* are known from the feces of Indian peacock species, *Pavo cristatus* from south Asia, particularly India, which is the area with the highest diversity of Indian peacocks species viz. *E. pavonina* (Banik & Ray, 1961); *E. mandali* (Banik & Ray, 1964); *E. pavonis* (Mandal, 1965); *E. mayurai* (Bhatia & Pande, 1966) and *E. patnaiki* (Ray, 1966)

have been described. In addition, Amoudi (1988) described two new species of coccidia; *Eimeria riyadhae* and *Eimeria arabica* from the faeces of local Indian peacocks reared in the experimental agriculture station of king Saud University, Saudi Arabia. Likewise, Alyousif and Al-Shawa (1998) further examined several *Eimeria* species such as *Eimeria mayurai* (Bhatia & Pande, 1966), *Eimeria pavonina* (Banik & Ray, 1961) and *Eimeria pavonis* (Mandal, 1965) and they also gave a description of *Eimeria mutica* and *Eimeria kharjensis* as a new eimerian species from the faeces of the local green peacocks, *Pavo muticus* of the Al-Kharj area in central Saudi Arabia. Recently, Elshahawy (2010) described a new species of coccidia, *Eimeria pavaegyptica* from the faeces of local Indian peacocks reared in the zoological park of El-Gharbia governorate, Egypt.

In the present paper, I describe the oocysts of these two new species of the genus *Eimeria* and compare them morphologically with other similar species of *Eimeria* infecting another member of the same host genus.

MATERIALS AND METHODS

During July 2014, Fresh faecal droppings of 16 identified, free range adult white peacocks, *P. cristatus* were collected from the zoological park of El-Gharbia governorate, in central Egypt and brought to the laboratory, Department of parasitology, Faculty of Veterinary Medicine, South Valley University, for coprological examination. The samples were examined by direct faecal smear method and simple flotation technique to detect parasitic oocysts (Soulsby 2005). Each fecal sample was transferred to a 20-ml plastic container with a screw cap, diluted and mixed with the same volume (1 g diluted with 1 ml) of 3% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) solution. The samples were kept for a week at room temperature (23–25°C) for sporulation. Twenty five sporulated oocysts and their inner structures were examined after

concentration by flotation using a saturated sodium chloride solution for 5 min at 1,500 RPM (Brown & Neva 1983). Morphological observations were performed according to Duszynski and Wilber (1997) and Wilber *et al.* (1998). These morphological observations included several oocyst characters such as length (L), width (W) and their ranges and ratio (L/W), micropyle (M), micropylar cap, oocyst residuum (OR) and polar granule (PG). In addition, the sporocyst characters observed included L, W and their L/W, Stieda body (SB), substieda body (SSB), sporocyst residuum (SR), sporozoites (SP), refractile bodies (RBs) and nucleus (N) in SP. The measurements were performed by using a binocular microscope Leica equipped with a calibrated ocular micrometer. All measurements in the text are given in micrometers (μm) and are means followed by the ranges in parentheses. Photomicrographs and line-drawings were made with a binocular microscope Leica leitz Biomed and using a digital camera Wild MPS 32 and Wild M3X, respectively. The species identification was based on the complex morphology of the sporulated eimerian oocyst (Pellerdy, 1965; Levine, 1985; Soulsby, 2005).

RESULTS

Two *Eimeria* species were found. After examination, measurements and photomicrographs of the studied oocysts were compared to the eight *Eimeria* species already described from *Pavo cristatus* (Banik & Ray, 1961; 1964; Mandal, 1965; Bhatia & Pande, 1966; Ray, 1966; Amoudi, 1988; Alyousif & Al-Shawa, 1998; Elshahawy, 2010), as well as to dozens of *Eimeria* species described from other bird species in the family Phasianidae (Rommel, 2000; Smith *et al.*, 2003). These comparisons convinced me that both of the forms found were new species, which described below.

***E. pavota* n. sp.**

Type-host: *Pavo cristatus* Linnaeus, white peacock.

Type-locality: El-Gharbia Governorate, Egypt (30° 52' 51.6" N, 31° 3' 36" E).

Prevalence: 6/16 (37.5%).

Site of infection: Not investigated

Type-material: Oocysts are kept in 10% aqueous buffered formalin (v/v) and deposited in the Parasitology Collection, in the Department of Animal Parasitology, at FVM, located in QENA governorate, EGYPT. Phototypes and line drawings are deposited at the same location. The repository number is Pavo 2.

Sporulation time: 3 days at RT (23°C).

Etymology: The specific name was derived from the scientific name of the host.

Description (Figs. 1, 1a, 2)

Sporulated oocyst

Oocyst shape: ellipsoidal; number of walls: 2; wall thickness: 1.0 (0.9–1.3); wall characteristics: outer wall smooth, ~4/5 of total thickness and appears bicolored, outermost portion dark brown, innermost

portion yellow to red-brown, inner wall dark. M usually visible, ~1.7 wide, usually medial, but sometimes slightly displaced to one side. LxW (n=25), 23.9x16.5, (19.5–25x14.5–20); L/W ratio, 1.5 (1.2–1.8); OR present with an average diameter of ~3 and it consists of one small homogenous globule is found near the bottom of the oocyst and above the sporocyst. One PGs present, ellipsoidal in shape, 1.6x2.5 (2–3x1–2.5).

Distinctive features of oocyst: M usually visible, one prominent PGs, distinct OR, and thin bicolored outer wall, with a dark inner wall.

Sporocyst and sporozoites

Sporocyst: ovoidal, rounded at end opposite SB; LxW (n=25), 14.3x7.3, (12–16.5x5.5–8); L/W ratio, 2.3 (1.9–2.7); SB, present, knob-like; SSB and PSB, absent; SR, present; SR characteristics: several dozens of dispersed

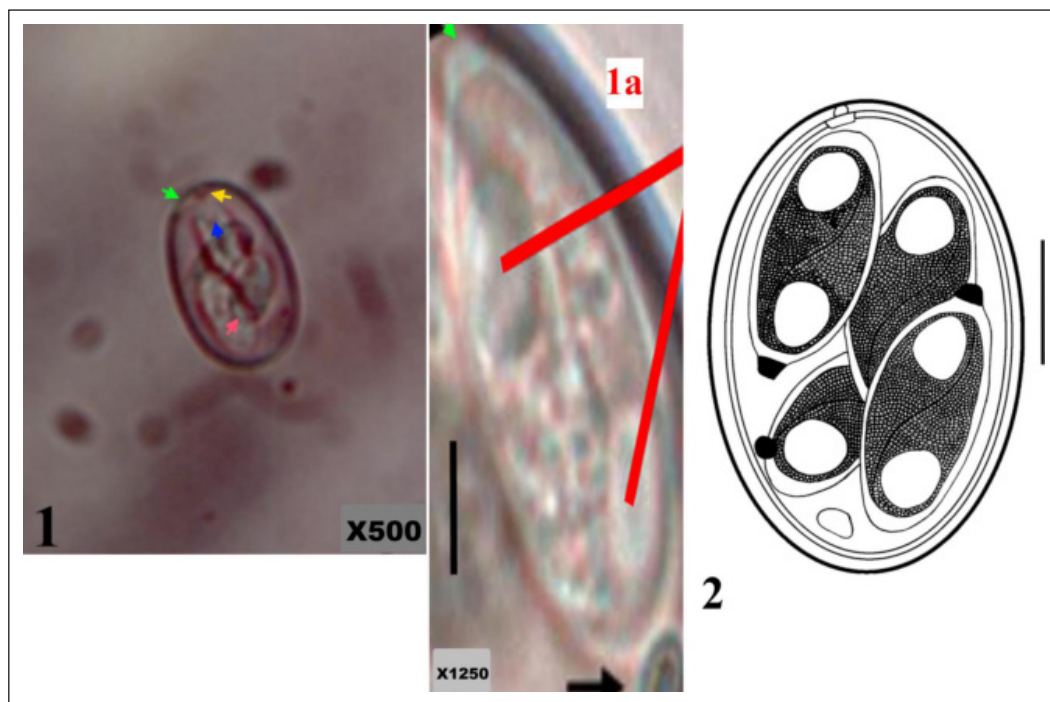


Figure 1. Photomicrographs of living, sporulated oocyst of *Eimeria pavota* n. sp. recovered from the faeces of white peacocks in Egypt. Oocyst showing polar granule (yellow arrow), residuum (pink arrow), large refractile body (blue arrow) and micropyle (green arrow) (X500).

Figure 1a. Photomicrographs of sporulated oocyst showing the characteristics of sporocyst; large refractile body (red line), Stieda body (green arrow) and polar granule (black arrow) (X1250).

Figure 2. Composite line drawing of a sporulated oocyst of *Eimeria pavota* n. sp. Bar = 10 µm.

granules; SP have one posterior large RB, subspheroid 3.9x2.9 (3–5x2–4); a thin membrane encloses the contents of each sporocyst.

Distinctive features of sporocyst: knob-like SB, granular SR, one large RB in each SP, and contents enclosed within a thin membrane.

Remarks:

The metrical characteristics of the sporulated oocysts of the new species are compared with the eight eimerians known from Indian peacocks as well as with *Eimeria mutica* and *Eimeria kharjensis* from green peacock (Table 1).

The sporulated oocysts of the new species are most similar to those of *Eimeria mayurai*, but differ in many ways; those of *E. pavota* sp. n., they have clear micropyle, distinct OR, nipple-like SB, granular SR, one large RB in each SP, and contents enclosed within a thin membrane, whereas those of *Eimeria mayurai* have a small micropyle and lacks an oocyst residuum.

The primary differences between *E. pavota* and the recently described *E. pavoegyptica* from Indian peacock from Egypt, revolve around the morphological features of the oocysts, (smaller size for *E. pavoegyptica* vs. larger size for *E. pavota*), in the presence of micropyle and ovoidal-shaped sporocyst with only one large refractile granules.

Among the *Eimeria* species with ellipsoidal oocysts, *E. pavota* n. sp., can be easily discerned from *E. riyadhae* by its considerably smaller size and the presence of M and OR, both absent in *E. riyadhae*. Moreover, *E. pavota* possess one large RB in each SP, while the oocysts of *Eimeria kharjensis* are characterized by the presence of M with a dome-shaped cap and absence of OR. Additionally, oocyst dimensions of all other eimerian species from the host genera are distinctly different.

E. muta from wild rock ptarmigans, *Lagopus muta islandorum* (Skirnisson & Thorarinsdottir, 2007) also closely resemble the present new species in terms of the shape of the oocyst, wall characteristics and in the

presence of M, but it mainly differs from this newly species in having one to three prominent PGs and by the absence of OR. These above-mentioned morphological peculiarities and the different host genus make *E. pavota* a clearly separate form.

On the basis of the abovementioned differences, in addition to the geographic isolation of the hosts, I consider this newly discovered species, described here for the first time, to be a new coccidian and I propose to name it *E. pavota*.

***Eimeria egyptica* n. sp.**

Type-host: *Pavo cristatus* Linnaeus, white peacock.

Type-locality: El-Gharbia Governorate, Egypt (30° 52' 51.6" N, 31° 3' 36" E).

Prevalence: 3/16 (18.8%).

Site of infection: Not investigated

Type-material: Oocysts are kept in 10% aqueous buffered formalin (v/v) and deposited in the Parasitology Collection, in the Department of Animal Parasitology, at FVM, located in QENA governorate, EGYPT. Phototypes and line drawings are deposited at the same location. The repository number is Pavo 3.

Sporulation time: 4 days at RT (23°C).

Etymology: The specific name was derived from the country, Egypt, where the species was discovered.

Description (Figs. 3, 3a, 4)

Sporulated oocyst

Oocyst shape: subspherical; number of walls: 2; wall thickness: 1.3 (1.2–1.6); wall characteristics: outer wall rough, ~2/3 of total thickness and appears bicolored, outermost portion light green, innermost portion red-brown, inner wall dark. M absent. LxW (n=25), 14x20, (12–17x18–22); L/W ratio, 1.1 (1.0–1.3); OR present with an average diameter of ~2.5 and it consists of one small homogenous globule is found in the middle. One PG present, round in shape, 1.7x1.2 (3–1x2–0.8).

Distinctive features of oocyst: M absent, usually one prominent PGs, OR present, smooth, bicolored outer wall, with light brown inner portion, and a dark inner wall.

Table 1. Comparative data of eimerian species described from Pavo hosts

Eimeria species	Host	oocyst				Sporocyst				Locality	Authors
		Shape	Size (µm)	M	MC	OR	Shape	Size (µm)			
<i>Eimeria mandali</i>	<i>Pavo cristatus</i>	Spherical	14–20 x 14–18	+	-	-	No data	6–12 x 4–8	India	Banik and Ray (1964)	
<i>Eimeria mayurai</i>	<i>P. cristatus</i>	Ellipsoidal	23–27 x 13–16	+	-	-	Ovoid	10–13 x 5–7	India	Bhatia and Pande (1966)	
<i>Eimeria pavonina</i>	<i>P. cristatus</i>	Ovoid	20–28 x 16–20	+	-	+	Boat shaped	6–16 x 4–8	India	Banik and Ray (1961)	
<i>Eimeria pavonis</i>	<i>P. cristatus</i>	Ovoid	20–25 x 18	+	-	-	Ellipsoidal	12.1–15 x 6.6	India	Mandal (1965)	
<i>Eimeria patnaiki</i>	<i>P. cristatus</i>	Spherical	17–19 x 13–17	-	-	+	Lennon shaped	6.4–8.5 x 3.2–4.8	India	Ray (1966)	
<i>Eimeria rigadhae</i>	<i>P. cristatus</i>	Ellipsoidal	27–30.5 x 20.5–25	-	-	-	Ovoid	11–14.5 x 6.5–8	Saudi Arabia	Amoudi (1988)	
<i>Eimeria arabica</i>	<i>P. cristatus</i>	Spherical	17.5–21.5 x 17.5–21.5	-	-	-	Ovoid	9.5–12 x 4–6.5	Saudi Arabia	Amoudi (1988)	
<i>Eimeria mutica</i>	<i>Pavo muticus</i>	Subspherical	22.4–25 x 16.2–18.7	+	-	-	Elongate-ovoid	12–14.2 x 5.4–6.7	Saudi Arabia	Alyousif and Al-Shawa (1998)	
<i>Eimeria kharjensis</i>	<i>P. muticus</i>	Broadly ellipsoidal	19–21.5 x 16.2–18.7	+	+	-	Elongate-ovoid	11.9–13.5 x 5.4–6.8	Saudi Arabia	Alyousif and Al-Shawa (1998)	
<i>Eimeria pavaegyptica</i>	<i>P. cristatus</i>	Ellipsoidal	13–16 x 10–12.9	-	-	+	Boat-shaped	9–11 x 4–4.7	Egypt	Elshahawy (2010)	
<i>Eimeria pavota</i>	<i>P. cristatus</i>	Ellipsoidal	23.9 x 16.5	+	-	+	Ovoid	14.3 x 7.3	Egypt	This paper	
<i>Eimeria egyptica</i>	<i>P. cristatus</i>	Subspherical	14 x 20	-	-	+	Ovoid	14.4 x 8.0	Egypt	This paper	

M: micropyle; MC: micropylar cap; OR: residuum

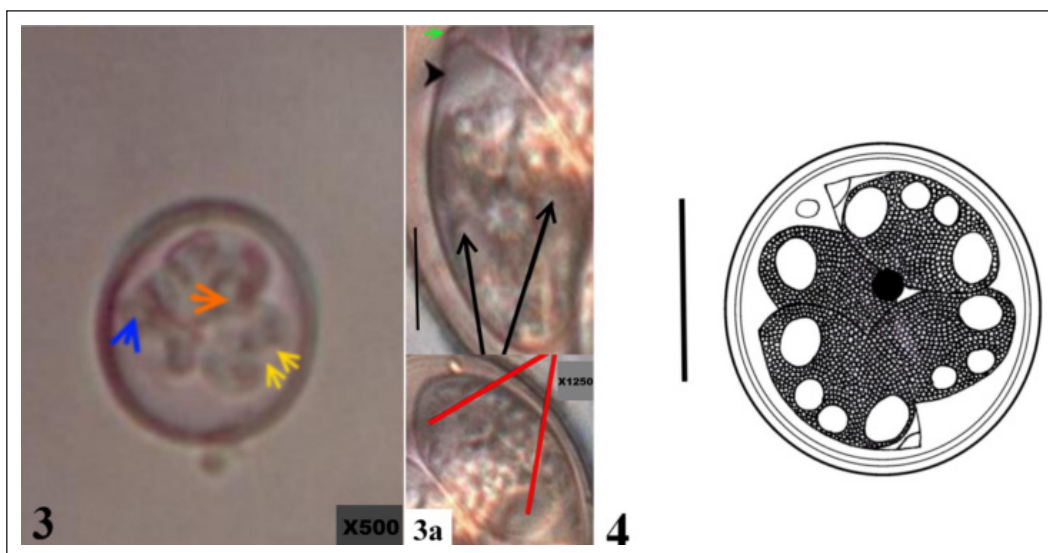


Figure 3. Photomicrographs of living, sporulated oocyst of *Eimeria egyptica* n. sp. recovered from the faeces of white peacocks in Egypt. Oocyst showing polar granule (blue arrow), residuum (orange arrow) and small refractile body (yellow arrow) (X500).

Figure 3a. Photomicrographs of sporulated oocyst showing the characteristics feature of sporocyst; Stieda body (green arrow), Sub Stieda body (headed arrow), small refractile body (black arrow) and large refractile body (red lines) (X1250).

Figure 4. Composite line drawing of a sporulated oocyst of *Eimeria egyptica* n. sp. Bar = 10 μ m.

Sporocyst and sporozoites

Sporocyst: ovoidal, rounded at end opposite SB; LxW (n=25), 14.4x8.0, (12–15.5x6.5–9); L/W ratio, 1.8 (1.6–2.2); SB, present, nipple-like; SSB, present; PSB, absent; SR, present; SR characteristics: large, scattered granules, obscure SP detail; SP have two RBs, one ellipsoidal 3.9x2.9 (3–5x2–4) and one round, ~2.5, often difficult to see under the dense SR granules.

Distinctive features of sporocyst: nipple-like SB, prominent SSB, large, granular SR and two RBs, one large, ellipsoidal, another smaller, round, in each SP.

Remarks:

The subspherical oocysts of *E. egyptica* resemble *Eimeria mutica* (Alyousif and Al-Shawa, 1998). Various morphological characters, however, distinguish these coccidians; the wall color is different (bicolored outer wall with a dark inner wall in *E. egyptica*, yellowish wall in *Eimeria*

mutica); oocyst sizes are different (14x20 in *E. egyptica*, 22.4x16.2 in *Eimeria mutica*); the presence of M (present in *E. mutica*, absent in the new species); the sporocyst form is different (ovoidal in *E. egyptica*, elongate-ovoid in *Eimeria mutica*); and a prominent SSB is present in *E. egyptica* but is absent in *Eimeria mutica*.

Oocysts of *E. egyptica* differ from *E. mandali* by the absence of micropyle and the presence of OR and a SSB. Additionally, it differs from *E. arabica* by the presence of OR; it's comparatively smaller size and the presence of a SSB. Furthermore, *E. patnaiki* can readily be differentiated by comparatively larger oocyst, lacking a SSB and lemon-shaped sporocysts. Likewise, the presence of a nipple-like SB with two different-sized RB in each SP and a uniformly granular SR covering the entire SP can easily differentiate the new parasite from *E. patnaiki*, which possess a vestigial SB with several small scattered SR granules within the sporocysts.

The ovoidal-shaped oocyst form of *E. pavonina* and *E. pavonis* distinguishes these species from the sub-spherical shaped oocyst form of *E. egyptica*. Furthermore, the absence of M and the presence of SSB also differentiate *E. egyptica* from *E. pavonina* and *E. pavonis*, which all have a M and lack SSB. Moreover, the measurements of *E. egyptica* oocysts do not overlap with the size ranges reported for both of the abovementioned species. Therefore, these morphological features are distinct and I consider the coccidian described herein as new to science.

DISCUSSION

Eimeria spp. are coccidian parasites that infect a wide range of vertebrate hosts (McDonald & Shirley, 2009). With more than 1300 described species (Duszynski *et al.*, 2000), the genus is one of the most species eukaryotic taxa. Pathogenic eimerian species that cause severe clinical disease and economic loss in poultry and production animals have been well characterized (Aarathi *et al.*, 2010; Taubert *et al.*, 2010).

Remarkably, eimerian parasites of birds are generally considered to be highly host specific not only under natural conditions (Hiepe & Jungmann, 1983) but also in farmed birds (Rommel, 2000). Traditionally, identification of *Eimeria* species has been based largely on sporulated oocyst morphology but also on host species, pathology and geographic distribution (Duszynski & Wilber, 1997; Tenter *et al.*, 2002).

To date, eight species of *Eimeria* have been reported from Indian peacock, *Pavo cristatus* from various localities in India, Saudi Arabia and Egypt [Banik & Ray 1961 (*E. pavonina*); Banik & Ray 1964 (*E. mandali*); Mandal 1965 (*E. pavonis*); Bhatia & Pande 1966 (*E. mayurai*); Ray 1966 (*E. patnaiki*); Amoudi 1988 (*E. riyadhae* and *E. arabica*) and Elshahawy 2010 (*E. pavoegyptica*)]. Furthermore, *E. mutica* and *E. kharjensis* have been reported from

green peacock *P. muticus* in Saudi Arabia (Alyousif & Al-Shawa, 1998). The low scientific effort in various parts of the world, however, suggests that much of the eimerian diversity remains to be discovered as very few hosts have been surveyed.

In the present study, morphological data was used to describe *E. pavota* and *E. egyptica* n. sp. found in the faeces of white peacock in Egypt. Future studies on coccidia within peacocks and other Galliformes in Egypt should continue to provide new species, and once more extensive surveys are completed, our understanding of the biogeography of these parasites will become clearer.

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