DETECTION OF *EHRLICHIA MURIS* IN A YELLOW-NECKED MOUSE (*APODEMUS FLAVICOLLIS*) IN CENTRAL SLOVAKIA

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The aim of this work was to examine the occurrence of various tick-borne pathogens of the genera *Rickettsia, Anaplasma* and *Ehrlichia* in small terrestrial mammals in selected localities of Central Slovakia. We detected presence of *Ehrlichia muris* in the spleen of a yellow-necked mouse (*Apodemus flavicollis*) trapped in Malá Lehota. This is the first evidence of a monocytic ehrlichia, *E. muris* in Central Europe as well as in the yellow-necked mouse.

E. muris AS145 strain was isolated as a new infectious agent from the spleen of a wild mouse, *Eothenomys kageus*, trapped during a routine monitoring of rickettsial agents in Japan in 1983 (*1*). Like other ehrlichiae, *E. muris* AS145 strain is a small, gram-negative, pleomorphic, obligate intracellular bacterium causing splenomegaly, lymphadenopathy, ruffled fur, inactivity, and anorexia in laboratory mice (*1, 2*). Other isolates of *E. muris* were obtained from the mice *A. speciosus*, *A. argenteus)* and the tick *Haemaphysalis flava*.

The antibodies against *E. muris* were detected in dogs, wild monkeys, bears, deer, and boars, but not in *R. norvegicus* rats (*2*). *E. muris* was found in *Ixodes persulcatus* ticks in the Baltic region of Russia (*3*) and in the West Siberia, Russia (*4*), where the prevalence of 8.8% ± 2.5% of this bacterium was detected.

In October 2006, a total of 30 small terrestrial mammals (the order *Rodentia*) e.g.19 yellow-necked mice (*A. flavicollis*), 9 bank voles (*Clethrionomys glareolus*), and 2 wood mice (*A. sylvaticus*) were live-trapped in four localities near the villages of Lutila (48° 37´N, 18°50´E), Malá Lehota (48°30´N, 18°34´E), Horná Ves (48°40´N, 18°54´E), and Hodruša Hámre (48°29´N, 18° 46´E) in the Central Slovakia. The localities near Malá Lehota, Horná Ves, and Hodruša Hámre represent woodland areas with forests of Carpatian oak-hornbeam vegetation type and moderately cool montane climate. The locality near Lutila is agricultural countryside with wide lanes, trees and low bush, and moderately warm intramontane basin climate. Spleens were taken from the animals, stored in 70% ethanol and used for DNA isolation using DNeasy Tissue Kit (Qiagen). Three different PCRs were used for the detection of eubacteria, ehrlichiae/anaplasmae, and rickettsiae. The PCR for eubacteria amplified a part (470 bp) of the 16 S rRNA gene using primers GA1B and 16S8FE designed from the respective sequence of *E. ruminantium* (*5*). The PCR for ehrlichiae/anaplasmae amplified a part (298 bp) of the 16 S rRNA using primers Ehr521 and Ehr790 designed from the respective sequence of *Anaplasma phagocytophilum* (*6*). This PCR detected besides *A. phagocytophilum* also *A. marginale* and *E. chaffeensis* (*7*). The PCR for rickettsiae amplified a part (381 bp) of the *gtlA* gene using primers RpCS.877p and RpCS.1258n designed from the respective sequence of *R. prowazekii* (*8*). PCR products were separated by 1.2 % agarose gel electrophoresis and visualized by ethidium bromide staining. The sequencing was performed by Macrogen, Inc., Korea. The obtained sequences were compared with those available in databases using the BLAST Program of the National Center for Biotechnology Information, Bethesda, USA (www.ncbi.nlm.nih.gov/blast). Phylogenetic and sequence analyses were conducted using the MEGA version 3.1 (*9*).

Only one adult male yellow-necked mouse trapped in Malá Lehota was positive by the PCR for eubacteria, but negative by the PCRs for ehrlichiae/anaplasmae and

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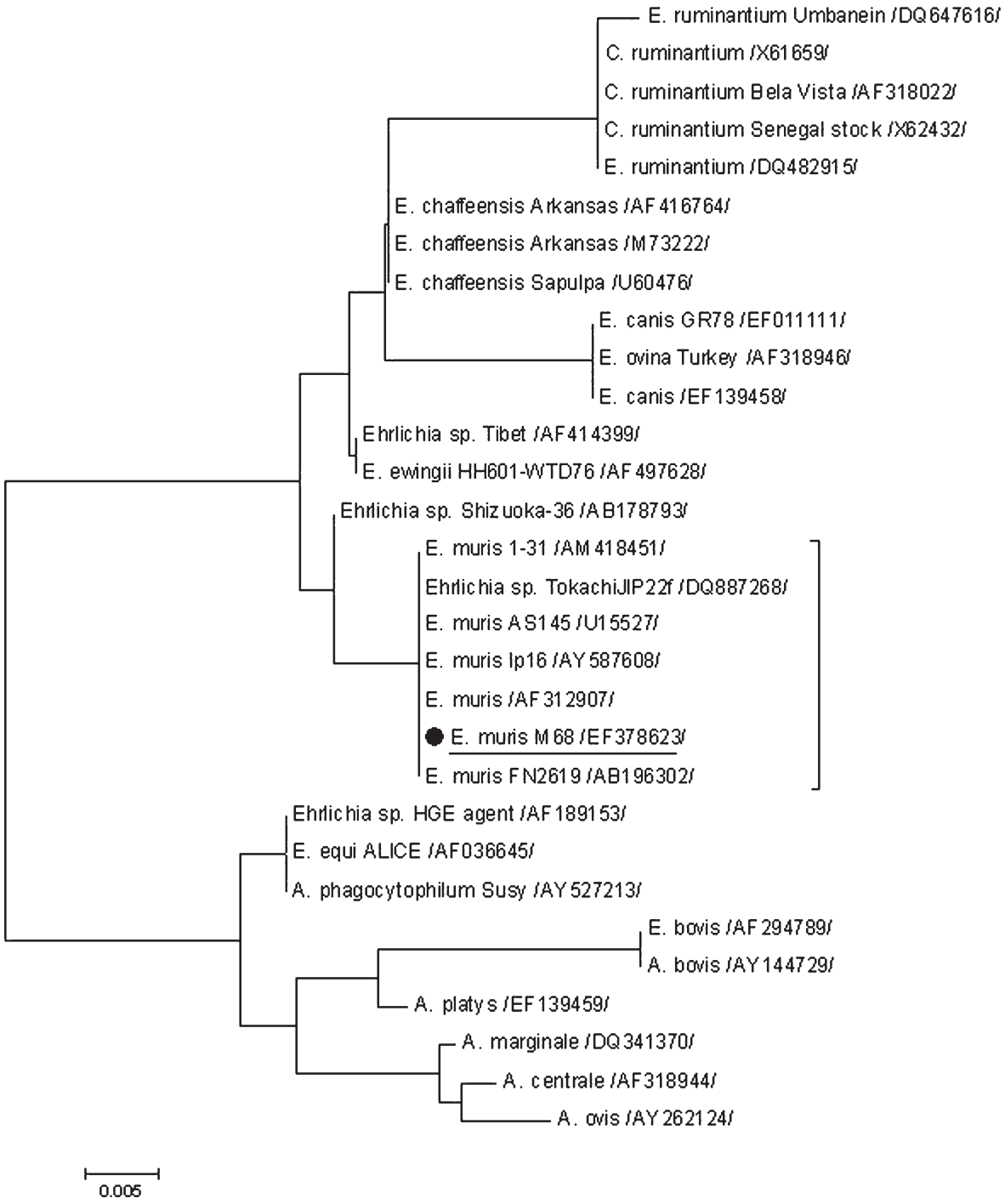
rickettsiae. In spite of the negativity in PCR for ehrlichiae/anaplasmae, the obtained DNA sequence matched those of the other *E. muris* isolates. Phylogenetic analysis revealed that the new isolate of *E. muris* (Acc. No. EF378623-underlined) clustered together with five other *E. muris* isolates Acc. Nos. AB196302, AF 312907, and AY587608 with the identity of 99.8%, U15527 with the identity of 99.5%, and AM418451 with the identity of 100% (figure).



Thus, our results demonstrated that the 16S rRNA PCR using Ehr521-Ehr790 primers was not generally suitable for detection of all isolates of *E. muris*.

This study represents the first molecular evidence of the occurrence of *E. muris* in a yellow-necked mouse and in Central Europe as well. This monocytic ehrlichia is supposed to be associated with different species of wild mouse as host and *Ixodes* and *Haemaphysalis* ticks as vectors. It may occur not only in Asia but also in Europe. Further studies are necessary to answer the question how *E. muris* coexists with other tick-borne microorganisms and to identify its tick vectors in Central Europe.

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