

## Blood parasites of mound-building mouse, *Mus spicilegus* Petényi, 1882 (Mammalia, Rodentia)

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**ABSTRACT.** Mound-building mice, *Mus spicilegus*, were studied for the blood parasites in Eastern Slovakia, vicinity Kechnec village near Košice town (Košická kotlina basin, 21°14' E, 48°33' N) during years 2002–2005. Overall, 251 specimens were examined. The parasites were detected using microhematokrit centrifugation technique and on the Giemsa's method stained blood smears and light microscopy. The parasites were found in 3.57% of specimens; 1.20% of mice were infected with *Bartonella* sp., 2.39% were infected with *Babesia* piroplasms. No *Hepatozoon* hemogregarines and trypanosomes were observed. The intensity of infection with *Bartonella* was low, less than 0.01% of erythrocytes were invaded, the percent of the erythrocytes with *Babesia* sp. was less than 0.01%. The morphological description and measurements of parasites were made using the „Analysis” software combined with a video camera and a microscope. The mean size of *Bartonella* sp. bacteria's were 0.8×0.3 μm, range 0.4–1.5×0.1–0.9 μm, *Babesia* sp. occurred in pear-shaped and ring-like forms, 1.00–1.27 μm in diameter, and 0.98–1.27 μm in size, respectively. The regular form of four cells – “maltese cross” was not noticed. This is the first record infection of *Mus spicilegus* with blood parasites.

**Key words:** *Mus spicilegus*, *Babesia* sp., *Bartonella* sp.

### Introduction

Mound-building mice *Mus spicilegus* is the common Mediterranean rodent, that have a wide geographical range extending from South-eastern Austria, through Romania to Northern Ukraine [1,2]. In the former period due to its morphological similarity to the house mouse *Mus musculus* Linnaeus, 1758 and to the Macedonian mouse, *M. macedonicus* Petrov et Ružič, 1983, many false reports were published regarding the distribution and habitat preferences of mentioned above species. However, due to the ethological feature, the study using enzyme electrophoresis subsequently enabled the development of morphological and morphometric tools to identify individual species. Once genetic studies were made based on sequencing of segments of the mitochondrial DNA (mtDNA), the divergence of *M. spicilegus* from other *Mus* species was confirmed [3–6].

The wild mouse-like rodents and other small mammals can play an important role in distribution of the human pathogens (parasites, viruses, bacteria caused streptococcal infections, tularaemia, spirochaetosis, leptospirosis, etc.). The ability to be a vector and reservoir of human pathogens also relates to mound-building mice. Originally it was thought that *M. spicilegus* had occupied steppe grassland habitats, however, due to the extension of farming, mound-building mice are mainly found in agricultural fields. The specific nidobiology and subterranean placement of nests of *M. spicilegus* differ from other Central European rodents. This fact may considerably affect the composition of parasitofauna. It is presented that the number and composition of ectoparasitofauna differ from other rodent genera [7,8]. Furthermore, the fact that this species neighbours close to human activities, may be the reason for special epidemiological and economical significance. The communities of blood

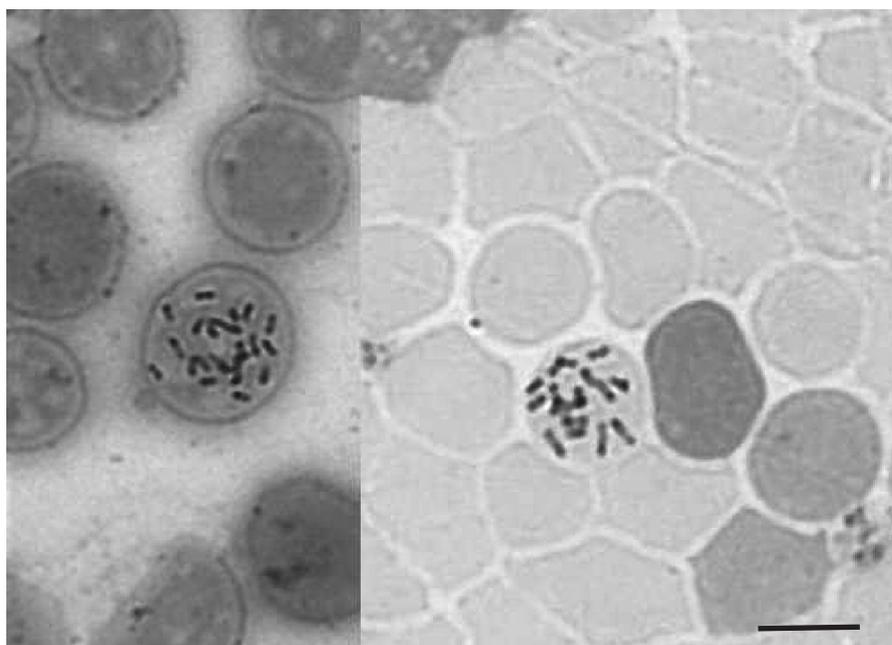


Fig. 1. *Bartonella* sp. found in the blood of *Mus spicilegus*. Scale bar: 5  $\mu$ m.

parasites of *Apodemus* genus mice and many Microtidae rodents are already quite well described [9–11]. The hematozoa of *Mus* genus nonetheless are not well known, although mice *Mus musculus* and *Mus domesticus* are very common all over the world [12]. From the blood parasites family of *Mus* genus, only the *Trypanosoma musculi* was investigated so far and is known as a parasite found in every region on the Earth [13]. The data regarding other common small rodent blood parasites – bacteria's from genera *Bartonella* and *Anaplasma*, *Hepatozoon* hemogregarines and *Babesia* piroplasms are fragmented.

## Materials and methods

Mound-building mice were trapped live in the vicinity Kechnec village near Košice town (Eastern Slovakia, Košická kotlina basin (21°14' E, 48°33' N) during years 2002–2005; the majority of animals were caught in 2004 (55 individuals) and in 2005 (48 specimens). The mice were lured by Swedish bridge metal traps and sunflower as a bait. Totally, 251 specimens of mound-building mice were examined. The mice were trapped in autumn and winter mainly (e.g., in November – 56 specimens, in December – 19, and October – 15), some mice in spring (in March – 17 specimens), near their store mounds. The blood was taken from the anaesthetised rodents using the heart puncture method. During section studies, the blood was taken immediately from the heart in euthanized mice. The

blood smears were dried and stained by the standard Giemsa's method (fixation in methyl alcohol, staining for 1 hour in Giemsa's stain diluted [1:5] in phosphate buffer, pH 7.2). The slides were examined under a light microscope. For each slide, 50 fields were examined at  $\times 1500$  magnification, using an oil-immersion objective. Additionally, for trypanosomes detection, the microhematocrit centrifugation method was used. Measurements of parasites were made using the „Analysis” software combined with a video camera and a microscope. The morphological features taken into consideration and morphometric parameters were used after other investigations of haemoparasites [10].

## Results

In total, 251 *Mus spicilegus* mice were investigated. The blood parasites were found in 9 (3.58%) specimens. Three (1.19%) mice were infected with *Bartonella* sp., and 6 (2.39%) mice were infected with *Babesia* piroplasms. No *Hepatozoon* hemogregarines and trypanosomes were found. The intensity of infection with *Bartonella* sp. was very low usually, less than 0.01% of erythrocytes were invaded. Bacteria's were visible on blood smears as dark-blue or black comma-like bodies. Their mean size was  $0.8 \times 0.3 \mu\text{m}$ , range  $0.4\text{--}1.5 \times 0.1\text{--}0.9 \mu\text{m}$  (Fig. 1).

The mean intensity of infection with *Babesia* sp. was in less than 0.01% of erythrocytes. The parasites were small, mostly of the ring-like and

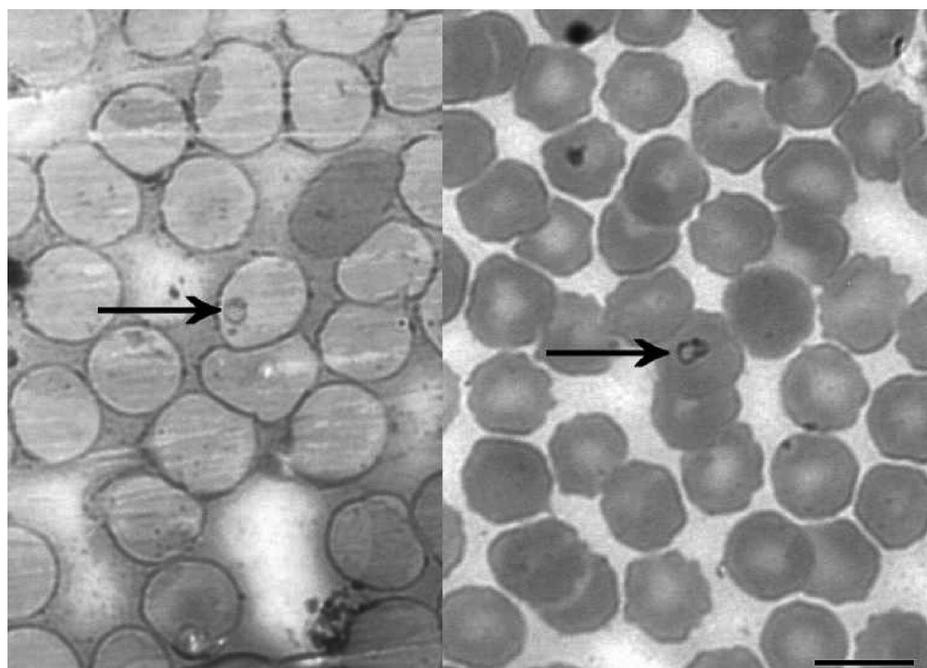


Fig. 2. Merozoites of *Babesia* sp. in the erythrocytes of *Mus spicilegus*. Scale bar: 5  $\mu$ m.

pear-shaped form (Fig. 2) (the morphological terminology after Mehlhorn and Schein [14]). The ring-like forms were 1.00–1.27, mean  $1.15 \pm 0.19$   $\mu$ m in diameter, pear-shaped 0.98–1.27, mean  $1.19 \pm 0.07$   $\mu$ m in size. Usually one parasite was seen in an erythrocyte. The regular form of four cells – “maltese cross” characterised as the “small” *Babesia* species, was not noticed. The infected animals did not show any noticeable pathological symptoms.

## Discussion

The prevalence of infection of *M. spicilegus* with blood parasites found – *Babesia* sp. and *Bartonella* sp. – was very low. Although the methods used have the limited efficiency and in the planned study molecular detection would show bigger prevalence, this result is significant in comparison to infection prevalence in other rodents from *Microtus* and *Apodemus* genera. *Babesia* piroplasms has the level comparable with *Babesia* infection in other small mammal species [15]. The foregoing dependences were observed both under the study conducted at the same time on the same areas as well as in the longer period on other populations of the *Apodemus* spp. mouse (Karbowski and Stanko, unpublished). The probable reason can be the relatively poor community of ectoparasitofauna of hosts, the vectors and reservoir of haemoparasites, described elsewhere [16]. On the other side relative rich mite

and flea fauna were recorded in nests [8,17]. The vast difference between abundance of fleas and mites in nests and/or in hair of *M. spicilegus* can probably be influenced by the grooming effect [8], several individuals (2–19, usually 5–6) live in association with a mound during wintering, usually from September until April [17,18, Stanko, unpubl.]. Due to the low prevalence of *M. spicilegus* infection with blood parasites, it was impossible to find any statistically significant relationships between infection and sex, age, season and other ecological or biological factors. *Babesia* piroplasms isolated from *M. spicilegus* are smaller than typical forms which occur in other rodents, being 1.5–3.0  $\mu$ m in size [10] – more similar to smaller, non-identified *Babesia* piroplasms found in dogs [19]. For this reason, without molecular study we can classify these parasites as *Babesia microti*-like. As mentioned above, the identification of *Bartonella* bacteria is impossible using standard light microscopy methods. *Bartonella* infection was found in *Mus* mice in Sweden only and identified as *Bartonella grahamii* [20]. So far, the following species: *B. birtlesii* in *Apodemus* mice [21], *B. tribocorum* in *Rattus norvegicus* [22], *B. grahamii* and *B. taylorii* in *Myodes glareolus* [23], *B. doshiae* in *Apodemus sylvaticus* [24] are described in rodents. A significant fact was the lack of infection with trypanosomes. These parasites are widespread in *Mus musculus* mouse and other small rodents [11]. Their absence is an additional

confirmation for the biological and ecological separateness of mound-building mouse.

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