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Blood parasites of small mammals (*Chionomys nivalis, Myodes glareolus*) living in montane zone Biele plesá, High Tatras

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Abstract. The study is focused on the investigation of the prevalence of blood parasites of the genera *Hepatozoon* and *Trypanosoma* (*Herpetosoma*) in two vole species; snow vole *Chionomys nivalis* and bank vole *Myodes glareolus* at the localities Dolina Bielych plies. 2,9% of individuals of *Ch. nivalis* were infected by *Hepatozoon* sp. and the same percentage of snow voles had *Trypanosoma* sp. in their blood. The prevalence of *Hepatozoon* sp. within the *M. glareolus* population was 6,7%. Similar results were discovered in samples collected during 2009 and 2010 at the same locality. At another locality, in Brestová, no parasites were found.

Key words: *Chionomys nivalis, Myodes glareolus,* voles, *Hepatozoon, Trypanosoma (Herpetosoma),* blood parasites

Introduction

Two species of rodents, the snow vole *Ch. nivalis* and the bank vole *M. glareolus* coexist in the selected locality (Dolina Bielych plies Valley, High Tatras). While the bank vole is a common species in various habitats, living in temperate forests at various altitudes, the snow vole is a species specific to rocky habitats (Janeau and Aulagnier 1997; Baláž and Ambros 2010). Although it is not bound strictly to high mountains, suitable habitats with poor vegetation cover and much rocky area are mostly found in alpine zones. Biele plesá lies on the border between the alpine and sub-alpine vegetation zone. The Bank vole is a very common species with a broad area of abundance. It is easily trapped and particularly suitable for study. In the blood of both vole species, eukaryotic parasites of the genera *Hepatozoon* and *Trypanosoma* (resp. subgenus *Herpetosoma*) can be found (Janeau and Aulagnier 1997; Mehlhorn 2008). The prevalence of blood parasites is investigated relatively well in *M. glareolus* (Molyneux 1969; Laakkonen *et al.* 2001; Noyes *et al.* 2002; Rigó *et al.* 2016). The Snow vole is rarer and thus less investigated. Snow vole are a good indicator of heavy metal pollution (Metcheva *et al.* 2008; Janiga *et al.* 2012) and in this study we investigated whether there is a correlation between such contamination and the presence of a parasite.

The prevalence of the parasite genera *Hepatozoon* and *Trypanosoma* in rodents (*Ch. nivalis, M. glareolus*) is compared in two localities (Dolina Bielych plies Valley, High Tatras, and Brestová, Western Tatras) in this study. Samples were collected during the summer and autumn of 2016 and compared to samples collected there in 2009 and 2010 as well as those collected near Brestová in 2009 and 2010. In the infected individuals contamination levels are measured by spectrometer and compared.

The goals of this study were: (1) Examination of blood smear slides by microscope to record the prevalence of blood parasites of the genera *Hepatozoon* and *Trypanosoma* within the population of snow vole and bank vole in the Dolina Bielych plies Valley and the population of snow vole from the locality Brestová. (2) To compare contamination levels in healthy and infected individuals of *Ch.nivalis* from the Dolina Bielych plies Valley.

Material and Methods

The study took place at two localities, Dolina Bielych plies Valley, High Tatras (GPS coordinaes 49.224469 N, 20.216681 E; 1,612 m a.s.l.) and Brestová, Western Tatras (GPS coordinates 49.224752 N, 19.679453 E; 1,934 m a.s.l.). Samples from Dolina Bielych plies were collected between September 2009 and December 2010, and again between July and November 2016. In Brestová, sampling took place only between 2009 and 2010.

Sampling

Samples of blood for smears and tail bones for spectrometry analysis were collected. Animals were trapped using Sherman traps filled with hay or fresh grass. This was important for thermoregulation especially in colder months. Oatmeal and small piesces of apple were used for bait and as source of food. Traps were laid 4-10 m apart though all microhabitats included (rocky screes with all sizes of stones, beneath dwarf pines, vegetation of alpine meadows, tussocks of grass). The traps were checked twice per day; early in the morning, when the majority of animals were caught (as they are more active overnight), and in the evening, in case additional animals were

trapped during the day. There were two vole species of interest, snow vole (*Ch. nivalis*) and bank vole (*M. glareolus*). The species, sex, age (distinguished adults, sub-adults and juveniles) and sexual activity was determined if possible. Morphometric measurements including weight of trapped voles, and ear, foot, tail, and body length, were taken and recorded.

After completing these measurements, a blood sample was taken. Voles were narcotized by inhaling Isoflurane applied to cotton wool. While the animal was narcotized, blood was taken using the retro orbital sampling method and bleeding was stopped by applying cotton wool. Blood smears were prepared by smearing a drop of the blood from the capillary on a clean slide. Ideally, two smears were prepared for each individual.

While the animal was narcotized, a portion of tail (max 0.05 cm) was clipped to analyze for heavy metal contamination in its bones. The bone sample was stored in a test-tube with ethanol. Animal were marked by clipping the fingers to identify individuals in the case of future re-trapping.

Following all measurements and samplings, the voles were released close to the location in which they were trapped.

Laboratory examination

Smear slides must be stained before examination. The method described in Doubek *et al.* (2003) was used. Stained smears can be viewed by microscope using a lens with a 40x magnification, or, for more detail, a lens with 100x magnification.

Vertebrae taken from the tail were screened by the ED-XRF Spectrometer delta. The concentration of nine elements: S, Cl, K, Ca, Cr, Mn, Zn, Rb, Mo, Ba, Pb was investigated. Only samples of *Ch. nivalis* were used for this analysis. The spectrometer measured each concentration three times for each sample and the average was calculated automatically. In total, 31 samples of tail bones were analyzed.

Statistical analysis

Average and standard deviations of parameters obtained from the spectrometer were calculated using the statistical functions of MS Excel and Libre Office Calc.

Results

The prevalence of parasites

The prevalence of parasites was determined by the number of detected parasites in the blood smear in the total number of examined individuals. During the research period, 99 individuals of *Ch. nivalis* were caught between the Brestová and Dolina Bielych plies valley (DBP) locations, and 33 individuals of *M. glareolus* were caught in the DBP. No *M. glareolus* individuals were caught at the Brestová site. The total prevalence (%) of parasite species in voles is stated in Table 1, categorized by time and locality.

The intensity of infection was very low in all cases, and the detection of parasites in the blood smears was sporadic (less than 1 per 1,000 erythrocytes).

Brestová 2009/2010

In this locality there were 29 individuals of *Ch. nivalis* caught in total, but no *M. glareolus* individuals were caught. The presence of blood parasites was not detected in the examined blood smears.

Dolina Bielych plies 2009/2010

Out of a total of 49 investigated individuals (*Ch. nivalis* and *M. glereolus*), three positive samples were found. The total prevalence of parasite species (*Trypanosoma* sp. and *Hepatozoon* sp.) in voles was 6.1%.

One adult male of *Ch. nivalis* was infected by *Trypanosoma* (*Herpetosoma*) sp. This vole was captured during the sample collection in September 2016. *Hepatozoon* sp. was found in the blood of a male of *Ch. nivalis* (age not determined), trapped in August. Only one adult female of *M. glareolus* was infected by *Hepatozoon* sp. It was trapped during the last sampling in November 2016.

Contamination

Concentration values of the elements measured in infected individuals of *Ch. nivalis* were compared with reference values. Concentrations of S, Cl, K, Ca, Cr, Mn, Zn, Rb, Mo, Ba and Pb was measured in 29 non-infected individuals of *Ch. nivalis* (Table 2). Concentration of selected pollutants remained within the standard range and values measured in infected individuals did not differ significantly.

Discussion

This finding represents the first recorded occurence of *Trypanosoma* sp. and *Hepatozoon* sp. in snow voles in Slovakia. *Trypanosoma* sp. was previously found in *Ch. nivalis* in Austrian Alps (Mahnert 1970), were 3.8% of population was infected.

Prevalence of parasites within the population

The parasites were found at the Dolina Bielych plies Valley location during the 2009 – 2010 sampling period, as well as during last sampling season

	Locality		
	Brestová 2009/2010	**DBP 2009/2010**	**DBP 2016**
Hepatozoon sp.			
Ch. nivalis	0	0	2.9
M. glareolus	-	11.1	6.7
Trypanosoma sp.			
Ch. nivalis	0	5.6	2.9
M. glareolus	-	0	0

Table. 1. Prevalence of parasites (%) in the localities during all years of research (DBP- Dolina Bielych plies).

Element	Average (non-inf.)	SD	CHN m Hepatozoon	CHN m Trypanosoma
S	5274	9.37	5272	5278
Cl	1031	549.2	805	1267
K	1312	1296	656	1340
Ca	1418	1081	944	928
Cr	27	10.1	25	24
Mn	59.7	37.9	52	52
Zn	73.45	26.32	80	60
Rb	16	4.09	16	16.7
Mo	7.44	1.02	7.3	7.4
Ba	64	21	68	64
Pb	15.7	3.67	13	16

Table. 2. Contamination levels of *Ch. nivalis*. Measured in ppm (CHN - *Ch. nivalis*; m - male).

in 2016. Abundance and long-term survival at this locality were confirmed.

The prevalence of *Hepatozoon* sp. was approximately the same as the prevalence of *Trypanosoma* sp. 11.11% of individuals were infected, similar to the results of Hamšíková *et al.* (2016), where 11.45% of bank voles from Slovakia and Czech Republic carried the *Hepatozoon* parasite. In Hungary, Rigó *et al.* (2016) show an infection rate of 17%.

There are two related rodent species at the same locality, belonging to the same family, and the transmission of parasites by vectors from one population to another one could be expected. Despite the fact that both species can host *Trypanosoma* subgen. *Herpetosoma*, those parasites were found only on the smears of *Ch. nivalis*. This can be explained by the strong host specificity of the parasite. According to Molyneux (1969) and Noyes *et al.* (2002), *T.* (*H.*) *evotomys* is a species of *M. glareolus* and this *Trypanosoma* cannot infect any other vertebrate species. Similarly, Šebek (1975) records *T. evotomys* in *M. glareolus* from Czechoslovakia and Karbowiak *et al.* (2005) records cases from Poland. *Trypanosoma* found in *Ch. nivalis* must therefore belong to other species. The parasites were not classified into species, but if the host specificity of the subgenus *Herpetosoma* is accepted as a common rule, the parasite found in snow voles must also exhibit a host specificity to *Ch. nivalis*. According to our findings, *T.* (*H.*) *evotomys* of *M. glareolus* is not present at the Dolina Bielych plies valley location.

Another host specificity is reported in the *Hepatozoon* genus; the only vertebrate host species infected by *H. erhardovae* tends to be *M. glareolus* (Mehlhorn 2008; Rigó *et al.* 2016). Therefore, it is assumed that *Hepatozoon* found in snow voles at Dolina Bielych plies is of a different species.

In Brestová, only samples of *Ch. nivalis* were collected, and no parasites was found. This locality in the Western Tatras is distant from Biele plesá and we can assume geographical isolation from the population of parasite survival is prevalent. The entire meta-population is fragmented into small subpopulations and there are places with suitable habitats where no voles were found (e.g. Kolová Valley – Hrehová and Némethy - personal conversation), so the transmission and colonization by vectors for such a long distance, skipping the empty areas, is not possible. The samples were collected systematically during the whole year and smears were prepared in good quality. Overlooking a parasite while viewing the smears is less likely.

Wiger (1979) states that *Hepatozoon* sp. prevalence culminates in early summer. The only infected individual trapped in Dolina Bielych plies - the adult female - was, however, trapped in November. A season of peak prevalence does not exclude the possibility of finding a parasite late in the season. According to Laakkonen *et al.* (2011), dormant and slowly reacting stages (meronts) are found in organs (lung, spleen) during the whole year, while blood forms (gamonts) are visible only when arthropod vectors are active. Despite the cold weather of early winter, there were still some undetermined ectoparasites found in the fur of voles caught in November. Possible vectors transmitting parasites can be active.

Contamination

Wild animals are exposed to air pollutants and heavy metals from their natural habitats (Kalas *et al.* 2000). Environmental pollution has the potential to change the ecological interaction between species, including the relationship between a parasite and its host (Eeva and Klemola 2013).

Mechanisms of host defense are negatively influenced and make a host more sensitive to parasite infection. Alternatiely, there may be an increasing population of proper hosts and intermediate hosts (Lafferty and Kuris 1999). Some research results suggest that this pollution would lead to the deterioration of resistance to parasite infections (Tersago *et al.* 2004), especially endo-parasitic nematodes (Jalčová and Dvorožňáková 2014) and ectoparasites (Eeva and Klemola 2013).

Results of the experiment do not record any significant variation in the concentration of toxic elements, many of which are heavy metals. There were neither indications to suggest the ability of a parasite to affect the host's metabolism, nor opposite dependence when highly contaminated individuals are more vulnerable to infection by a parasite.

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