

# Systematic and Ecological Survey on Coccidians (Apicomplexa: Eucoccidida) in European Ground Squirrel (*Spermophilus citellus* L.) (Rodentia: Sciuridae) from Bulgaria

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**Abstract:** The study based on 109 examined coprological samples from the European ground squirrel (*Spermophilus citellus*) from 10 localities in Bulgaria shows the presence of 4 species of Eucoccidians: *Eimeria citelli* Kartchner & Becker, 1930 (Prevalence: 92.7%), *E. callospermophilli* Henry, 1932 (Prevalence: 66.6%), *E. cynomysis* Andrews, 1928 (Prevalence: 14.6%) and *Klossia* sp. (Prevalence: 6.3%). Data on coccidian invasion, depending on the age and sex of the hosts, as well as the problem of mixed invasions, are discussed. A more detailed morphological and biometrical description of *Klossia* sp. oocysts is given and the problem of parasitism of this species in vertebrate hosts is also discussed.

**Key words:** Coccidia, *Eimeria*, *Klossia*, *Spermophilus citellus*, prevalence, ecology

## Introduction

In a previous publication we have presented the results of our investigation on the intestinal eucoccidians of European ground squirrel (*S. citellus*) (GOLEMANSKY, KOSHEV 2007). They were based on 14 animals examined from 3 localities in Bulgaria and 3 species of eucoccidians were identified: *Eimeria citelli* KARTCHNER & BECKER, 1930 (prevalence: 86%), *E. callospermophili* HENRY, 1932 (71%) and *E. cynomysis* ANDREWS, 1928 (35%).

The last two years, within the framework of some ecological projects, we have collected new reach material of coprological samples from *S. citellus*, originated from some new localities in Bulgaria. The investigation of these materials gave new information about the coccidian invasion of *S. citellus* depending on the age and sex of the examined animals.

Furthermore, a new interesting coccidian parasite was observed in some animals from two different localities – *Klossia* sp., which parasitological status in vertebrates is debatable to now.

The aim of the proposed article is to present the results of our last investigation of coccidian invasion in *S. citellus* in Bulgaria and, on the basis of our previous and present investigation, to give more detailed information on some ecological parameters of this invasion.

## Material and Methods

For the present investigation faecal samples from new 95 specimens of *S. citellus* were collected from 10 different localities in Bulgaria during the period

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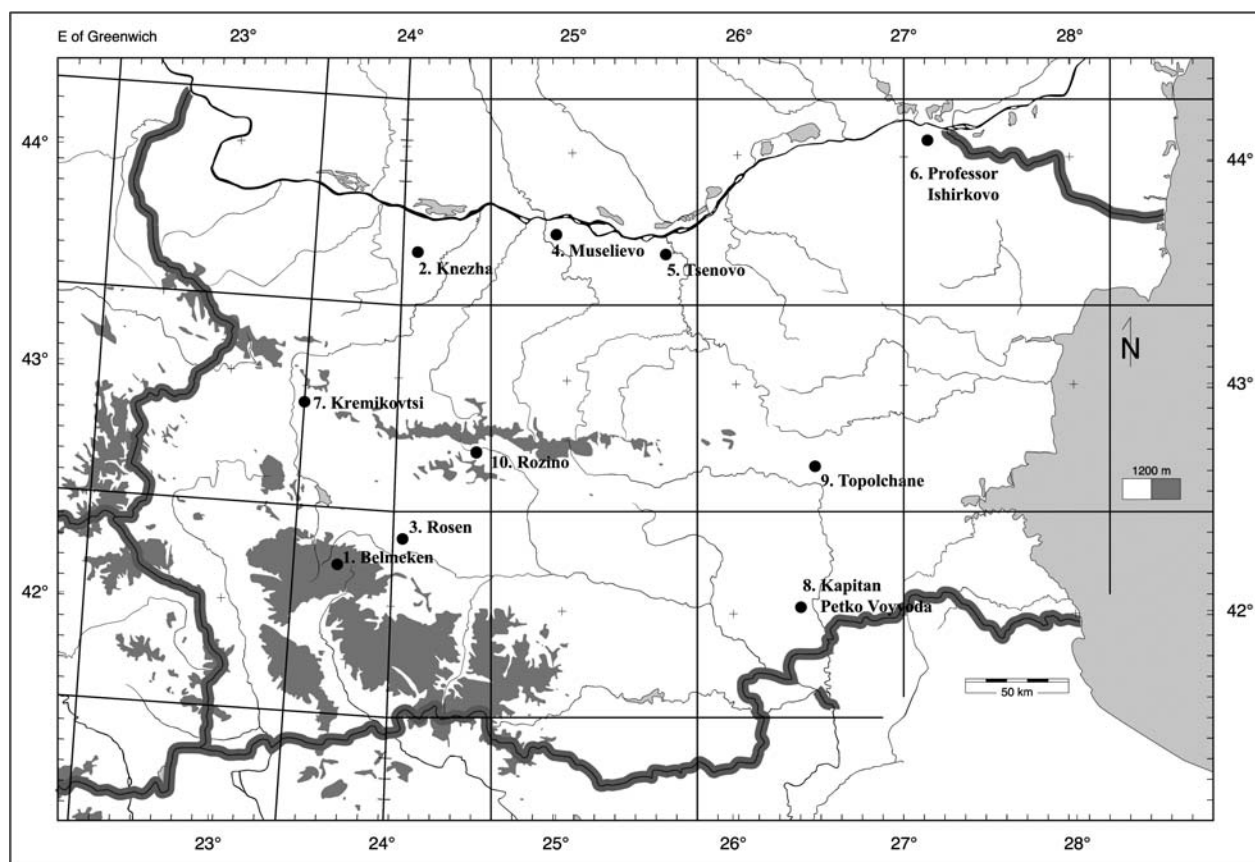


Fig. 1. Topographic location of investigated populations of *Spermophilus citellus*.

2007 – 2008 (Table 1, Fig. 1). For general analysis of coccidian status of *S. citellus* in Bulgaria in the table are included also 14 animals, studied for our previous publication (GOLEMANSKY, KOSHEV 2007), all the more that some samples were collected during the last two years from the same localities. The localities, where the material was collected twice or more in the last 3 years are marked in the Table 1 with the letters *a*, *b*, *c*.

The laboratory investigation for oocyst detection was made by the method of Fulleborn. It was informative for calculation of prevalence of examined Ground squirrels with *Coccidia*. With the purpose of assessment of invasion intensity of the animals with different species of coccidians we accepted a comparative scale for the number of observed oocysts on 100 visual sites of the microscope at a magnification with objective 12.5 x and ocular 10 x. The accepted degrees of invasion were: very low – from 1 to 5 oocysts in 100 visual sites; low – from 6 to 20 oocysts; medium – from 21 to 50 oocysts and high

– more than 50 oocysts. Microphotographs of coccidian oocysts were made by a digital microphotocamera ‘Olympus E-500’ with a light microscope ‘Zeiss’ (NU-2). All measurements in the text and in the tables are in microns.

The statistical analysis about different age, sex, prevalence and intensity was made with Median Test which was recommended from RÓZSA *et al.* (2000) and NEUHÄUSER, POULIN (2004) for parasitological studies. The total coccidian intensity of four parasites was analysed with Quantitative Parasitology 3.0. (REICZIGEL, RÓZSA 2005). All other analyses were performed by Statistic for WINDOWS 7.0. Release 2004.

## Results and Discussion

The present study, based on more abundant material of 95 new specimens of *Sp. citellus* from 10 different habitats in Bulgaria, proves the presence of 3 species of eucoccidians, observed and reported

**Table 1.** Localities, number and age of examined *Spermophilus citellus* from Bulgaria. Legend: ♂ – male; ♀- female; Ad – adultus; Sad – subadultus; juv – juvenus  
 Note: 1a, 2a and 3 – Localities explored before by GOLEMANSKY, KOSHEV (2007).

N	Localities	Time of collection	Examined animals			Invaded animals		
			Number	Age of animals	Sex of animals	Number	Age of animals	Sex of animals
1a	Belmeken Peak (Rila Mountain)	14-17.07.2006	2	2 Sad	2 ♀	2	2 Sad	2 ♀
1b	Belmeken Peak (Rila Mountain)	11.06-04.09.2007	7	4 Ad, 3 juv	2 ♂, 5 ♀	5	2 Ad, 3 juv	1 ♂, 4 ♀
1c	Belmeken Peak (Rila Mountain)	28-29.07.2008	12	12 juv	6 ♂, 6 ♀	9	9 juv	4 ♂, 5 ♀
2a	Knezha (Pleven district)	27.04-31.07.2006	11	9 Ad, 2 Sad	5 ♂, 6 ♀	9	7 Ad, 2 Sad	5 ♂, 4 ♀
2b	Knezha (Pleven district)	21.06.-19.09.2007	18	15 Ad, 1 Sad, 2 juv	11 ♂, 6 ♀	16	13 Ad, 1 Sad, 2 juv	10 ♂, 5 ♀
2c	Knezha (Pleven district)	01-06. 05.2008	7	5 Ad, 2 Sad	2 ♂, 5 ♀	5	3 Ad, 2 Sad	2 ♂, 3 ♀
3	Rosen (Pazardzhik district)	08.08.2006	1	1 Sad	1 ♀	1	1 Sad	1 ♀
4	Muselievo (Pleven district)	27.06.2007	12	9 Ad, 1 Sad, 2 juv	9 ♂, 3 ♀	11	8 Ad, 1 Sad, 2 juv	8 ♂; 3 ♀
5	Tsenovo (Ruse district)	28.06.2007	5	1 Ad, 3 Sad, 1 juv	1 ♂, 4 ♀	5	1 Ad, 3 Sad, 1 juv	1 ♂, 4 ♀
6	Professor Ishirkovo (Silistra district)	24-25.08.2008	4	4 Ad	4 ♂	4	4 Ad	4 ♂
7	Kremikovtsi (Sofia district)	14.05-20.08.2008	9	9 Ad	2 ♂, 7 ♀	9	9 Ad	2 ♂, 7 ♀
8	Kapitan Petko voyvoda (Haskovo district)	18.06.2008	8	8 Ad	5 ♂; 3 ♀	7	7 Ad	5 ♂; 2 ♀
9	Topolchane (Sliven district)	19.06.2008	5	3 Ad, 2 Sad	2 ♂, 3 ♀	5	3 Ad, 2 Sad	2 ♂, 3 ♀,
10	Rozino (Plovdiv district)	07-20.06.2008	8	3 Ad, 3 Sad, 2 juv	2 ♂, 6 ♀	8	3 Ad, 3 Sad, 2 juv	2 ♂, 6 ♀
	Total		109	70 Ad, 17 sad, 22 juv	52 ♂, 57 ♀	96	60 Ad, 17 Sad, 19 juv	47 ♂, 49 ♀

in our previous publication (GOLEMANSKY, KOSHEV 2007): *Eimeria citelli* KARTCHNER & BECKER, 1930, *E. callospermophilli* HENRY, 1932 and *E. cynomisis* ANDREWS, 1928. But in 6 animals from two new localities we have observed also oocysts of an identified species of Coccidia, considered by us as *Klossia* sp. In our earlier publication we have done morphometric characteristics of oocysts of tree *Eimeria* species and their prevalence. The finding of oocysts of *Klossia* sp. in some of the studied animals is an interesting fact and we present below a more detailed description of their morphology and biometry, according our data.

### Systematic part

#### *Klossia* sp. (Fig.2-6).

*Morphology of the oocysts.* Oocysts subspherical, with double colourless or pale yellow wall. The exocyst

is slowly rough, about 3.2 – 3.5 micr. thick. Oocysts dimensions vary from 32.0 – 51.2 x 27.5 – 48.0 micr. (Mean: 40.4 x 34.23 micr.) (Table 2). Rarely we have observed some spherical oocysts but their number was very limited and may be an optical artifact. Compact oocyst residuum was not observed but in the sporulated oocysts 1-5 polar granules were present (Fig. 2-5).

Sporocysts round, with thin single wall. The number of sporocysts vary from 6 to 21. Their diameter is relatively constant and vary from 10.2 to 12.8 micr. As a rule the number of sporocysts was higher in the big oocysts. Sporocyst residuum present and formed by many granules dispersed between the sporozoites. The four sporozoites were oval, with well visible vacuole (Fig. 4-6).

*Prevalence.* The oocysts of *Klossia* sp. were observed in 6 Ground squirrels from two different

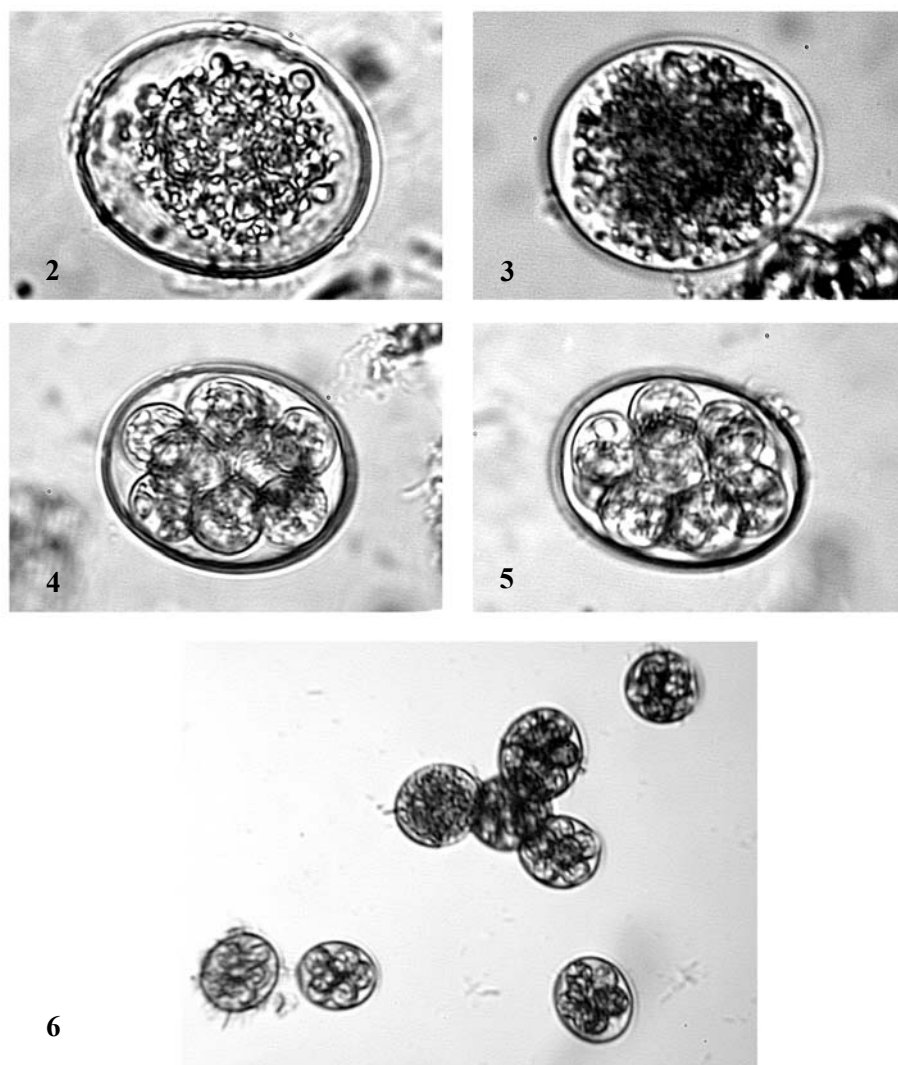


Fig. 2-6. *Klossia* sp. 2, 3 – unsporulated oocysts; 4, 5 – sporulated oocysts; 6 – free sporocysts.

**Table 2.** *Klossia* ssp.: oocyst and sporocyst (SP) dimensions and their variations.

Characters	Mean (µm)	M	SD	SE	CV	Min	Max	N
Length	40.04	38.8	4.43	0.79	11.06	32	51.2	31
Width	34.23	33.6	4.18	0.75	12.21	27.5	48	31
Length/Width	1.17	1.16	0.07	0.01	5.98	1.09	1.29	31
SP (diameter)	11.29	11.2	0.44	0.09	3.90	10.2	12.8	25

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

localities: Kapitan Petko voyvoda village (South Bulgaria) and Tsenovo village (North Bulgaria). The total prevalence of the invasion with *Klossia* sp. was 6.25% but the prevalence in both localities was relatively high: 20% in the animals from Tsenovo village and 50% in the Ground squirrels from Kapitan Petko voyvoda village.

*Remarks.* Although the taxonomy and systematic of Coccidia has been discussed by many authors so far, there are not an accepted agreement up to now. In our study we accept that the genus *Klossia* belong to family Klossiidae, suborder Adeleina, order Eucoccidida (MEHLHORN, SHEIN 1984).

At first the species of genus *Klossia* were considered as parasites of invertebrate animals, mainly snails but LEVINE, IVENS, KRUIDENIER (1955) have described two species of *Klossia* from two species of Mammals in USA: *K. perplexens* from Deer mouse (*Peromyscus maniculatus*) and *K. variabilis* from Western Big-eared Bat (*Corhyrorhinus rafinesquii*). Later oocysts of *Klossia* sp. were observed in faeces of other Mammals also by MULLIN, COLLEY (1972) (H: *Rathus whiteheadyi*), GOLEMANSKY, YANKOVA (1973) (H: *Apodemus agrarius*), GOLEMANSKY (1975) (H: *Vulpes vulpes*), SEVILLE *et al.* (1992) (H: North American Mammals), etc. BARNARD, ERNST, DIXON (1974) founded similar oocysts in faecal samples from Cotton rat (*Sigmodon hispidus*) in USA, but they identified it as *Adelina* sp. Recently KVICEROVA (2008) and KVICEROVA *et al.* (2008) have found free coccidian oocysts like those of *Klossia* sp. in faeces of *Spermophilus citellus* from Czech Republic and Slovakia, but they also identified it as *Adelina* sp. The cited authors did not give more detailed morphological description of the observed oocysts. It is

quite possible the observed oocysts of *Klossia* sp. in *S. citellus* to be of a pseudoparasite of the omnivore Ground squirrel but we can not exclude either the possibility of a real parasitism of *Klossia* sp. in vertebrate animals and particular in *S. citellus*.

### Ecological part

Faecal samples collected from 109 *S. citellus* from 10 different localities in Bulgaria showed that 96 samples were positive for presence of coccidian oocysts, an infestation percentage of 88.05% (Table 1). KVICEROVA (2008) and KVICEROVA *et al.* (2008) reported 100% prevalence of *Eimeria* infection in *S. citellus* in Czech Republic and Slovakia. Similar high values of coccidians prevalence in other species of *Spermophilus* was observed also by STANTON *et al.* (1992) for *S. elegans elegans* – 69 %, by SEVILLE and STANTON (1993) for *S. richardsonii*–60%, etc. Twenty-nine percent of the squirrels harbored 1 coccidian species, 57.5% had 2 species and 13.5% had 3 species. The most of infected animals (68) were invaded with more than one *Eimeria* sp. (70.8%).

*E. citelli* (92.7%) and *E. callospermophilli* (66.6%) were more frequently observed *Eimeria* species in *S. citellus* (Table 3). The four parasites have significant difference about their intensity of invasion ( $\chi^2 = 200.58$ ,  $df = 3$ ,  $p = 0.001$ ). Most animals were infected with very low and low degree of invasion. Such situation with one or two predominated coccidian parasites was reported also for other *Spermophilus* species (STANTON *et al.* 1992; WILBER *et al.* 1994).

Many studies in North America describe that coccidian species may be a common and stable

**Table 3.** Intensity of coccidian invasion of *Spermophilus citellus* (n=96). Note: The percentage was higher than 100 because more than one animal was infected with more than one parasite.

Species	Intensity								N	%
	Very low		low		medium		high			
	n	%	n	%	n	%	n	%		
<i>E. citelli</i>	63	70.8	20	22.5	1	1.1	5	5.6	89	92.7
<i>E. callospermophilli</i>	55	85.9	4	6.3	1	1.6	4	6.3	64	66.6
<i>E. cynomysis</i>	8	57.1	3	21.4	2	14.3	1	7.1	14	14.6
<i>Klossia</i> ssp.	4	66.7	2	33.3	0	0	0	0	6	6.25

assemblage in host populations in *Spermophilus* sp. and closely related genera may be infected by similar species complexes of *Eimeria*. This multiple host capability may be an effective strategy for parasites with a direct life cycle and immobile infective stage without dispersal agents (STANTON *et al.* 1992). However the case with *S. citellus* is deeply different. It was distributed in the most western part of *Spermophilus* range in Eurasia with small contact zone with *S. suslicus* on the territory of Moldova and Ukraine.

Within examined *S. citellus*, no significant difference (chi-square,  $p > 0.05$ ) in prevalence of infection by the coccidian species and mixed coccidian invasion occurred between sexes (Table 4 and 5). Therefore the sexes were combined because infection did not differ significantly with sexes. Absence of sex prevalence of coccidian infection was described for other *Spermophilus* species too (SEVILLE, STANTON 1993).

Subadults and juveniles *S. citellus* were more infected with *E. callospermophilli* and *E. cynomysis*. We found significant difference between different ages infected with *E. callospermophilli* ( $\chi^2 = 7.76$ ,  $df = 2$ ,  $p = 0.02$ ) and *E. cynomysis* ( $\chi^2 = 11.29$ ,  $df = 2$ ,  $p = 0.003$ ) (Table 4). These two parasite species have relatively different prevalence in different ages of *S. citellus*.

We did not find adults and subadults invaded with *E. citelli* + *E. cynomysis* and *E. callospermophilli* + *E. cynomysis*. There are significant differences between ages infected with mixed parasites from *E. citelli* + *E. callospermophilli* + *E. cynomysis* ( $\chi^2 = 13.88$ ,  $df = 2$ ,  $p = 0.001$ ) (Table 5).

The fact that juveniles are infected with Eimerians more than the adults is debatable. The dominant hypothesis is that immunity was developed, and adults should have a lower prevalence of infection than juveniles. But evidence in the literature regarding immunity to spermophiline eimerians is contradictory (STANTON *et al.* 1992, WILBER *et al.* 1994, FULLER 1996). Resistance to infection with *E. callospermophilli* has been demonstrated in *S. lateralis* after 5 inoculations with 10 000 oocysts (TODD, HAMMOND 1968), but in the same study *S. armatus*, *S. richardsoni*, and *S. variegatus* showed no immune response. In general, spermophiline immunogenic response to eimerians seems quite variable (WILBER *et al.* 1994). Decisive for age prevalence and resistance to infection will be large sample size and artificial infection (STANTON *et al.* 1992).

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**Table 4.** Prevalence of different type of coccidian invasion depending of sex and age of animals of *Spermophilus citellus* (n=109). Legend: ♂ – male; ♀- female; Ad – adultus; Sad – Subadultus; Juv – juvenus;

Species	Sex				Age						Total	
	♂	%	♀	%	Ad	%	Sad	%	Juv	%	Number	%
<i>E. citelli</i>	43	83	46	80.7	54	77.1	16	94.1	19	86	89	81.7
<i>E. callospermophilli</i>	33	63	31	54.4	40	57.1	13	76.5	13	59	64	58.7
<i>E. cynomysis</i>	5	9.6	9	15.8	2	2.86	4	23.5	8	36	14	12.8
<i>Klossia</i> ssp.	4	7.7	2	3.5	5	7.14	1	5.88	0	0	6	5.5
n	52		57		70		17		22		109	

**Table 5.** Prevalence of different type of mixed coccidian invasion depending on sex and age of animals of *Spermophilus citellus* (n=109). Legend: ♂ – male; ♀- female; Ad – adultus; Sad – Subadultus; Juv – juvenus

Mixed invasion	Sex				Age						Total	
	♂	%	♀	%	Ad	%	Sad	%	Juv	%	Number	%
<i>E. citelli</i> + <i>E. callospermophilli</i>	25	48.1	21	36.8	33	47.1	7	41.2	6	27.3	46	81.7
<i>E. citelli</i> + <i>E. cynomysis</i>	1	1.9	1	1.8	0	0	0	0	2	9.1	2	58.7
<i>E. callospermophilli</i> + <i>E. cynomysis</i>	2	3.8	1	1.8	0	0	0	0	3	13.6	3	12.8
<i>E. citelli</i> + <i>E. callospermophilli</i> + <i>E. cynomysis</i>	3	5.8	7	12.3	1	1.4	4	23.5	5	22.7	10	5.5
<i>E. citelli</i> + <i>Klossia</i> ssp.	2	3.8	2	3.5	4	5.7	0	0	0	0	4	6
<i>E. citelli</i> + <i>E. callospermophilli</i> + <i>Klossia</i> ssp.	1	1.9	1	1.8	1	1.4	1	5.9	0	0	2	3
SUM	34		33		39		12		16		67	

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## Систематични и екологични изследвания на кокцидиите (Apicomplexa: Eucoccidia) на европейския лалугер (*Spermophilus citellus* L.) (Rodentia: Sciuridae) в България

В. Големански, Й. Кошев

### (Резюме)

В резултат на изследването на 109 копрологични проби от европейски лалугер (*Spermophilus citellus*) от 10 местообитания в България е установено наличието на 4 вида кокцидии: *Eimeria citelli* Kartchner & Becker, 1930 (Екстензивност на инвазия – ЕИ: 92,7%), *E. callospermophilli* Henry, 1932 (ЕИ: 66,6%), *E. cynomysis* Andrews, 1928 (ЕИ: 14,6%) и *Klossia* sp. (6,3%). В статията са обсъдени резултатите от кокцидийната инвазия в зависимост от възрастта и пола на гостоприемниците, както и проблемът за смесените инвазии. По-подробни морфологични и биометрични сведения са представени за *Klossia* sp. и е дискутиран проблемът за паразитизма на този вид при гръбначните животни.