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LETTER TO THE EDITOR

Ectotherm vertebrates as a new potential reservoir of murid gammaherpesvirus 4

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Herpetic viruses have been selected and have diversified in the course of evolution, resulting in the emergence of viruses capable of persistent infection in several animal species. These viruses also include murid gammaherpesvirus 4 (MHV-68) with its characteristic ability to reactivate from a latent form, which can result in the rapid spread of viral infection in nature (*1*).

MHV-68 virus is a natural pathogen of free-living muroid rodents, isolated from *Myodes glareolus* (*2*). However, neutralizing antibodies were also found in sera from animals of other species living in the same biotope with infected rodents (*3*). The shedding of the virus by breast milk, urine, saliva, and tears was experimentally confirmed (*4*). All these data have led to the conclusion, that in nature, MHV-68 infects various rodent species belonging to different phylogenetically distant families, and to the hypothesis that the MHV-68 virus could be transmitted from infected rodents to other animals living in the same biotopes.

The presence of the virus was confirmed indirectly by a virus neutralization test in sera of, wild house mouse (*Mus musculus*), hare (*Lepus europaeus*), domestic cat (*Felis catus*), red fox (*Vulpes vulpes*), domestic dog (*Canis familiaris*), wild boar (*Sus scrofa*), cattle (*Bos taurus*), domestic goat (*Capra hircus*), mouflon (*Ovis musimon*), sheep (*Ovis aries*), European roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), horse (*Equus ferus*), and directly by a PCR reaction in ticks (Ixodida) (*5*). Recently, three different species of bats (*6*) and birds belonging to the family *Paridae* were confirmed as a reservoir of MHV-68 (*7*).

A significant finding during the ecological study of this virus was the discovery that ticks play an important role in its circulation in nature (*8*). These experimental data fulfill the transmission criteria that define an arthropod-borne virus (arbovirus), the largest group of viruses. Before this finding, the African swine fever virus was the only DNA virus recognized as an arbovirus.

Several species of ticks (belonging to the genera *Dermacentor, Ixodes, Haemaphysalis, Hyalomma* and *Amblyomma*) were collected from bodies of lizards and

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Abbreviations: MHV-68 = murine gammaherpesvirus 68; L = larva; N = nymph

a tortoise from various countries of Central (Slovakia, Hungary, Slovenia), Eastern (Romania) and Southern Europe (Albania, Bulgaria, Croatia, North Macedonia, Serbia), and from two countries in Central Asia (Tajikistan and Kyrgyzstan).

Genomic DNA was isolated from the samples of 82 ticks using the alkaline hydrolysis method (9). Of the total number of ticks collected, 39 were assigned to the species *Ixodes ricinus* (6 ♂, 12 ♀, 14 N - nymph, 7 L - larva), 20 to the species *Dermacentor reticulatus* (9 ♀, 11 ♂), 20 to the genus *Haemaphysalis* (7 ♀, 10 N, 3 L); in one case, the species was determined as *Haemaphysalis punctata*. In the genus *Hyalomma*, we included two ticks of different species (*Hyalomma marginatum rufipes* ♀ and *Hyalomma aegyptium* ♂). The genus *Amblyomma* was represented by one tick (1 N).

Using a nested PCR targeting the ORF50 gene of MHV-68 (7), virus DNA was detected in 20 samples of isolated genomic DNA from ticks. The presence of MHV-68 was confirmed in 13 samples (5 ♀ and 8 ♂) of the total number of 20 ticks of the *Dermacentor reticulatus* species collected in October 2017 from the Dobrohošt region (Slovakia), which represents 65% of the total number. The prevalence of the MHV-68 virus was slightly higher in males than in females. The presence of the virus was also confirmed in the 3 samples (2 ♀ and 1 ♂) from 14 ticks of the *Ixodes ricinus* collected in Retovje, Slovenia; from the juvenile stage of the tick nymph of the genus *Amblyomma*; in the species *Haemaphysalis punctata* collected from the Munţii Măcinului site in Romania; in the nymphal stage of a tick of the genus *Haemaphysalis* from the Togut Toto site in Kyrgyzstan and in an adult male tick of the genus *Hyalomma aegyptium* from the Kotë area in Albania. Since the presence of MHV-68 DNA was confirmed in some ticks attached to captured reptiles and amphibians, we investigated its presence in the blood of these animals as well.

No traps or snares were used during the capture of animals. Both the lizards and the tortoise were captured during the day and night randomly with bare hands during the mapping of the herpetofauna (observing and recording the number of animals) living in the given localities. Frogs were caught during the night in selected water sources. Blood sampling from the ventral abdominal or tail vein was performed using insulin syringes. Totally, twenty-four DNA samples (13 ♂, 10 ♀, and 1 unspecified gender) were from animals caught in the spring, summer, and early autumn between 2014 and 2017. None of the captured specimens were harmed, and they were released again in the same place after sampling. Genomic DNA was extracted from 33 blood samples using a NucleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol.

Nine DNA samples were from blood taken from lizards of the genus *Eremias* (7 ♂ a 1 ♀) caught in Kyzyl Tuu in Kyrgyzstan and one lizard of the species *Eremias nikolski* caught in Sambuli in Tajikistan in spring and early autumn during 2016–2017.

The presence of MHV-68 was confirmed by nested PCR in a sample of genomic DNA isolated from the tortoise *Testudo hermanni*, caught in the Kotë area in the territory of Albania.

We also detected MHV-68 DNA in blood samples from two *Podarcis muralis* lizards from the Logje area in Slovenia and the Baba Mountains, in North Macedonia; from the lizard *Algyroides nigropunctatus* from the Syri i Kaltër area in Albania, and also from the lizard, *Lacerta agilis* caught near the Patinský Channel in Slovakia.

Altogether, the presence of MHV-68 DNA was confirmed in 5 of 33 blood samples (prevalence 33%) from reptiles and amphibians from different geographical areas in Europe and Asia.

Our results support the hypothesis that ticks of the genera *Ixodes, Dermacentor, Haemaphysalis, Hyalomma,* and *Amblyomma* can play a key role in the spread of MHV-68 in nature. Confirmation of the presence of MHV-68 DNA in ticks and their hosts of the ectotherms, suggests another new source of MHV-68 in nature, and repeatedly confirm the importance of the role of ticks in the spread of this virus in common biotope with these animals.

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