



## First Detection of *Eimeria* (Apicomplexa: Eimeriidae) in *Emys Orbicularis* in Azerbaijan



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### Abstract

EUROPEAN pond turtle, *Emys orbicularis* are an essential component of freshwaters and marshes cenoses and evaluation of parasites in their populations is essential for the management of conservation processes. Coccidiosis is the most common intestinal infection in free-living and captive reptiles. The aim of this study was to characterize the presence of *Eimeria* sp. in European pond turtle. Faecal samples were examined for the presence of coccidian oocysts by the standard flotation technique with saturated sodium chloride (NaCl; sp.gr. 1.18). A Leica DM1000 microscope equipped with the Leica DFC425 camera and ImageScope™ Image Analysis Software was used for identification of found coccidia. Copro-parasite examinations revealed the presence of two types oocysts of *Eimeria* in one of six fecal samples (16.6%) analyzed by flotation technique: *E. gallaeciensis* and *E. emydis*. In addition, this is the first report of *Eimeria* in *E. orbicularis* in Azerbaijan. The morphological and statistical parameters of both species were determined and a comparative analysis was carried out with data from other authors. The presence of Coccidian can be the cause of enteritis and death in young tortoises. The source of coccidiosis infection in European pond turtle could be attributed to contamination of food and water.

**Keywords:** Parasite, *Eimeria*, oocyst, morphology, *Emys orbicularis*.

### Introduction

Pond turtles (family Emydidae) are semi or fully aquatic turtles, with 53 currently recognized species [1] that comprise about 16% of global turtle species richness. In the fauna of Azerbaijan, this genus was represented by only one species: European pond turtle, *Emys orbicularis*, Linnaeus, 1758. This species is wide distributions and high abundance in Azerbaijan. These turtles play a major role in the stability of biodiversity of freshwaters and marshes cenoses. They prevent

organic pollution of the reservoir by feeding on algae and they are the food of birds of prey and mammals. Distributed in all geographical regions of Azerbaijan (except Nakhchivan Autonomous Republic) [2].

Parasitism in wild animal populations is recognized as a fundamental factor that can affect fertility and survival. Parasitic infections in reptiles are generally asymptomatic, but they can also cause severe disease leading to mortality in turtles [3]. Although imperceptible most of

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the time, parasitic infection can significantly reduce existing populations [4, 5]. Therefore, the evaluation of the parasite communities in wild animal populations is important for conservation management [4]. Intestinal coccidiosis is very common in free-living and captive reptiles [6]. Coccidia are among the most common gastrointestinal parasite species in tortoises and vary among species [7].

The gastrointestinal tract, in freshwaters turtles as well as in other organisms, might be rightly considered as an ecosystem where bacteria, viruses, protozoa, fungi and endoparasites co-exist [8]. This study aimed to provide baseline data on parasitic composition, through method in cloacal and faecal samples of live loggerhead European pond turtle, thus supporting the existing literature of protozoa parasite of this species.

### **Material and Methods**

A total of 6 specimens of *E. orbicularis* were collected by hand in the Agzibircala Lake, Shabran District in the north-east of Azerbaijan Republic. The turtles were housed individually in glass aquaria and, immediately after defecation, the fresh faeces of each individual animal was placed into 4% (w/v) potassium dichromate solution ( $K_2Cr_2O_7$ ). Faecal samples were examined for the presence of coccidian oocysts by the standard flotation technique with saturated sodium chloride (NaCl; sp.gr. 1.18) [9]. A Leica DM1000 microscope equipped with the Leica DFC425 camera and ImageScope™ Image Analysis Software was used for identification of found coccidia. The sample was then observed under a microscope at  $\times 200$  and  $\times 400$ . Morphology and morphometry of oocysts were evaluated according to the standards published by Duszynski and Wilber (1997) [10].

All measurements (25 sporulated oocysts) were given in micrometres ( $\mu\text{m}$ ) as the mean  $\pm$  standard deviation followed by the ranges in parentheses.

### **Results**

Copro-parasite examinations revealed the presence of two types oocysts of *Eimeria* in one of six fecal samples (16.6%) analyzed by flotation technique: *E. gallaeciaensis* and *E. emydis*.

*E. emydis* oocyst pear-shaped,  $24.02 \pm 0.04 \times 16.37 \pm 0.19$  ( $19.4-27.8 \times 15.2-19.2$ ), shape index (length/width ratio)  $1.45 \pm 0.02$  (1.2-1.7), with smooth, single-layered wall (1.1 thick),

which is slightly thinner at the pointed end (table 1). Micropyle and polar granule absent. Oocyst residuum present at the opposite side of sporocysts, consisting of an aggregate of numerous granules. Sporocyst ellipsoid,  $7.92 \pm 0.1 \times 5.15 \pm 0.11$  ( $6.76-9.53 \times 3.55-6.77$ ), shape index  $1.55 \pm 0.03$  (1.1-2.2), with single-layered wall. Stieda body is present. Sporocyst residuum present as loose cluster of small granules. Sporozoite elongate. Each sporozoite contains a large spherical or ellipsoid refractile body at the broad end, with a smaller one, usually at narrow end. Nucleus usually visible in the middle of sporozoite (Fig.1.A).

*E. gallaeciaensis* oocyst ovoid-ellipsoid,  $21.0 \pm 0.51 \times 19.5 \pm 0.25$  ( $19.0-23.5 \times 17.7-21.5$ ), shape index  $1.08 \pm 0.01$  (1.03-1.15), with smooth, single-layered wall, 1.14 thick (table 1). Micropyle and polar granule absent. Oocyst residuum present at the opposite side of sporocysts, consisting of irregular cluster of unbounded granules usually surrounding a vacuolar-like structure. Sporocysts ellipsoid,  $7.92 \pm 0.19 \times 5.48 \pm 0.11$  ( $6.7-9.24 \times 4.65-6.3$ ), with smooth thin wall; shape index  $1.44 \pm 0.04$  (1.18-1.67). Conical Stieda body present at 1 end of sporocyst. Sporocyst residuum present, normally as loose cluster of small granules. Sporozoite elongate. Each sporozoite contains a large, ellipsoid, refractile body at 1 end and another, normally smaller (and not always discernible), at other end. Nucleus sometimes visible in the middle of sporozoite (Fig.1.B).

In addition in the fecal samples *Eimeria*-infected turtle, were found eggs of *Ascaris* spp. (Nematoda, Ascaridida). The eggs were elliptical in shape, beige in color, and measured  $63-78 \mu\text{m}$   $42-50 \mu\text{m}$  with a length-to-breadth ratio of 1.56–1.87:1. The eggshell was thick and an ovoid egg cell was found within it (Fig.1.C).

### **Discussion**

*Eimeria* is a large genus, with over 1800 species identified to date [11]. Despite exquisite host specificity of individual species, the genus as a whole has a highly diverse host range and affects members of all vertebrate classes [11]. As the replication stages of schizogony and gametogony occur within host cells, infection with *Eimeria* species results in cellular destruction and pathology to the susceptible host. The usual site of this replication is within epithelial cells lining the intestinal tract. This can lead to clinical symptoms of gastrointestinal dysfunction such as diarrhoea, dehydration and failure to gain weight [12].

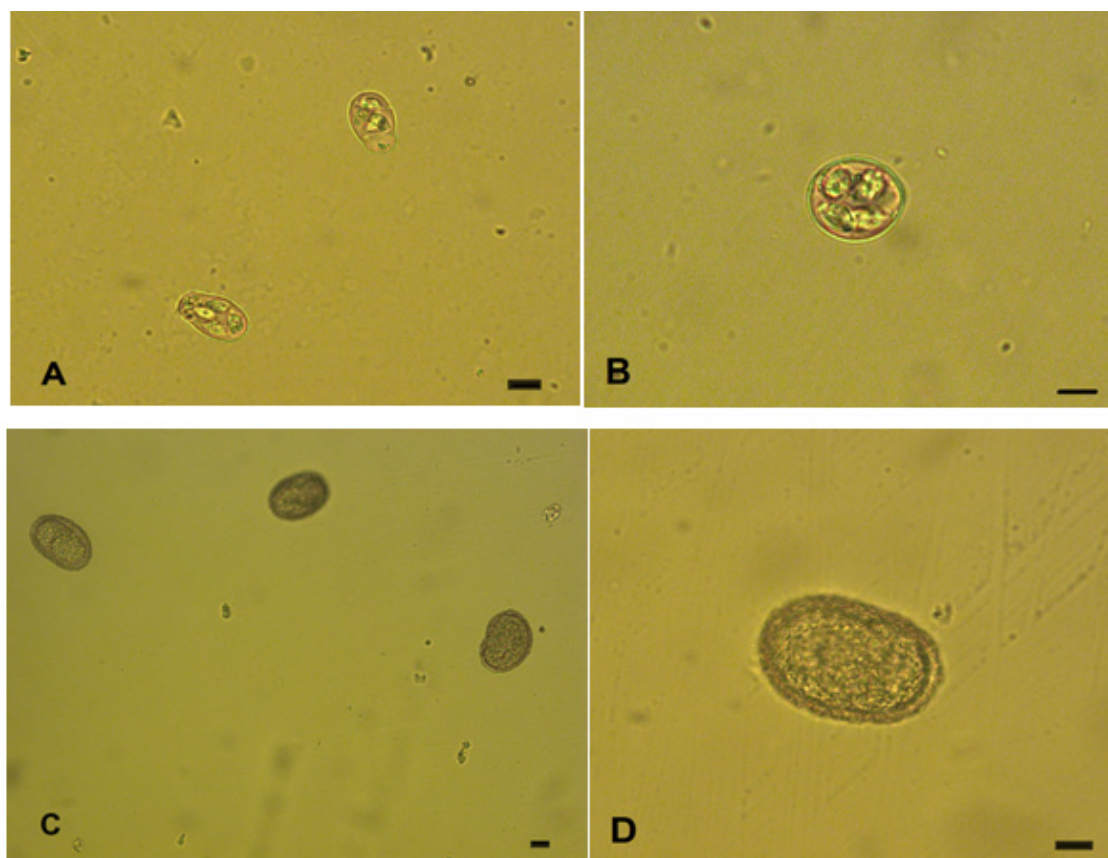


Fig. 1. Photomicrographs of sporulated oocysts of eimerians and eggs of nematode collected from the feces of *Emys orbicularis*: A- *E. emydis*, B- *E. gallaeciensis*. C, D- eggs of nematode of *Ascaridia* spp. Bar = 10  $\mu$ m.

TABLE 1. *Eimeria* species reported in literature and in this study from *Emys orbicularis* in Azerbaijan

Eimeria species	Oocyst					Sporocyst				
	Shape	L $\times$ W range	L/W ratio	OR	PG	Shape	L $\times$ W range	L/W ratio	SB/SSB	SR
<i>E. delagei</i>	ovoid	16-22x17	1.3	Y	N	curved spindle-shaped	7-8 x nd	nd	N	N
<i>E. gallaeciensis</i>	subspherical to lightly ovoid-ellipsoid	19.3x16.0	1.2 (1.1-1.3)	3.8x3.0	N	ellipsoid	9.7 x 5.1	1.9 (1.7-2.0)	Y	Y
<i>E. emydis</i>	clearly ovoid, rarely pear-shaped	22.6 x 17.0	1.3 (1.2-1.5)	4.5 x 4	N	ellipsoid	11.4 x 6.0	1.9 (1.6-2.2)	Y	Y
<i>E. mitraria</i>	miter-shaped	12.1 x 9.5	1.3 (1.1-1.5)	N	N	ellipsoid	4.5 x 3.3	1.3 (1.0-1.7)	Y	Y
<i>E. emydis</i>	pear-shaped	24.02x 16.37	1.45 $\pm$ 0.02 (1.2-1.7)	Y	N	ellipsoid	7.92 x 5.15	1.55 (1.1-2.2)	Y	Y
<i>E. gallaeciensis</i>	ovoid-ellipsoid	21.0x 19.5	1.08 $\pm$ 0.01 (1.03-1.15)	Y	N	ellipsoid	7.92x5.48	1.44 (1.18-1.67)	Y	Y

L = length ( $\mu$ m); W = width ( $\mu$ m); OR = oocyst residuum; PG = polar granule/s; SB/SSB = Stieda body/substieda body; SR = sporocyst residuum; Y = present (yes); N = absent (none); nd = not determined in the description

The first report on eimerians from *Emys orbicularis* in Europe was focused on describing *E. delagei* (as *Coccidium delagei*) from the intestine of 1 turtle maintained under captive conditions in a laboratory of the Sorbonne University [13]. Later were described 3 eimerians from the feces of European pond turtles from Galicia (NW Spain): 2 new species (*E. gallaeciensis* and *E. emydis*) and *Eimeria mitraria* [14].

All three species of turtles (*Emys orbicularis*, *Mauremys caspica* and *Testudo graeca*) which occur in Azerbaijan have been examined for coccidia. Only in *T.graeca* was found coccidia genus of Cryptosporidium, Eimeria and Isospora [15].

### Conclusions

This is the first report of *E.emydis* in European pond turtle. The presence of Coccidian can be the cause of enteritis and death in young tortoises. The source of coccidiosis infection in European pond turtle could be attributed to contamination of food and water.

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### Conflicts of interest

There are no conflicts to declare.

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## الكشف الأول عن الكوكسيديا في سلحفاة البرك في أذربيجان

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### الخلاصة

تعد سلحفاة البرك الأوروبية، *Emys orbicularis*، مكوناً أساسياً في مستوطنات المياه العذبة والمستنقعات، ويعتبر تقييم الطفيليات في مجموعاتها أمراً ضرورياً لإدارة عمليات الحفظ. الكوكسيديا هي العدوى المعوية الأكثر شيوعاً في الزواحف الأسيرة والتي تعيش بحرية. كان الهدف من هذه الدراسة هو توصيف وجود *Eimeria* sp. في السلحفاة البركة الأوروبية. تم فحص عينات البراز للتأكد من وجود البويضات الكوكسيديا بواسطة تقنية التعويم القياسية بكلوريد الصوديوم المشبع (NaCl; sp.gr. 1.18). تم استخدام مجهر Leica DM1000 Leica DFC425 وببرنامج تحليل الصور ImageScope™ لتحديد الكوكسيديا الموجودة. كشفت فحوصات الطفيليات المشتركة عن وجود نوعين من بويضات الإيميريا في واحدة من ست عينات برازية (٦٦٪). تم تحليلها بتقنية التعويم *E.galaeciaensis* و *E.emydis* بالإضافة إلى ذلك، هذا هو التقرير الأول لـ *Eimeria* في *E.orbicularis* في أذربيجان. تم تحديد المعلمات المورفولوجية والإحصائية لكلا النوعين وتم إجراء تحليل مفران مع بيانات من مؤلفين آخرين. يمكن أن يكون وجود الكوكسيديا سبباً لالتهاب الأمعاء وموت السلحفاة الصغيرة. يمكن أن يعزى مصدر عدوى الكوكسيديا في السلحفاة الأوروبية إلى تلوث الغذاء والماء.

الكلمات المفتاحية: الطفيلي، الإيميريا، البويضة، الشكل، *Emys orbicularis*