

## LETTER TO THE EDITOR

### Molecular detection of murine gammaherpesvirus 68 (MHV-68) in bats from Mexico

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More than 1,400 species of bats (representing approximately 20% of mammalian species diversity) can be found almost all over the world (Mammal Diversity Database 2019). Nowadays, bats have been intensively studied animals since they represent important natural reservoir hosts for an increasing number of zoonotic pathogens with the ability to cross species barriers. A wide range of virus species from different families have been isolated from or detected in bats, including rabies virus, tick-borne encephalitis virus, SARS coronavirus, MERS coronavirus, Ebola virus, Dengue virus, Hendra virus, Nipah virus, West Nile virus, Zika virus, Yellow fever virus, and Chikungunya virus. While bats generally harbor these viruses with no clinical symptoms of infection, they can cause serious diseases in humans, sometimes with fatal outcomes. Moreover, phylogenetic data indicate that infections now restricted to humans, such as mumps and measles, may have their origins in bats (1, 2).

The way bats live and their behavior (migratory patterns, high population density, roosting behaviors, extreme longevity, food choices and feeding habits) increase

the probability of inter-species transmission of various pathogens. The transmission of pathogens from bats to other animal species or humans occurs by direct contact with infected animals or their body fluids. Bat infectious agents can also be transmitted indirectly via vectors. One of the most important vector group for viruses are blood-feeding insects (ticks and mosquitoes) (1, 3).

The *Herpesviridae* family is a wide group of DNA viruses extensively disseminated in nature. Members of this virus family are present in most vertebrates (mammals, birds, and reptiles) (4). Many different herpesviruses representing all three subfamilies (*Alphaherpesvirinae*, *Betaherpesvirinae*, *Gammaherpesvirinae*) have also been recently isolated from bats (5). However, knowledge of the occurrence of herpesviruses in bats is still incomplete.

Murine gammaherpesvirus 68 (MHV-68) is a natural pathogen of small free-living murid rodents, isolated in Slovakia in 1976 (6). MHV-68 could be transmitted from infected rodents to other animals living in the same biotope as well as to livestock and household animals. The study performed in this context has brought evidence of virus-neutralizing (VN) antibodies against MHV-68 in the serum of fallow deer, wild boars, deer, sheep, foxes, mouflons and also in humans. Molecular detection of MHV-68 in different tick species suggested that ticks can act as vectors of MHV-68. The respiratory tract, however, appears to be the main gateway for MHV-68. During the

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**Abbreviations:** EBV = Epstein-Barr virus; KSHV = Kaposi's sarcoma associated herpesvirus; MHV-68 = murine gamma-herpesvirus 68; nPCR = nested PCR; VN = virus-neutralizing

Bat	239	GCTGCAGAACCCAGAAGAAATCATTAACCTGGACCCTCTACACAATACCACTGAACCCTG	298
MHV-68	68495	GCTGCAGAACCCAGAAGAAATCATTAACCTGGACCCTCTACACAATACCACTGAACCCTG	68554
Bat	299	TTTGTATCAAATGTTTTTCAGAGGCGGTGACAAACCCCTCTAAAAAAGATGGCCTTCTTC	358
MHV-68	68555	TTTGTATCAAATGTTTTTCAGAGGCGGTGACAAACCCCTCTAAAAAAGATGGCTTTCTTC	68614
Bat	359	TTTCAACATGGTCTTTTCAGGACTGTCTCCGAGAGCTCGGCCAGAGACCACTTATGAACC	418
MHV-68	68615	TTTCAACATGGTCTTTTCAGGACTGTCTCCGAGAGCTCGGCCAGAGACCACTTATGAACC	68674

Fig.1

Sequence of the ORF 50 PCR product of MHV-68 isolated from bat  
Arrows indicate point mutation.

infection, the virus is excreted via the respiratory tract excretes, saliva, tears, and urine (reviewed in 7).

Intranasal inoculation of laboratory mice with MHV-68 leads to a productive infection of lung epithelial cells. This lymphotropic virus spreads via lymph nodes to the spleen, where it establishes lifelong latency, predominantly in B-cells. MHV-68 is biologically and genetically closely related to human gammaherpesviruses - Epstein-Barr virus (EBV) and Kaposi's sarcoma associated herpesvirus (KSHV). Like EBV and KSHV, reactivation of MHV-68 virus can occur when the host's immune system is weakened, which may lead to the development of various malignancies (reviewed in 8). Interestingly, MHV-68 is capable of infecting and replicating in various cell lines derived from human tissues. All the above-mentioned information implies a cross-species transmission of MHV-68 with potentially serious consequences for the infected animals or humans (reviewed in 9).

Recently, VN antibodies against MHV-68 were detected in four blood samples from the lesser mouse-eared bat (*Myotis blythii*) and in one blood sample from the western barbastelle bat (*Barbastella barbastellus*) originating from Muránska Planina in Slovakia. The sample with the highest VN antibody titer (*M. blythii*) was found to be MHV-68 positive in nested PCR (nPCR).

In addition, MHV-68 DNA was also detected by nPCR in a blood sample collected from the noctule bat (*Nyctalus noctula*) captured in Kharkiv, Ukraine (10). These results suggested that MHV-68 may be widespread in several bat species and also distributed in different geographical areas.

The aim of the presented study was to look for the presence of MHV-68 DNA in liver tissue samples from 30 bats of six different species (*Pteronotus parnellii* - 8 males and 9 females, *Pteronotus personatus* - 1 male and 1 female, *Pteronotus davyi* - 4 males and 3 females, *Leptonycteris verbabuenae* - 2 males, *Mormoops megalophylla* - 1 male,

*Balantiopteryx plicata* - 1 female). All the specimens were collected in the region of Chamela, Jalisco, Mexico. Total genomic DNA was isolated from liver homogenates using the commercial Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. The presence of MHV-68 DNA was investigated by nPCR targeting the ORF50 gene (described in 10). We confirmed the presence of the ORF50 sequence in one sample from the lesser long-nosed bat (*L. verbabuenae*). Nested PCR product was then purified and sequenced with the inner primers for the ORF50 gene-specific reaction using a commercial sequencing service. Sequence analysis revealed homology to the ORF50 sequence of MHV-68 strain WUMS (Acc. No. U97553.2), except for one point mutation at position 68,608 (WUMS sequence). The original nucleotide thymidine was replaced by cytosine in the sequence of the PCR product (Fig. 1). This point mutation caused amino acid substitution in the protein encoded by ORF50 - leucine at position 328 was replaced by proline.

This is the first time that MHV-68 has been found in bat outside Europe - on the American continent. This finding suggests that MHV-68 is a globally widespread herpesvirus capable of frequent inter-species transmission, probably thanks to suitable arthropods vectors.

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