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Molecular Screening of European Wild Rodents for Tick-Borne Encephalitis Virus

***Philippe Gil de Mendonça*[1*], *Ana-Maria Benedek*[2], *Martina Jurčovičová*[3]**

[1] Institute of Comparative Tropical Medicine and Parasitology, Ludwig Maximilian University, D–80802 Munich, Germany
[2] Department of Ecology and Environment Protection, Lucian Blaga University, RO– 550337 Sibiu, Romania
[3] Institute of Mountain Biology, Zilinska University, SK–05956 Tatranska Javorina, Slovakia

Abstract: A total of 377 rodents from Germany, Slovakia and Romania were inspected for ticks and screened by real–time RT–PCR for tick–borne encephalitis virus. All ticks found on rodents were immature stages. *Apodemus flavicollis, A. sylvaticus, A. uralensis* and *Clethrionomys glareolus* hosted *Ixodes ricinus*, whereas *Pitymys subterraneus* hosted *I. trianguliceps*. All rodent ear biopsies tested negative for TBE virus despite this virus reaching a relatively high prevalence in questing ticks in some of the areas surveyed.

Key words: Yellow–necked mice, *Apodemus flavicollis*, co–feeding transmission, TBEV, real–time RT–PCR

Introduction

It is commonly believed that a parasitic arthropod vector has to feed on a reservoir host presenting with systemic infection in order to acquire a pathogen from that host. However, it was shown that, under laboratory conditions, ticks may become infected by a virus while feeding on a non–viraemic host (Labuda *et al*. 1996). Indeed, tick saliva contains immune–modulating factors (Jones *et al*. 1992) which permit the survival of a pathogen in a locally immune–depressed skin area restricted around the tick bite. A non–infected tick feeding near an infected (and infective) tick may thus become infected in turn by the virus present in that locally immune–depressed skin area (Labuda *et al*. 1996). In laboratory experiments, yellow–necked mice and bank voles immune to TBEV (tick–borne encephalitis virus) were nevertheless able to act as transmission hosts thanks to co–feeding transmission between ticks (Labuda *et al*. 1997). Since ticks feeding on rodents are often clustered on the ear flap (Fig. 1), and since this pattern was observed in yellow–necked mice, which were relatively abundant in a few endemic areas, it was proposed that this species may act as a non–systemic amplification host for TBEV in nature (Randolph *et al*. 1999).

The present study therefore aimed to screen ear skin biopsies from wild rodents (with emphasis on the yellow–necked mouse) caught in known or suspected endemic areas for TBE virus.

Materials and Methods

Rodents were caught in live–traps from 2007 to 2009 in known (Germany) or suspected (Slovakia and Romania) endemic areas for TBEV. They were inspected for ticks and the latter ones were identified to the species level. A small skin biopsy (approx.

[*]Corresponding author: P. G. de Mendonça, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1, Tzar Osvoboditel blvd., BG–1000 Sofia, Bulgaria; E-mail: philippe.mendonca@iber.bas.bg

5 mm^2) was collected by ear punching or ear notching, and rodents were immediately released at the place of capture thereafter. Skin samples from Germany and Slovakia were immediately placed in 1 mL RNA-Later® (Ambion, Austin, TX) and incubated for 12 h at 4 °C before being stored at –32 °C. Romanian samples were immediately fixed and stored in 80% ethanol, as it was demonstrated that TBEV RNA is very efficiently preserved in that way (P.G. de Mendonça, unpublished). RNA was extracted from each sample using RNeasy® Mini Spin Columns (QIAGEN, Hilden, Germany) following the manufacturer's instructions. RNA samples were screened for TBE virus by real–time RT–PCR (Schwaiger, Cassinotti 2003). Positive and negative controls were included in each RT–PCR run.

Results

Five rodent species were caught: the yellow–necked mouse (*Apodemus flavicollis*), the wood mouse (*A. sylvaticus*), the pigmy mouse (*A. uralensis*), the bank vole (*Clethrionomys glareolus*) and the European pine vole (*Pitymys subterraneus*).

Tick identification: *Apodemus flavicollis, A. sylvaticus, A. uralensis* and *Clethrionomys glareolus* hosted *Ixodes ricinus*, whereas *Pitymys subterraneus* hosted *I. trianguliceps*. All ticks found on rodents were immature stages.

Molecular diagnosis of TBE virus: All positive controls produced a typical positive amplification signal (S–shaped curve), whereas all negative samples produced a negative signal (flat horizontal line). All 377 rodent samples tested negative for TBE virus by real–time RT–PCR. Species, numbers, and place of origin are given in Table 1.

Table 1. Rodent species, number of individuals screened for TBE virus by real–time RT–PCR, and goegraphical origin of the samples.

SPECIES	N	ORIGIN
Apodemus flavicollis	324	Germany
Apodemus sylvaticus	10	Germany & Romania
Apodemus uralensis	3	Romania
Clethrionomys glareolus	1	Germany
Pitymys subterraneus	39	Slovakia
TOTAL	377	

Discussion

Although prevalence estimates for TBE virus in questing vector ticks may reach values as high as 1.8% in some of the areas where rodent samples were collected (de Mendonça, unpublished), all ear biopsies analysed as part of this study were negative for this pathogen, despite real–time RT–PCR being an extremely sensitive detection method. The fact that each positive control yielded a strong and typical positive signal indicates that both reverse transcription and PCR amplification were successful in each RT–PCR run. Considering that experimentally infected yellow–necked mouse skin remains positive for several days (Labuda *et al*. 1997), and that European pine voles, which are highly susceptible to TBEV infection, commonly develop high levels of viraemia (Labuda *et al*. 1993), one would expect a few samples to test positive if these rodents were 'the principal amplifying hosts' of this virus in nature, as was proposed by Randolph *et al*. (2002). However, there are major differences between the experiments performed in the laboratory and the actual tick infestation observed in nature. Many ticks were placed in retaining chambers thus preventing grooming in experimental animals (Labuda *et al*. 1993, 1996, 1997), whereas most rodents harbour very few if any tick under natural conditions (de Mendonça 2003, 2005), and self–grooming and social grooming are very efficient at removing ticks (de Mendonça 2003, 2005). Furthermore, most ticks found on small rodents are larvae, very few are nymphs, and adults are extremely rare (de Mendonça 2003, 2005). This point is very important because TBEV prevalence is extremely low in larval ticks, it may reach 0.9% in nymphs from Bavaria (de Mendonça, unpublished), and reaches its maximum value in adult ticks. Most cases of heavy tick infestation on yellow–necked mice actually occur when an unfortunate mouse walks through a 'tick nest', thus 'harvesting' many larvae at once. In such a case however, the potential for co–feeding transmission of TBEV between ticks is very low due to the extremely low prevalence of TBEV in larvae. In conclusion, the present study found no supportive evidence for TBEV transmission by co–feeding on yellow–necked mice under natural conditions in confirmed endemic foci. The actual part of this species in the epidemiology of TBEV under natural conditions should therefore be critically reviewed.

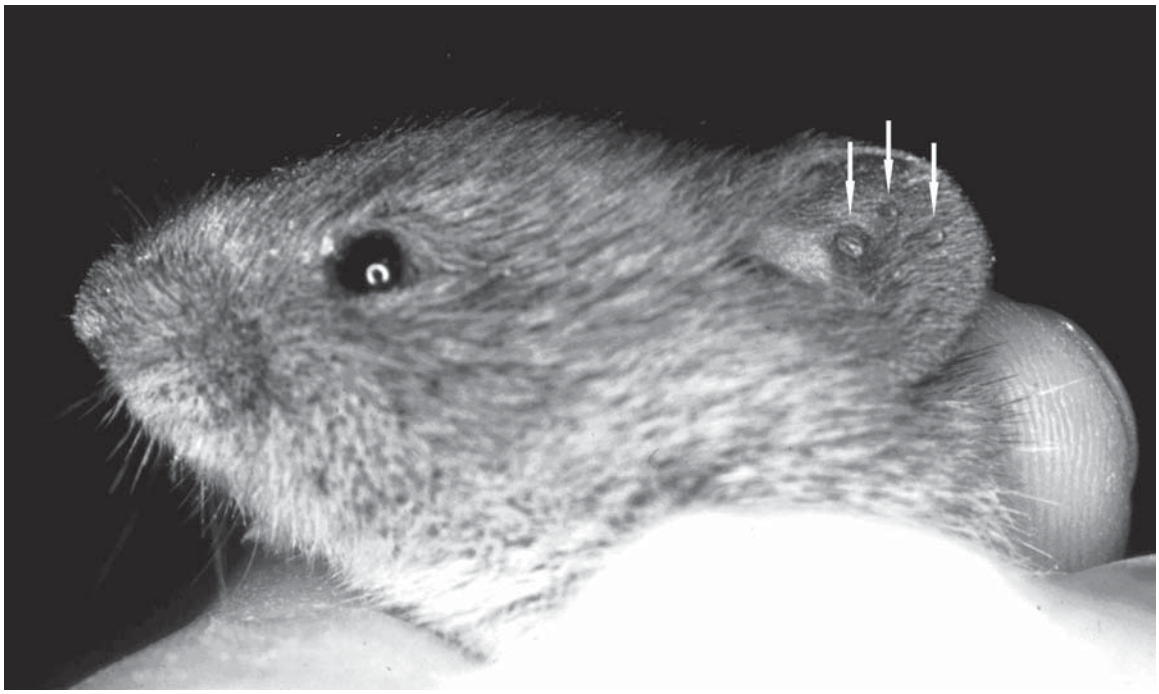

Fig. 1. Immature ticks (arrows) are often attached to the ear flap of small rodents.

Individual contributions

Rodent trapping and tissue sampling: PGM, MJ and AMB.
RNA extraction: PGM and AMB.
Molecular diagnosis (RT–PCR): PGM.

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