

Coccidian Parasites (Eucoccidia: Eimeriidae) in European Ground Squirrel (*Spermophilus citellus* L., 1766) (Rodentia: Sciuridae) from Bulgaria

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Abstract: Three eucoccidian parasites from the genus *Eimeria* were observed in the European ground squirrel *Spermophilus citellus* from Bulgaria: *E. citelli* KARTCHNER & BECKER, 1930, *E. callospermophilli* HENRY, 1932, and *E. cynomysis* ANDREWS, 1928. Data on their morphology and prevalence in the infected animals are given. *E. citelli* and *E. callospermophilli* were observed in great number in tree sub-adult animals and the authors considered that the coccidiosis was one of the limiting factors for the population density of *S. citellus* in Bulgaria.

Key words: Coccidia, *Eimeria*, *Spermophilus citellus*, morphology, prevalence

Introduction

From the genus *Spermophilus* 31 recent species are known, inhabiting Europe, Asia and North America. The subject of our study – the European ground squirrel (*S. citellus*) is distributed in Southeastern Europe in two main basins: Pannonian and Balkan, which are divided by the Carpathians (RUZIC 1978). In Bulgaria the species is patchy in the plane and with small isolated mountain population in the Vitosha mountain, the Rila mountain, Western and Central Balkan mountains (PESHEV *et al.* 2004). The populations of *S. citellus* in Europe are not numerous, with decreasing numbers, therefore the species is included in the IUCN Red List of Threatened species as “Vulnerable” (AMORI 1996).

The studies on the coccidian parasites of the

European ground squirrel are scanty. The first data for the observation of 2 *Eimeria* species in *S. citellus* from Europe was published by PELLERDY and BABOŠ (1953). They found 2 *Eimeria* species in the studied animals from the Bakony mountain: *E. bilamellata* Henry, 1932, and *E. citelli* KARTCHNER and BECKER, 1930. According to PELLERDY and BABOŠ (1953) and PELLERDY (1974) *E. citelli* produced catharal enteritis in the small and the large intestines of the infected *S. citellus*. Four years later RYŠAVÝ (1957) observed 100% coccidian infection in 5 examined animals from Czechoslovakia, caused by *Eimeria citelli* KARTCHNER and BECKER, 1930 and *E. eubeckery* HALL and KNIPLING, 1935. In a critical revision of the taxonomy and the nomenclature of *Eimeria* spp. in

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the tribe Marmotini (Sciuridae) WILBER *et al.* (1998) consider that in *S. citellus* the following 3 valid species occur: *E. citelli* KARTCHNER and BECKER, 1930, *E. callospermophili* HENRY, 1932, and *E. cynomysis* ANDREWS, 1928.

The aim of the proposed study is to present our results of the investigation on the Coccidian parasites of *S. citellus*, especially on the morphology of their oocysts and their prevalence in the examined *S. citellus* from Bulgarian populations.

Material and Methods

For the proposed study the fecal samples from 14 living animals were investigated. The animals were collected from 3 different populations in the country by special live-traps and kept in individual cages for defecation. The localities of the studied animals, the time of the collection, their number and age are presented in the Table 1.

The fecal samples were kept before the laboratory investigation in 2.5% of $K_2Cr_2O_7$ at a room temperature (21-23°C). The laboratory investigation for oocysts was made by the Fülleborn method. The sporulation of the oocysts was studied at a room

temperature. The microphotographs of the oocysts were made by a digital microphotocamera "Olympus E-500" with a microscope "Zeiss", (NU-2). All measurements are in microns (μm). The statistical analysis of the oocyst variation was performed using the computer program STATISTICA, version 5.0.

Results and Discussion

The following 3 species of *Eimeria* were found in the studied animals of *Spermophilus citellus*:

Eimeria citelli KARTCHNER and BECKER, 1930.

Fig. 1a, 2.

Oocyst and sporocyst morphology. The oocysts are sub-spheroid, rarely round, colourless and without micropyle. The oocyst's wall is smooth, about $1\mu m$ thick. One or two polar granules are sometime present in the sporulated oocysts. A compact granulated oocyst residuum is always present. The sporocysts are elliptical, narrowed at one pole, with a very small Stiedae body. A granular sporocyst residuum is dispersed between the sporozoites.

The size variation of 36 measured oocysts of *E. citelli* from one infected animal are present in Table 2.

Table 1. Localities, number and age of the studied *Spermophilus citellus*.

Localities	Time of collection	Number of animals	Age of animals
Belmeken Peak, (Rila Mountain)	14-17.07.2006	2	Subadults
Rosen, (Pazardzhik district)	08.08.2006	1	Subadult
Knezha, (Pleven district)	27.04-31.07.2006	11	9 adults 2 subadults
TOTAL		14	9 adults 5 subadults

Table 2. *Eimeria citelli*: oocysts dimensions and their variations.

Characters	Mean	M	SD	SE	CV	Min	Max	N
Length	21.28	21.15	1.82	0.3	8.55	16.2	25.6	36
Width	17.99	17.95	1.84	0.3	10.22	14.5	22.4	36
Length/Width	1.19	1.17	0.09	0.02	7.56	1.06	1.53	36

Abbreviations: M - median; SD - standard deviation; SE - standard error of mean; CV - coefficient of variation in %; N - number of oocysts.

Prevalence. *E. citelli* was observed in 12 of 14 examined animals (86%).

***Eimeria callospermophili* HENRY, 1932.** Fig. 1b, 3.

Syn.: *Eimeria citelli* KARTCHNER and BECKER, 1930, pro parte: RYŠAVÝ, 1957, p.333.

Oocyst and sporocyst morphology. The oocysts are subspheroid or round, colourless and without micropyle. The oocyst wall is smooth, about 0.7 µm thick. In the sporulated oocysts 1-3 polar granules are present. A granulated oocyst residuum is also present. The dimensions of subspheroid oocysts vary: 15.4-19.2 x 14.5-17.6 µm (L/W: 1.0-1.1). The round oocysts dominate the subspheroid oocysts and their diameter varies from 14.4 to 17.6 µm. The sporocysts are pear-shaped, with a small Stiedae body. A light and finely granulated sporocyst residuum is dispersed between the sporozoites. The

sporocyst dimensions vary: 8-9.6 x 6-6.5 µm.

Prevalence. *E. callospermophili* was found in 10 of 14 examined animals (71%), always in mixed infection with *E. citelli*. In three subadult animals we observe an intensive mixed infection with *E. citelli* and *E. callospermophili* – more than 50 oocysts in 1 field of view with ocular 12.5x and objective 10x and we suppose that coccidiosis is one of the limiting factors of the population density of *S. citellus* in Bulgaria.

Remarks. RYŠAVÝ (1957) observed two kinds of oocysts of *E. citelli* in *S. citellus* from Czechoslovakia: ellipsoidal or subspherical, the dimensions of which vary: 17.1-22.8 x 13.2-19 µm (mean: 19 x 15.2 µm), and round oocysts with a diameter from 13.3 to 19 µm (mean: 17.1µm). We consider that the smaller round oocysts belong to *E. callospermophili* and not to *E. citelli*.

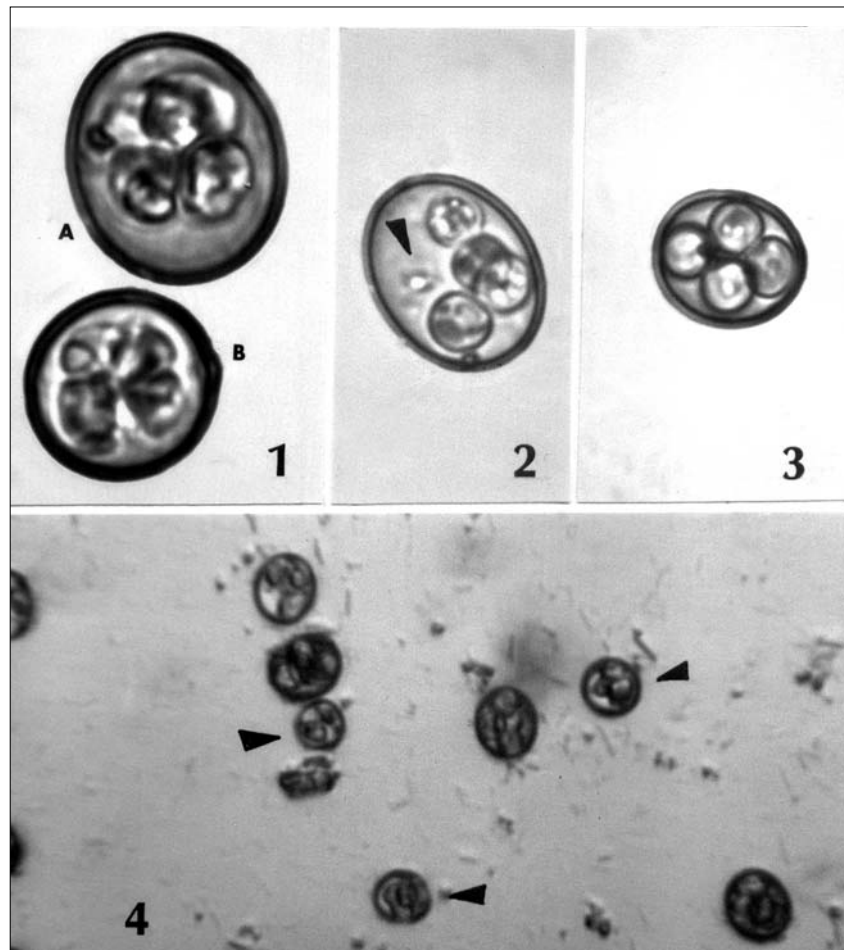


Fig. 1-4. *Eimeria* spp. from *Spermophilus citellus*.

1. *Eimeria citelli* (a) and *E. callospermophili* (b) (1400 x); 2. *E. citelli* with polar granule (arrow) (1000 x); 3. *E. callospermophili*, sporulated oocyst (1000 x); 4. Mixed infection with *Eimeria citelli* and *E. callospermophili* (arrows) (500 x).

***Eimeria cynomysis* ANDREWS, 1928.** Fig. 5-8.

Syn.: *E. bilamellata* HENRY, 1932: PELLERDY and BABOŠ, 1953, p. 167.

Oocyst and sporocyst morphology. The oocysts are ovoid, rarely ellipsoidal, pale-brown or pale-yellow with a thick bilayered wall. The outer layer is rough. The oocyst wall is about 1.8-2.5 µm thick and semitranslucid. At the one pole of the sporulated oocyst a well-visible micropyle is present (5-8 µm), sometimes with a structure like micropyle cap (Fig. 6). But in two animals we observed also some oocysts without micropyle, even after the sporulation (Fig. 7, 8). In the sporulated oocysts a compact oocyst residuum is present, sometime a polar granule is also visible. The oocyst dimensions vary in large diapason: 32.2-40.0 x 25.6-29.4 µm. The sporocysts are ellipsoidal or ovoid, with well-formed Stiedae body. A dispersed granular sporocyst residuum is also present. The sporocyst dimensions vary: 16-19 x 12-14 µm.

Prevalence. *E. cynomysis* was observed in 5 of 14 examined animals (35%), but always in low oocyst number.

Conclusions

Despite of the limited numbers of examined animals from Bulgaria, 3 species of *Eimeria* were established in *S. citellus* from 3 natural populations. In the 14 studied animals (9 adults and 4 subadults) *Eimeria* oocysts were found in 12 animals (Total prevalence: 86%). Arranged in order of their prevalence, with highest one there were *E. citelli* (86%) and *E. callospermophili* (70%), while *E. cynomysis* was observed in 5 animals only with limited oocyst number (35%). Only 2 adult animals from the population of Knezha were free of parasites.

From the morphological point of view the presence of polar granule in some sporulated oocyst of *E. citelli* was interesting, at the same time with oocyst without polar granule. In the redescription of the species by WILBER *et al.* (1998) the attention was drawn to the lack of polar granules in the oocysts of *E.*

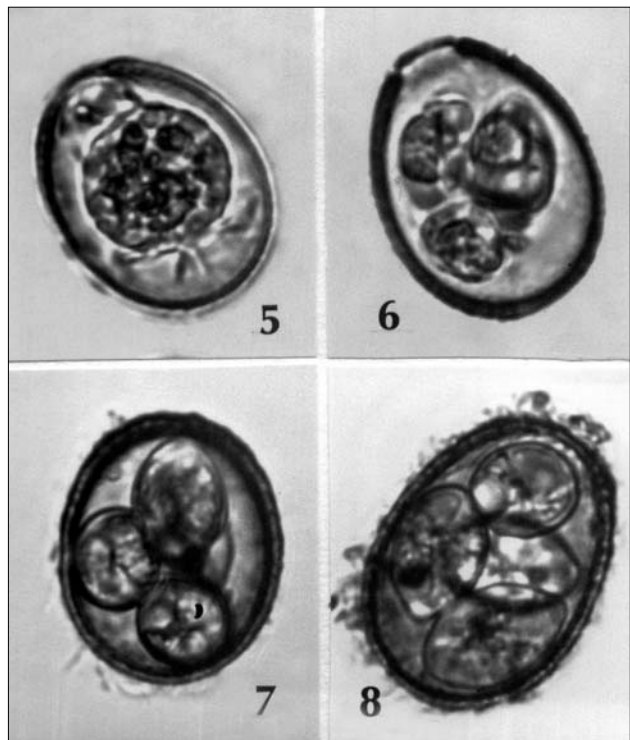


Fig. 5-8. *Eimeria cynomysis* from *Spermophilus citellus* (1100 x).

5. Unsporulated oocyst with micropyle; 6. Sporulated oocysts with micropyle; 7-8. Sporulated oocysts without visible micropyle.

citelli, but we consider this peculiarity taxonomically unimportant and it must be corrected.

The observations of intensive presence of oocyst in the faeces of 3 subadult animals is an indication confirming the opinion of PELLERDY and BABOŠ (1953) that the coccidiosis is one of the limiting factors for the population density of *S. citellus*.

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Кокцидии (Eucoccidia: Eimeriidae) на европейския лалугер (*Spermophilus citellus* L., 1766) (Rodentia: Sciuridae) в България

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(Резюме)

В европейския лалугер (*Spermophilus citellus*) от България са установени три вида кокцидии от род *Eimeria*: *E. citelli* KARTCHNER и BECKER, 1930, *E. callospermophilli* HENRY, 1932, и *E. cynomysis* ANDREWS, 1928. Дадени са данни относно тяхната морфология и разпространение в изследваните животни. В три неполовозрели животни е установена интензивна смесена инвазия с *E. citelli* и *E. callospermophilli* и авторите считат, че кокцидиозата е един от лимитиращите фактори върху популацията на *S. citellus* в България.

