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LETTER TO THE EDITOR

Molecular detection of murine gammaherpesvirus 68 (MHV-68) in *Haemaphysalis concinna* ticks collected in Slovakia

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Summary. – Murine gammaherpesvirus 68 (MHV-68) is a natural pathogen of murid rodents, which serve as hosts to *Haemaphysalis concinna* ticks. The occurrence of MHV-68 was investigated in a total of 47 *H. concinna* adult ticks collected on the vegetation in Gabčíkovo, situated in south-western Slovakia (47°54´0´´N, 17°35´0´´E), from May 2013 to May 2014. DNA from ticks was purified and screened by nested PCR targeting ORF50 of MHV-68 and the copy number of virus genome in ticks was determined by a real-time PCR assay specific for ORF65. The MHV-68 incidence in questing ticks was 38.3% (18/47) and the virus genome copy number per tick varied from $2x10^2$ to $9.6x10^3$. In this study, MHV-68 was documented for the first time in *H. concinna ticks*. Results expand previous data describing the occurrence of MHV-68 in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected in Slovakia, supporting the hypothesis that MHV-68 might be a new-found pathogen in ticks.

Keywords: murine herpesvirus 68; Haemaphysalis concinna ticks; nested PCR; Slovakia

As obligate blood-sucking ectoparasites of various terrestrial vertebrates, ticks are notorious for transmitting the widest variety of pathogens of any blood-sucking arthropod, causing numerous diseases in humans and animals. Ticks identified as pathogen vectors (less than 10%) belong to the genera *Ixodes, Haemaphysalis, Hyalomma, Amblyomma, Dermacentor, Rhipicephalus,* and *Boophilus* (*1,2*). *Haemaphysalis concinna* Koch (*Acari*:*Ixodidae*) is widely distributed in France, Germany, Poland, Hungary, Bohemia, Slovakia, Russia, Austria, in temperate Eurasia (*3*) as well as in China. *H. concinna ticks* have been found to transmit pathogens such as *Coxiella burnetii*, *Borrelia* genus spirochetes, *Rickettsia and Babesia* spp., *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis* as well as Russian-spring encephalitis and Crimean-Congo hemorrhagic fever virus (*4, 5, 6*). In some areas of Slovakia, *H. concinna* has been found to cooccur with *I. ricinus* and *D. reticulatus* ticks, which feed on small and medium sized mammals (*7*). Rodents are known to play a role in the enzootic cycles of nonviral pathogens, such as *Rickettsia* spp., *Ehrlichia* spp., *Francisella tularensis, Coxiella burnetii* and viruses such as hantaviruses, Tick-born encephalitis virus and Lymphocytic choriomeningitis virus. Notably, *Apodemus* spp. mice and *Myodes glareolus* exhibit infections with numerous tick-born viruses from the ticks that infest them (*8*).

During 2011–2014, we have reported the first data on MHV-68, originally isolated from bank voles (*M. glareolus*), as a potential pathogen in *I. ricinus* and *D. reticulatus* ticks, the most common free-living tick species in Slovakia

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Abbreviations: MHV-68 = murine gammaherpesvirus 68

(*9*). A total of 1.8% of immature *I. ricinus* ticks infesting *Lacerta viridis* green lizards (10 of 649 nymphs and 5 of 150 larvae) have been identified as virus-positive by molecular methods (*10*). Next, Kúdelová *et al.* (*11*) have shown MHV-68-positivity in about 23.3% (28/120) and 40% (125/312) of *D. reticulatus* adults collected in Gabčíkovo and Vojka nad Dunajom (47°58´35´´N, 17°22´50´´E), respectively. Thereto, an examination of the salivary glands, intestines and ovaries of *D. reticulatus* ticks identified live MHV-68, capable of replication in mammalian cells, in all organs (using an explantation/co-cultivation procedure), suggesting this virus is a potential arbovirus.

In this study, we used nested PCR method to examine a group of 47 adult *H. concinna* ticks collected over the vegetation in Gabčíkovo. DNA from ticks was isolated and screened for the presence of MHV-68 DNA by standard nested PCR targeting the ORF 50 gene of MHV-68 as previously described (*11*). The sequences of forward and reverse primers amplifying a 382-bp long nested PCR product were: ORF50/F1: 5'-AACTGGAACTCTTCTGTGGC-3'; ORF50/R1: 5'-GGCCGCAGACATTTAATGAC-3' and ORF50/F2: 5'-CCCCAATGGTTCATAAGTGG-3'; ORF50/R2: 5'-ATCAGCACGCCATCAACATC-3'). DNA of MHV-68 BAC and DNA samples of known negative *H. concinna* tick served as a positive control and an additional negative control. All PCR work performed complied with generally known strict protocols to control cross-contamination, such as pipetting the template in a separated PCR box and room and using a PCR mixture without template as a negative control. The nested PCR products were resolved on a 1.5% agarose gel and samples yielding PCR products of the expected size were determined to be MHV-68 positive (Fig. 1, lanes 2–4, 6–9, and 12–15). The MHV-68 occurrence in questing *H. concinna* ticks was 38.3% (18/47). Amplicons of nine randomly chosen MHV-68-positive ticks were purified using the PCR Clean-up System (Promega) and sequenced on both strands using a commercial sequencing service (BITCET). Comparing of sequences amplified from virus-positive ticks with the corresponding sequence of MHV-68 ORF 50 revealed nearly 100% identity by the BLAST program (www.ncbi.nlm.nih.gov/blast). Then, the copy number of viral genomes was determined in samples of all 18 virus-positive *H. concinna* ticks by a real-time PCR assay specific for ORF65 of MHV-68 using Maxima® SYBR Green PCR Master kit (Lambda Life) and primers as previously described (*12*). Our results showed that the copy number of MHV-68 per *H. concinna* ticks varied from $2x10^2$ to $9.6x10^3$.

H. concinna ticks, known as reservoir for many viral and non-viral pathogens, often feed on small murid rodents, from which MHV-68 was originally isolated. Due to the nature of MHV-68, its ability to cause lifelong latent infection in host B-lymphocytes and to reactivate from latent infections, MHV-68 can exist for a relatively long time in the blood of murid rodents (*13*). In very early study, finding of neutralizing antibodies to murine herpesvirus in the serum of rodents, fallow deer (*Damadama*), wild boar (*Sus scrofa*), and red deer (*Cervuselaphus*) gave rise to a hypothesis that MHV-68 could be transmitted via ticks from rodents to other animals living in the same biotope (*14*). In the first molecular study in rodents, an approximate 34.4% prevalence of MHV-68 was detected by PCR in blood of free bank voles (M. *glareolus*) and yellow-necked field mice (*A. flavicollis*) trapped in Slovakia (*15*). In an early study on MHV-68 in adult ticks from Slovakia collected in Vojka nad Dunajom in autumn 2011 and spring 2012, about 9.7% (14/144) and 66.0% (111/168) of *D. reticulatus* ticks were found positive, respectively. Later on, about 23.3% incidence of MHV-68 (28/120) was detected in these ticks collected in Gabčíkovo in 2014 (*11*). Here, while examining *H. conccinna* ticks collected in the same locality in the time interval from 2013 to 2014, we confirmed virus presence in 18 out of 47 (40.4%) ticks.

In conclusion, our study expands previous finding of MHV-68 in *I. ricinus* nymphs and *D. reticulatus* adults, and *Haemophysalis concinna* represents the third tick species

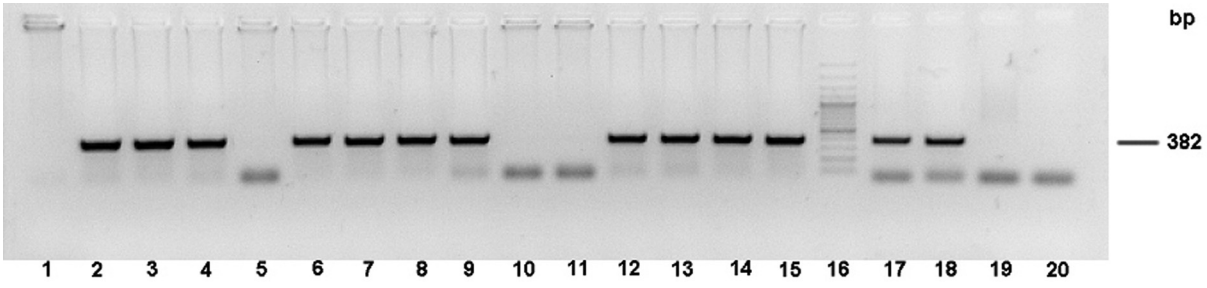

Fig. 1

Detection of MHV-68 in *H. concinna* adult ticks collected in Gabčíkovo from May 2013 to May 2014 using nested PCR

Lanes: 1–15 – ticks Nos. 1–15; 16 – 100 bp ladder (Fermentas); 17 – MHV-68 BAC DNA (nested PCR; positive control); 18 – MHV-68 BAC DNA (1. PCR with nested primers; positive control); 19 – no template (nested PCR; negative control); 20 – no template (1. PCR with nested primers; negative control).

found to be infected with MHV-68. Taken together, MHV-68 could be detected in some tick species in South-western Slovakia each year and collection season from 2011 to 2014. These findings support the hypothesis that ticks could play a role in MHV-68 circulation in nature. They also might suggest that MHV-68 is the first among known gammaherpesviruses to be detected in ticks. The experimental evidence of virus transmission between ticks and hosts and *vice versa,* using appropriate experimental tick-host-virus model, is needed to take a position on the idea that MHV-68 might be a novel arbovirus.

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References

1. Labuda M, Nuttall PA, Parasitology 129, 221–245, 2004. https:/doi.org/10.1017/S0031182004005220

2. Burri C, Schumann O, Schumann C, Gern L, Ticks Tick Borne Dis. 5, 245–251, 2014. https:/doi.org/10.1016/j.ttbdis.2013.11.007

3. Nosek J, Z. Parasitenkd. 36, 233–241, 1971.

4. Špitalska E, Kocianová E, Eur. J. Epidemiol. 18, 263–266, 2003. https:/doi.org/10.1023/A:1023330222657

5. Svitálková Z, Haruštiaková D, Mahríková L, Berthová L, Slovák M, Kocianová E, Kazimírová M, Parasites & Vectors 8, 276–279, 2015. https:/doi.org/10.1186/s13071-015-0880-8

6. Hamšíková-Svitálková Z, Haruštiaková D, Mahríková L, Mojšová M, Berthová L, Slovák M, Kocianová E, Vayssier-Taussat M, Kazimírová M, Parasites & Vectors 9, 2, 2016. https:/doi.org/10.1186/s13071-015-1287-2

7. Švehlová A, Berthová L, Sallay B, Boldiš V, Sparagano OAE, Špitalská E, Ticks TickBorne Dis. 5, 600–605, 2014. https:/doi.org/10.1016/j.ttbdis.2014.04.010

8. Estrada-Peña, de la Fuente J, Antiviral Res. 108, 104–128, 2014.

9. Kazimírová M, Hamšíková Z, Kocianová E, Marini G, Mojšová M, Mahríková L, Berthová L, Slovák M, Rosá R, Exp. Appl. Acarol. 69, 205–224, 2016. https:/doi.org/10.1007/s10493-016-0025-6

10. Ficová M, Betáková T, Pančík P, Václav R, Prokop P, Halásová Z, Kúdelová M, Microb. Ecol. 62, 862, 2011. https:/doi.org/10.1007/s00248-011-9907-7

11. Kúdelová M, Belvončíková P, Vrbová M, Kovaľová A, Štibrániová I, Kocáková P, Slovák M, Špitalská E, Lapuníková B, Matúšková R, Šupolíková M, Microb. Ecol. 70, 785–794, 2015. https:/doi.org/10.1007/s00248-015-0622-7

12. Guo H, Wang L, Peng L, Zhou ZH, Deng H, J. Virol. 83, 10582–10595, 2009. https:/doi.org/10.1128/JVI.00497-09

13. Rajčáni J, Kúdelová M, In Latency Strategies of Herpesviruses, Chapt. V, 102–136, 2007.

14. Mistríková J, Rašlová H, Mrmusová M, Kúdelová M, Acta Virol. 44, 211–226, 2000.

15. Klempa B, Mačáková K, Labuda M, Kúdelová M, Infect. Dis. Rev. 3, 31–33, 2001.