Chapter

Murine Gammaherpesvirus 68 (MHV-68), a Newly Discovered Tick Borne Virus

Marcela Kúdelová and Iveta Štibrániová

Abstract

MHV-68, closely related to human gammaherpesviruses (Epstein-Barr virus and Kaposi's sarcoma herpesvirus), is a natural pathogen of murid rodents commonly infested with ticks. After the first finding of MHV-68 in immature *Ixodes ricinus* ticks removed from wild green lizards, its occurrence was proved in free-living *Dermacentor reticulatus*, *I. ricinus*, and *Haemaphysalis concinna* ticks. Next, finding of live MHV-68 in salivary glands, intestine, and ovaries of *D. reticulatus* ticks strongly supported the idea that MHV-68 could be transmitted from infected to uninfected host via blood-feeding ticks. Recently, experimental transmission of MHV-68 between *I. ricinus* ticks and mouse and *vice versa* proved that MHV-68 could be vertically and horizontally transmitted from F0 to F1 tick generation, and thus, MHV-68 is a tick-borne virus (arbovirus). Therefore, ticks commonly attack humans transmitting important pathogens (e.g., tick-borne encephalitis virus and the Lyme disease spirochete); there is the speculation that MHV-68 can also infect humans *via* ticks. Earlier studies documented antibodies to MHV-68 in the sera of laboratory workers, hunters, and general population as well. In future, we need to carefully test whether people bitten by ticks are at real risk of infection with MHV-68 that normally infects murid rodents, and what effect it may have.

Keywords: MHV-68, gammaherpesvirus, blood-feeding tick, tick-borne transmission, arbovirus

1. Introduction

This review attempted to summarize the results of the work that contributed to the recognition of murine gammaherpesvirus 68 as a novel tick-borne virus in the context of to date known viruses found in ticks of species *Dermacentor reticulatus*, *Haemaphysalis concinna*, and *Ixodes ricinus* focusing on the territory where MHV-68 was first discovered.

2. MHV-68 and rodent gammaherpesviruses

Murine herpesvirus 68 (MHV-68 or γHV68) belonging to a group of dsDNA viruses of large genome was originally isolated from the bank vole *Myodes glareolus*

(formerly *Clethrionomys glareolus*) during a study on the ecology of arboviruses in Slovakia [1]. Four other murine gammaherpesviruses were isolated at the same time, two from bank voles and two from the yellow-necked field mouse *Apodemus flavicollis* trapped in west Slovakia. Later, three further gammaherpesviruses were isolated from the latter species in Bohemia and Slovakia [2]. Very early studies on murine herpesvirus neutralizing antibodies were identified in the sera of 20.7% individuals of five reservoir animal species (i.e., wood mice, bank voles, field voles, yellow-necked mice, and wild mice) [3]. By molecular methods, the presence of MHV-68 DNA was also confirmed in the blood of 34.4% of *M. glareolus* and *A. flavicollis* mice trapped in Slovakia [4]. Even, antibodies against MHV-68 have been detected in sera of at least 13 different mammalian species such as red deer (*Cervus elaphus*), fallow deer (*Dama dama*), hare (*Lepus europaeus*), wild boar (*Sus scrofa*), sheep, and foxes that share the biotope of infected rodents. Neutralizing antibodies to MHV-68 have also been detected in humans, but they are considered to reflect antigenic cross-reactions with human gammaherpesviruses (reviewed by [5]). Studies on MHV-68 *in vitro* showed that MHV-68 could replicate in as much as 16 cell cultures of different origins (e.g., mouse, chick, rabbit, hamster, mink, swine, monkey, and/or human origin, including T and B cells) [6]. The following molecular studies on MHV-68 genome including its full-length sequencing confirmed suggestions of a close genetic relationship of MHV-68 to primate saimiri herpesvirus-2 (SaHV-2) and human gammaherpesviruses—Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) [7, 8]. During the 1990s, the infection of mice with MHV-68 and some other murine gammaherpesviruses inducing lymphoproliferative diseases (LPD) have been intensively studied (reviewed in [9]). Compiling all the results, in the year 2000, MHV-68 was classified into a new species, as murid herpesvirus 4 (MuHV-4) (synonyms murine gammaherpesvirus 68 and mouse herpesvirus strain 68), the genus *Rhadinovirus* and the subfamily *Gammaherpesvirinae* [10]. Its genome contains 118,237 bp of unique sequence flanked by multiple copies of a 1213 bp terminal repeat. Of the 80 ORFs identified in the MHV-68 genome, 63 are homologs of SaHV-2 genes, all of which are also present in the KSHV genome and many of which are present in the EBV genome [8]. The discovery of MHV-68 provided a tractable laboratory infection model for investigating human gammaherpesvirus reactivation from latency as well as host immune response mechanisms involved in persistent infection associated with the development of malignancies such as Burkitt's lymphoma, Hodgkin's disease, and Kaposi's sarcoma [11–13]. A huge work was done on MHV-68 that is recently the most amenable animal model virus for studying the pathogenesis of human gammaherpesviruses [14].

It has been speculated rodent gammaherpesviruses are geographically widespread and may occur throughout the mouse and vole subfamilies [15]. The epidemiological surveys in the UK, Germany, France, and Peru found several other gammaherpesviruses in free-living rodents. Two novel gammaherpesviruses were isolated in the UK and France, one from the wood mouse (*Apodemus sylvaticus*), designated wood mouse virus (WMHV) (classified as MuHV-7), and the other from the white-toothed shrew (*Crocidura russula*) designated Brest herpesvirus (BRHV) [16, 17]. WMHV showed similarity with MHV-68 in the growth in cell culture and pathogenesis in its natural host, and its complete genome sequence was determined [17]. In the UK and Germany, the first gammaherpesvirus infecting house mice, *Mus musculus* (*Mus musculus* rhadinovirus 1 [MmusRHV1]), was described as a member of a newly discovered group of rodent herpesviruses. This virus, designed as MHV-68-like rodent gammaherpesvirus, is distinct from MHV-68 the most

probably diverged from the other gammaherpesviruses soon after the evolutionary separation of EBV-like lymphocryptoviruses from KSHV 8-like rhadinoviruses [18]. To date, the latest rodent gammaherpesvirus was isolated from pygmy rice rat (*Oligoryzomys microtis*), designated rodent herpesvirus Peru (RHVP). Analysis of its full-length genome sequence confirmed that it shares conserved genes and genome organization with MHV-68 and the primate gammaherpesviruses but is phylogenetically distinct from MHV-68 [19].

Although the MHV-68 belongs to the best-characterized murine gammaherpesviruses and it has been documented that is mainly transmitted in the rodent population via intranasal routes and through body fluids, such as saliva, urine, tears, breast milk, and also vertically, it is not yet fully understood how this virus spreads in nature (reviewed by [9]). Following intranasal inoculation of laboratory mice, the virus spreads to the lungs, and viremia appears due to virus replication in the alveolar epithelium and endothelial cells of alveolar septa. The productive virus growth within lung epithelium ceases at 7–10 days p.i. During the viremic phase, mature B cells as well as macrophages become infected. At the acute stage, an infectious mononucleosis-like syndrome develops, analogous to that induced by EBV, associated with the establishment of MHV-68 latency and splenomegaly. After primary infection, the MHV-68 spreads to host organs via blood, in which it remains for roughly 15 days. As with other gammaherpesviruses, it causes lifelong infection of its host. It establishes a long-term latency not only in B lymphocytes (spleen, lymph nodes) and macrophages but also in lung endothelial cells that may lead to lymphoproliferative disorders (LPDs) [20–23]. Besides LPDs, also solid tumors (lymphomas) were described in infected Balb/c mice [23, 24].

Furthermore, as typical for all herpesviruses, based on various conditions (stress, gravidity, immune deficiency, and others), the virus can reactivate to a state of repeated lytic infection and reappear in host blood. Taking into account the properties of MHV-68, including its extreme stability at a wide range of pH and temperature values and nature of its spreading (via urine, breast milk, and other body fluids), a relatively high host reinfection rate should be considered [25, 26]. This suggests that MHV-68 can exist for a relatively long time in the blood of murid rodents, which undoubtedly feed hard ticks, including spp. *Dermacentor* and *Ixodes*. Both tick species mentioned, the most common in Slovakia, were identified as vectors of many tick-borne pathogens. Based on these data, a hypothesis was formed suggesting that blood-feeding ticks might transmit the virus from infected to uninfected animal host.

3. Ticks and tick-borne pathogens

Rodents, including *Apodemus* spp. mice and *M. glareolus* from which some murine herpesviruses were isolated, were found displaying the infection along with numerous pathogens, viruses, and also nonviral pathogens (bacteria, protozoa, and helminths) from the ticks which fed on them. Rodents play a role in the enzootic cycles of the so-called tick-borne pathogens that are transmitted from the ticks to vertebrates of which most have a life cycle that requires passage through the vertebrate host, thus being important reservoirs for these pathogens [27–30]. The most extensively characterized viruses that have rodent hosts in the family Muridae are the members of the family Herpesviridae. These include mouse cytomegalovirus and rat cytomegalovirus, which are classified in the genus *Muromegalovirus* of the subfamily *Betaherpesvirinae* and MHV-68 of the family *Gammaherpesvirinae*.

Hard ticks are highly specialized obligate hematophagous ectoparasites of wild and domestic animals and humans. There are over 900 species of ticks in the world, and many of them are capable to transmit disease-causing pathogens, including viruses, thus having significant medical and veterinary impact by causing serious diseases in humans and animals [31–33]. Less than 10% of known tick species were identified to act as virus vectors. Many unique features of ticks make them inevitably suitable to host and to carry different viruses as well as act as long-term virus reservoirs. Among hard ticks, virus vectors have been found mostly in the genera *Ixodes*, *Haemaphysalis*, *Hyalomma*, *Amblyomma*, *Dermacentor*, and *Rhipicephalus*. Moreover, some tick species are known to be vectors of a few TBV species (e.g., *I. ricinus*, *A. variegatum*), while others can transmit many different TBV species (e.g., *Ixodes uriae* is the vector of at least seven TBVs) [33, 34].

In Europe, there are two important hard tick spp., *Ixodes* and *Dermacentor* (Acari: Ixodidae) [35], which act both as important arthropod vectors and reservoirs for a series of wildlife zoonotic pathogens such as bacteria (e.g., *Rickettsia* spp., *Coxiella burnetii*, *Anaplasma phagocytophilum*, *Ehrlichia* spp., *Borrelia burgdorferi sensu lato*, *Francisella tularensis*, and *Bartonella* spp.): protozoa (e.g., *Babesia* spp.) [36–38]: and viruses (e.g., tick-borne meningoencephalitis virus, Colorado tick fever virus, Kemerovo virus, Crimean-Congo hemorrhagic fever virus) [39, 40].

Tick-borne viruses (TBVs) belong to the largest biological group known as arboviruses with unique mode of transmission by blood-feeding arthropods (ticks, mosquitoes, sand flies, biting midges, etc.) to a susceptible vertebrate host. They are different from other viruses in their ability to replicate in both vertebrate and invertebrate cells. Tick-borne viruses are causative agents of several important human diseases. Since the discovery of the first tick-borne pathogenic virus which was identified as being responsible for severe encephalitis in sheep in 1918 [41], diversified TBVs with global distribution have been discovered and isolated belonging to at least 2 orders, 9 families, and 12 genera [42]. Most of them belong to orders Bunyavirales and Mononegavirales and families of Flaviviridae, Asfarviridae, Reoviridae, and Orthomyxoviridae [43]. In recent years, the rapid development of next generation sequencing (NGS) has boosted the discovery of novel TBVs, many of them still unassigned to families (reviewed by [44]). At present, more than 16 specific tick-borne diseases (TBDs) of humans and 19 TBDs of veterinary importance have been described [42, 45]. The tick-borne encephalitis virus (TBEV) is the most medically prominent and important arbovirus (arthropod-borne virus) in Europe and Northern Asia, causing more than 10,000 clinical cases of tick-borne encephalitis annually. Other relevant tick-borne viruses which cause encephalitis in humans are the Powassan virus, Tribeč virus, Kemerovo virus, and Colorado tick fever virus. Of all the routes of human infection by tick-borne viruses, those of TBEV have been described in the most detail. The latest emerging TBD, caused by Bourbon virus, was reported in 2014 [46].

With two exceptions, all arboviruses are RNA viruses. The only established DNA tick-borne virus, African swine fever virus (ASFV), belongs to the Asfarviridae family with a single genus *Asfivirus* [47]. The ASFV genome consists of a single molecule of linear, covalently close-ended, dsDNA varying in length from 170 to 190 kbp. ASFV is the causative agent of African swine fever. ASFV is maintained in the sylvatic transmission cycle of ticks in Africa [48, 49]. Widely distributed ixodid ticks in Europe such as *I. ricinus* and *D. reticulatus* are unable to support ASFV replication and presumably do not contribute to disease spread [50]. The spread of ASFV has been primarily caused by human activities including long-distance transport of livestock. The presence of a susceptible wildlife host, wild boar, has further complicated efforts to control the disease, and it is likely that it will continue to

spread across the continent. Recent transmission studies have demonstrated the evidence for a role of the hard ticks *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* in mechanical and transstadial transmission of the second DNA virus, lumpy skin disease virus (LSDV), a member of Poxviridae family of large enveloped viruses with linear double-stranded DNA [51, 52]. This virus belongs to the genus *Capripoxvirus*, causing lumpy skin disease of cattle in Africa and the Middle East [53, 54].

4. Findings of the MHV-68 in ticks

As mentioned above, MHV-68 is currently recognized as a natural pathogen of murid rodents that host blood-feeding ticks. The first evidence of MHV-68 in ticks was found in nymphs and larvae of *I. ricinus* feeding on 116 individuals of a temperate lizard species—the green lizard *Lacerta viridis* captured in the Slovak Karst National Park (48°57' N, 20°44′ E, ∼200 to 400 m above sea level). In this study [55], MHV-68 was detected in 10 of 649 nymphs and in 5 of 150 larvae, respectively. We found 15 of 799 (1.8%) nymphs and larvae as virus positive when 9.6% of green lizards fed at least one MHV-68-infected immature tick. These results provided two possible explanations. The first was that lizards could be infected via direct contact with jointly occupied holes and paths with rodents contaminated with infected animals. Although experimental data describing contact infection with MHV-68 *in vivo* are still missing, the routes of natural infection of murid rodents with this virus (intranasal or via body fluids) and relatively extreme stability of murine herpesvirus at wide range of pH and temperature [26] made contact infection with MHV-68 probable. The second possible source of MHV-68 infection of lizards represented feeding of infected hard ticks in the past (though in this study, no hard ticks were found on lizards). However, the following studies were evoked to obtain the evidence that the MHV-68 is able to escape from the gut after feeding and move through the tick to the salivary glands where it could be transmitted during a second feeding. Anyhow, finding of MHV-68 in immature *I. ricinus* ticks supported the hypothesis that ticks may play a mediating role in circulation of MHV-68 in nature.

In Slovakia, *Ixodes ricinus*, *Dermacentor reticulatus*, *Dermacentor marginatus*, *Haemaphysalis concinna*, *Haemaphysalis inermis*, and *Haemaphysalis punctata* tick species are common and widespread [56], where they had been found to be infected with numerous nonviral and viral pathogens [57–59]. In the following studies, three of the most common tick species in Slovakia were examined for the presence of MHV-68, *D. reticulatus*, *H. concinna*, and *I. ricinus*, to take a position on the hypothesis that the ticks could be a vector in the transmission of MHV-68 from infected wild mice to other mammals.

Dermacentor reticulatus (Fabricius, 1794) (Acari: Ixodidae) is the three-host meadow tick that parasitizes primarily wild and domestic mammals and, infrequently, humans. It is widespread throughout Europe and is expanding its range in several European countries [60, 61]. Recent comparative analyses have revealed changes in the distribution and abundance (almost doubled) of *D. reticulatus* ticks in some European countries, implying a higher risk of the transmission of tick-borne diseases. In Slovakia, the *D. reticulatus* tick had a focal distribution in Slovakia in the past [62], occurring mainly in the southwest and southeast along the Morava, Dunaj, and Latorica rivers. Of late, *D. reticulatus* has extended its former geographical distribution by at least 200 km further to the north and by approximately 300 m into higher altitudes up to 520 m above sea

level [63]. Rubel et al. [40] recently described geographical distribution of this tick in Europe.

D. reticulatus tick is associated with a number of different pathogens and currently considered the second most significant reservoir and vector of numerous pathogens causing bacterial, protozoal, rickettsial, and viral diseases in its hosts [38, 43, 64–68]. Its role in the transmission of disease to humans is currently small; however, it might play an important role in the maintenance of pathogens in enzootic cycles [69–72].

Until 2014, *D. reticulatus* tick was proven as a vector of only one viral pathogen, Omsk hemorrhagic fever virus, identified in Western Siberia [73]. However, two other viruses, Kemerovo virus and tick-borne encephalitis virus, have been identified in this tick collected in Western Siberia and Eurasia [74, 75]. In *Dermacentor* sp. several other tick-borne viruses were identified such as Colorado tick fever virus, Burana orthonairovirus, Lanjan virus, Razdan virus, Dhori virus, and Sawgrass virus (reviewed in [64]).

Kúdelová et al. [76] by nested PCR examined the presence of MHV-68 within a group of 432 adult *D. reticulatus* ticks collected near the river Dunaj in two sites in southwestern Slovakia from 2011 to 2014. Analyses showed MHV-68 positive as much as about 23.3% (28/120) and 40% (125/312) ticks from Gabčíkovo (47°54′00″ N, 17°35′00″E) and Vojka nad Dunajom (47°58′35″N, 17°22′50″E). The infecting virus was confirmed analyzing amplified products via sequencing. Thereto, the salivary glands, intestines, and ovaries of five females were examined for live MHV-68 using an explantation and cocultivation procedure used to achieve spontaneous reactivation of latent herpesviruses [77, 78]. These methods allowed to use tick organs to determine the presence of viruses capable of replicating in VERO cells and producing CPE. The VERO cells seemed to be a good choice because a relatively long cultivation was needed to properly detect the replication of the virus. A finite amount of virus from tick organs should be considered because it was impossible to predict virus dose in each tick. However, live MHV-68 capable of replication in mammalian cells was identified in all organs of two ticks (**Figure 1**) suggesting that MHV-68 found at least in salivary glands might be transmissible from infected to uninfected host. In the following study, MHV-68 was identified in *D. reticulatus* ticks collected from other two sites in Slovakia in 2014, while viral incidence in adult tick was 53.3% in a group of 30 ticks from Komárno (47°45′48″N 18°07′42″E) and 62.5% in a group of 40 ticks from Vysoká pri Morave (near the Morava river) (48°19′50.51″N, 16°54′15.38″E), respectively [79].

The next study on adult *D. reticulatus* ticks collected in Vojka nad Dunajom in spring 2013 provided the first evidence of MHV-68 transcripts in field-collected ticks suggesting that MHV-68 might replicate in their bodies. The transcripts of M3 gene (known to be expressed during both the lytic phase and latent infection of the animal host) were identified in as many as ten out of eleven questing ticks by nested RT-PCR method. As one might expect, the transcription of MHV-68 previously limited to evidence from tick organs after virus propagation in vitro was evidenced to have different amounts of M3 gene transcripts (**Figure 2**).

In this study, the amount of MHV-68 genome copies per *D. reticulatus* tick was identified in samples of 38 virus-positive ticks. An infectious dose of MHV-68 in ticks quantified by qPCR varied from 2.2×10^4 to 8.6×10^6 [80].

Haemaphysalis concinna (Koch, 1844) (Acari: Ixodidae) is the second most abundant tick species after *I. ricinus* collected from birds and third most abundant tick species flagged from vegetation in Central Europe. It is widely distributed in France, Germany, Poland, and alongside the rivers Danube and Morava in Hungary, Bohemia, Slovakia, and Austria, in Russia and temperate Eurasia, and in China [81]. In some areas of Slovakia, *H. concinna* has been found to co-occur with

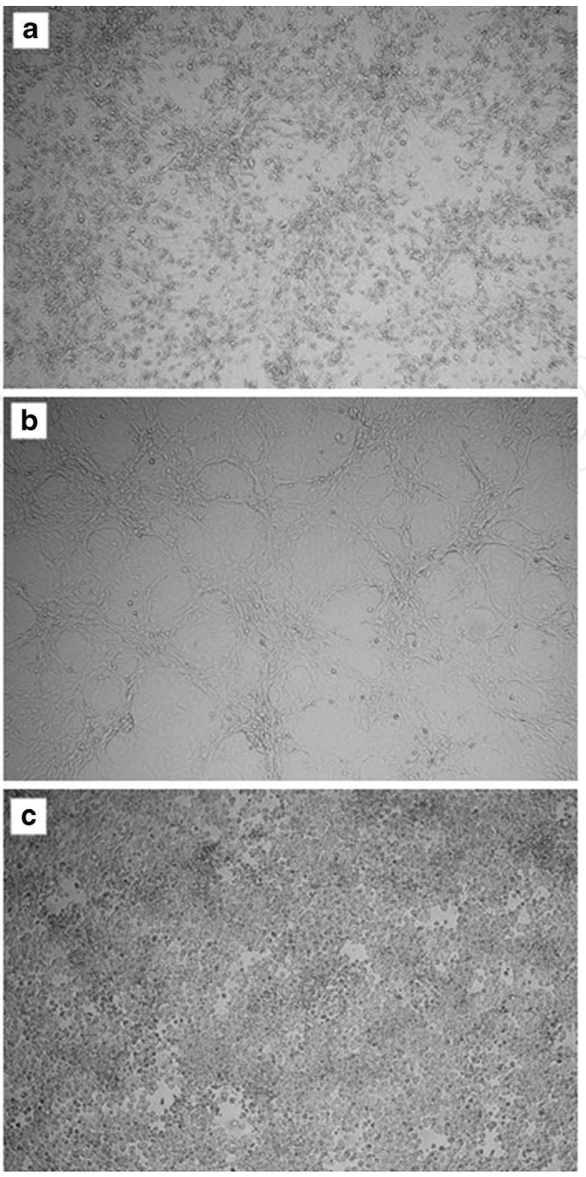

Figure 1.
Detection of infectious MHV-68 in the explanted salivary glands of the D. reticulatus *tick. Infectivity of MHV-68 as determined by plaque formation (CPE) in VERO cells 10 days after inoculation with explantation medium coming from salivary glands of tick No. 1 observed by light microscopy. Magnification, ×10, (b) uninfected VERO cells (negative control), (c) VERO cells infected with MHV-68 (MOI = 0.001 PFU/ml) (positive control); for details, see Kúdelová et al. [76].*

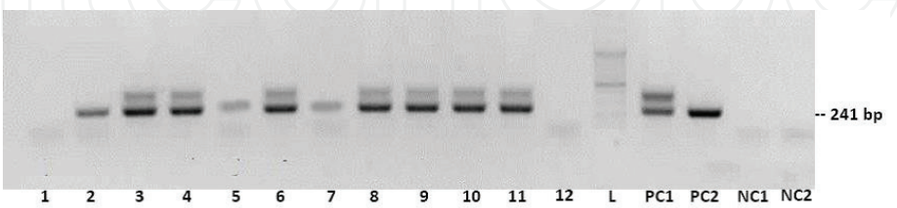

Figure 2.
Detection of M3 gene transcripts from MHV-68 in D. reticulatus *ticks collected in Slovakia in 2013 by nested RT-PCR. Lanes: 1–11—ticks nos. 49–59; 12—uninfected tick from the breeding station (negative control); L—100 bp plus DNA ladder (Thermo Fisher Sci); PC1—MHV-68 BAC DNA (nested PCR; positive control); PC2—MHV-68 BAC DNA (the first PCR with nested primers; positive control); NC1—no template (nested PCR; negative control); NC2—no template (the first PCR with nested primers; negative control); for details, see Kúdelová et al. [80].*

I. ricinus and *D. reticulatus* ticks which feed on small- and medium-sized mammals [82]. *H. concinna ticks* have been found to transmit nonviral pathogens such as *Coxiella burnetii*, *Borrelia* genus spirochetes, *Rickettsia* and *Babesia* spp., *Anaplasma*

phagocytophilum, and *Neoehrlichia mikurensis.* Two tick-borne viruses were established in *H. concinna* ticks, tick-borne encephalitis virus (TBEV), and Burana virus [83, 84]. In *Haemaphysalis* spp. also other viruses were identified such as Kyasanur Forest disease virus, three strains of Burana orthonairovirus, Nairobi sheep disease orthonairovirus, SFTS phlebovirus, Barur ledantevirus, Yongjia ledantevirus, New Minto virus, and to-date ungrouped viruses (Bhanja virus, Kaisodi virus, Silverwater virus, and Kwatta) [64]. In 2016, MHV-68 was firstly identified in adult *H. concinna* ticks collected in Gabčíkovo from May 2013 to May 2014. Virus incidence in ticks was 38.3% (18/47), and its genome copy number per tick varied from 2×10^2 to 9.6×10^3 [85].

The castor bean tick *Ixodes ricinus* (Linnaeus, 1758) (Acari: Ixodidae) is commonly found in Europe. Its distribution covers most of the continent, extending from Southern Italy up to northern Scandinavia. Rodents, natural hosts of MHV-68, are important hosts for several *Ixodes* ticks especially for larvae, to some extent for nymphs, and in the case of host-specific species also for adults. Therefore, following studies on MHV-68, a novel potential arbovirus concerned on *I. ricinus* ticks. As mentioned above, the first evidence of MHV-68 in ticks was found in immature *I. ricinus* ticks infesting *Lacerta viridis* green lizards, from which 1.8% (15/799) nymphs and larvae were virus positive [55]. It should be noted that ixodid tick species have multiple life stages with each feeding off a different host and often a different host species. *Ixodes* ticks are the most important arthropod disease vector, long been acknowledged as an important vector for a wide variety of pathogens of medical and veterinary importance, particularly the tick-borne encephalitis virus and the Lyme disease spirochetes of the genus *Borrelia* [86–88]. Their vectorial capacity is due to long-term coevolution with the pathogens that they transmit, an extended life span (up to years), and long-lasting blood feeding by all parasitic life stages [89]. *I. ricinus* ticks, considered as vectors and reservoir hosts, were collected from different localities in Slovakia [90]. As mentioned above, *Ixodes* ticks serve as reservoirs for a series of nonviral pathogens. Describing the results of their occurrence so far is beyond the scope of this review. One latest for all is recent study on diversity of *Coxiella*-like and *Francisella*-like endosymbionts, *Rickettsia* spp., and *Coxiella burnetii* in the tick populations, including *I. ricinus* ticks, collected in Slovakia [91]. Besides TBEV, several viruses were identified in *I. ricinus* ticks (e.g., Louping ill virus, Langat virus, Eyach virus, and at least 20 other viruses in *Ixodes* sp. ticks) [64, 92].

Recent studies assessing the occurrence of MHV-68 in *I. ricinus* ticks proved that nymphs collected from the vegetation (in Vysoká pri Morave; May 2014) [79] and also adult ticks could be infected with MHV-68. The viral incidence in adult ticks collected in the spring of 2014 near waterworks in Gabčíkovo was 38.1% (21/55), and the viral load varied from 1.5×10^3 to 2.85×10^4 genome copies per tick. These results suggest that the *I. ricinus* ticks became infected with MHV-68 from biting infected rodents; thus, *I. ricinus* ticks may also play a role in the spread of this virus in nature [93].

As described, there are a large number of pathogens found in ticks, but the low number of experimental transmission studies, that proved or disproved tick vector competence. To determine tick vector competence, the following conditions must be fulfilled: acquisition of the virus during blood-feeding on an infected host and transmission of the virus to a host by the tick after its molting to the next development stage. Vertical transmission of pathogens between generations of ticks has been observed (transovarial transmission) for viruses such as TBEV [94] and ASFV

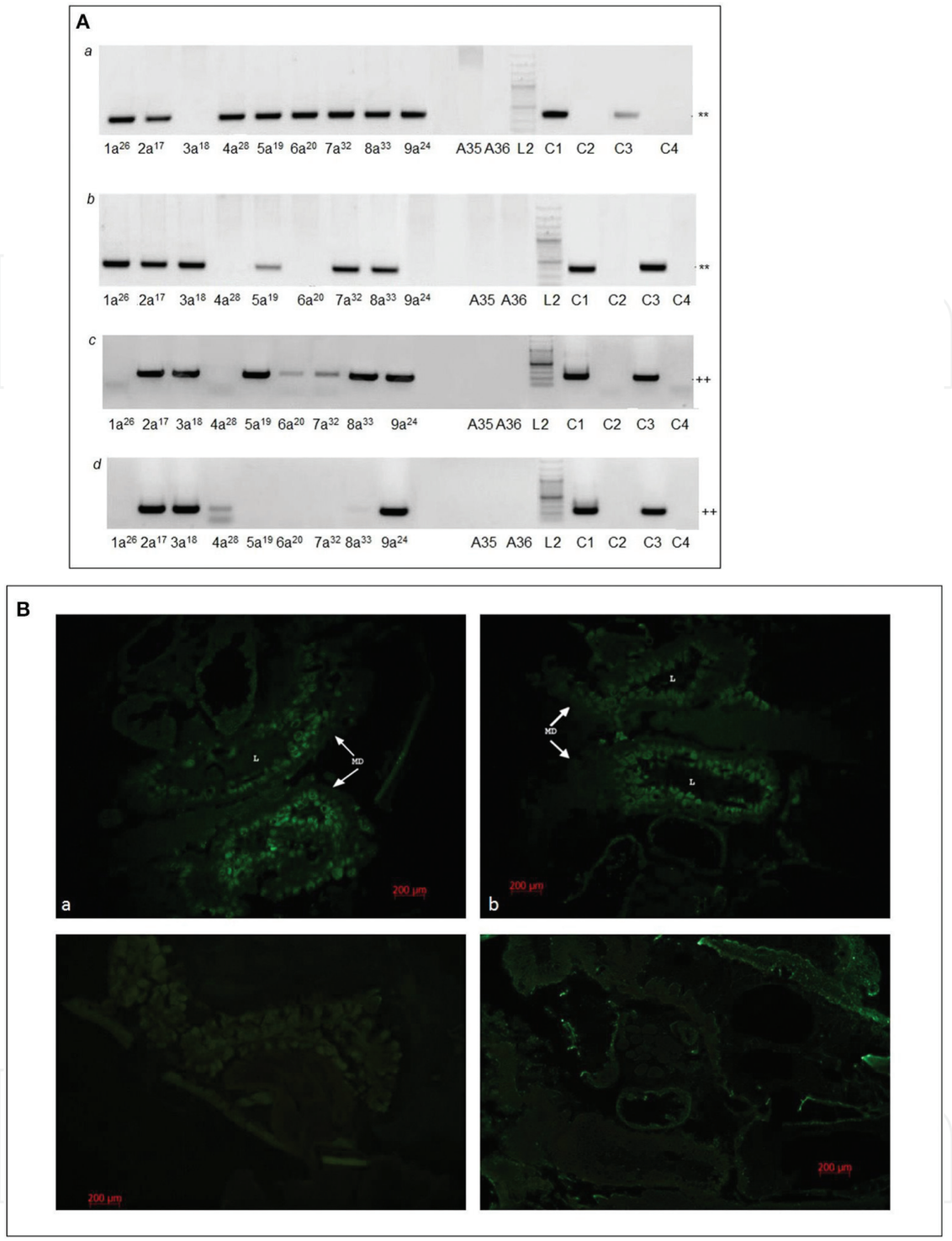

Figure 3.
MHV-68 detection in lung and spleen samples from mice infested with F1 infected adults and in F1-infected female ticks. (A) Lung (a,c) and spleen (b,d) samples of mice infested with F1-infected adults examined by nested PCR (a,b) and RT-PCR (c,d). Lanes 1a26, 2a17, 3a18, 4a28, 5a19, 6a20, 7a32, 8a33, and 9a24 samples of mice infested with F1-infected adult ticks; A35, A36, samples of control mice infested with F1-control adults. Lanes L2, C1–C4 as for ***Figure 1A****. ** Indicates MHV-68 ORF 50 gene nested PCR product of 382 bp; ++ indicates MHV-68 M3 gene nested RT-PCR product of 241 bp. (B) Semi-thin sections of frozen whole body of F1-infected females fed for 4 days. (a,b) F1-infected tick from mice 3a18 and 5a19 stained with anti-MHV-68 rabbit polyclonal serum; (c) uninfected tick (F6 generation of breeding) stained with anti-MHV-68 rabbit polyclonal serum; (d) F1-infected tick from mouse 3a18 stained with rabbit polyclonal serum against PB1-F2 protein of influenza virus A (H1N1) (negative control). MD, cells of midgut diverticula; L, lumen of midgut diverticulum. Scale bar, 200 µm; for details, see Hajnická et al. [96].*

[95]. Last but not the latest study of the MHV-68 in ticks submitted evidence of virus transmission via *I. ricinus* ticks. Hajnická et al. [96] studied experimental vertical and horizontal transmission of MHV-68 between *I. ricinus* ticks and their host—mouse—and vice versa investigating whether MHV-68 is a tick-borne virus. Uninfected *I. ricinus* ticks were shown to acquire the virus by feeding on experimentally infected laboratory mice. The virus survived tick molting, and the molted ticks transmitted the virus to uninfected laboratory mice on which they subsequently fed. MHV-68 was isolated from the tick salivary glands, consistent with transmission via tick saliva. The virus survived in ticks without loss of infectivity for at least 120 days and subsequently was transmitted vertically from one tick generation to the next, surviving more than 500 days. Furthermore, the F1 generation (derived from F0-infected females) transmitted MHV-68 to uninfected mice on which they fed, with MHV-68 M3 gene transcripts detected in blood, lung, and spleen tissue of mice on which F1 nymphs and F1 adults engorged. The presence of MHV-68 in the body of female tick of F1 generation was verified using anti-M3 monoclonal antibody (**Figure 3**). All results confirmed vertical transmission of MHV-68 in *I. ricinus* ticks. These experimental data fulfilled the transmission criteria that define an arthropod-borne virus (arbovirus).

5. Conclusions

Little is known of the natural history of MHV-68 that was discovered in 1980 to infect murid rodents trapped in Slovakia. About 20 years ago, the finding of neutralizing antibodies to MHV-68 in sera of at least 13 different mammalian species including humans sharing the same biotope with infected rodents gave rise to the hypothesis that MHV-68 might spread in nature also via tick biting. However, up to 10 years later, the first evidence of MHV-68 in ticks appeared, namely, in immature *I. ricinus* ticks, which feed on lizards. The following field studies have reported MHV-68 in free-living ticks of three species: *D. reticulatus*, *H. concinna*, and *I. ricinus*. They confirmed that MHV-68 belongs to few tick-borne viruses that have been detected in three tick species. Taking into account the nature and pathogenesis of MHV-68, it is not surprising that its incidence in ticks depends on, among other factors, in particular its incidence in natural host—murid rodents. Recent experimental transmission study submitted inevitable evidence that MHV-68 is capable of transmitting from infected to uninfected hosts via *I. ricinus* ticks; thus, MHV-68 is a novel arbovirus. This finding is of importance because herpesviruses were till now believed not to infect arthropods, and vector-mediated transmission of herpesviruses was unreported hitherto [97]. More interestingly, MHV-68 is the first herpesvirus and also a gammaherpesvirus among tick-borne viruses known to date. Further studies are needed to determine if neutralizing antibodies to MHV-68 detected 20 years ago in mammals sharing the same biotope with infected rodents are the result of tick-borne transmission of MHV-68 in nature and whether humans are at risk of infection.

Acknowledgements

This work was supported by the joint grant agency of the Slovak Ministry of Education and Slovak Academy of Sciences VEGA (#2/087/17) and by the Slovak Research and Development Agency (#APVV-15-0474).

Author details

Marcela Kúdelová* and Iveta Štibrániová
Department of Viral Immunology, Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

*Address all correspondence to: virukude@savba.sk

References

[1] Blaškovič D, Stančeková M, Svobodová J, Mistríková J. Isolation of five strains of herpesviruses from two species of free-living small rodents. Acta Virologica. 1980;**24**:468

[2] Kožuch O, Riechel M, Leššo J, Remeňová A, Labuda M, Lysý J, et al. Further isolation of murine herpesviruses from small mammals in southwestern Slovakia. Acta Virologica. 1993;**37**(1):101-105

[3] Mistríková J, Kožuch O, Klempa B, Kontseková E, Labuda M, Mrmusová M. New findings on the ecology and epidemiology of murine herpes virus isolated in Slovakia. Bratislavské Lekárske Listy. 2000;**101**(3):157-162

[4] Klempa B, Mačáková K, Labuda M, Kúdelová M. Detection of murine herpesvirus (MHV) among free-living rodents by nested PCR. Reviews of Infectious Diseases. 2001;**3**:31-33

[5] Wágnerová M, Chalupková A, Hrabovská Z, Ančicová L, Mistríková J. Possible role of different animal species in maintenance and spread of murine gammaherpesvirus 68 in the nature. Acta Virologica. 2015;**59**(01):14-19. DOI: 10.4149/av_2015_01_14

[6] Svobodová J, Blaškovič D, Mistríková J. Growth characteristics of herpesviruses isolated from free living small rodents. Acta Virologica. 1982;**26**: 256-263

[7] Efstathiou S, Ho YM, Hall S, Styles CJ, Scott SD, Gompels UA. Murine herpesvirus 68 is genetically related to the gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri. The Journal of General Virology. 1990;**71**(6): 1365-1372

[8] Virgin HW IV, Latreille P, Wamsley P, Hallsworth K, Weck KE, Dal Canto AJ, et al. Complete sequence and genomic analysis of Murine gammaherpesvirus 68. Journal of Virology. 1997;**71**:5894-5904

[9] Rajčáni J, Kúdelová M. Murid herpesvirus 4 (MHV-4): An animal model for human gammaherpesvirus research. In: Minarovits J, Gonczol E, Valyi-Nagy T, editors. Latency Strategies of Herpesviruses. Berlin; Heidelberg; New York, NY: Springer; 2007. pp. 102-136

[10] Van Regenmortel MH, Fauquet CM, Bishop DH. Herpesvirus family. In: Virus taxonomy: Classification and nomenclature of viruses. 7th ICTV report. San Diego, New York, London, Tokyo: Academic Press; 2000. pp. 220-226

[11] Kúdelová M, Rajčáni J. Gammaherpesviruses and oncogenesis. In: Gluckman TR, editor. Herpesviridae: Viral Structure, Life Cycle and Infection. USA: Nova Science Publishers, Inc; 2009. pp. 1-283. Book Series: Virology Research Progress. ISBN: 978-1-60692-947-6. Chapt VIII. p. 187-226

[12] Mesri EA, Feitelson MA, Munger K. Human viral oncogenesis: A cancer hallmarks analysis. Cell Host & Microbe. 2014;**15**:266-282. DOI: 10.1016/j.chom.2014.02.011

[13] Speck SH, Ganem D. Viral latency and its regulation: Lessons from the gammaherpesviruses. Cell Host & Microbe. 2010;**8**:100-115. DOI: 10.1016/j.chom.2010.06.014

[14] Barton E, Mandal P, Speck SH. Host control of Gammaherpesviruses: Lessons from the mouse. Annual Review of Immunology. 2011;**29**:351-397

[15] Nash AA, Dutia BM, Stewart JP, Davison AJ. Natural history of murine

γ-herpesvirus infection. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2001;**356**:569-579. DOI: 10.1098/rstb.2000.0779

[16] Blasdell K, McCracken C, Morris A, Nash AA, Begon M, et al. The wood mouse is a natural host for Murid herpesvirus 4. The Journal of General Virology. 2003;**84**:111-113

[17] Hughes DJ, Kipar A, Milligan SG, Cunningham C, Sanders M. Characterization of a novel wood mouse virus related to murid herpesvirus 4. The Journal of General Virology. 2010;**9**: 867-879

[18] Ehlers B, Kuchler J, Yasmum N, Dural D, Voigt S, et al. Identification of novel rodent herpesviruses including the first gammaherpesvirus of *Mus musculus*. Journal of Virology. 2007;**81**: 8091-8100

[19] Loh J, Zhao G, Nelson CA, Coder P, Droit L, et al. Identification and sequencing of a novel rodent gammaherpesvirus that establishes acute and latent infection in laboratory mice. Journal of Virology. 2011;**85**: 2642-2656

[20] Rajčáni J, Blaškovič D, Svobodová J, Čiampor F, Hučková D, Staneková D. Pathogenesis of acute and persistent murine herpesvirus infection in mice. Acta Virologica. 1985;**29**:51-60

[21] Stewart JP, Usherwood EJ, Ross A, Dyson H, Nash AA. Lung epithelial cells are a major site of murine gammaherpesvirus persistence. The Journal of Experimental Medicine. 1998; **187**:1941-1951

[22] Sunil-Chandra NP, Efstathiou S, Arno J, Nash AA. Virological and pathological features of mice infected with murine gamma-herpesvirus 68. The Journal of General Virology. 1992; **73**(Pt 9):2347-2356

[23] Sunil-Chandra NP, Arno J, Fazakerley J, Nash AA. Lymphoproliferative disease in mice infected with murine gammaherpesvirus 68. The American Journal of Pathology. 1994;**145**:818-826

[24] Mistríková J, Rajčáni J, Mrmusová M, Oravcová I. Chronic infection of Balb/c mice with murine herpesvirus 72 is associated with neoplasm development. Acta Virologica. 1996;**40**: 297-301

[25] Rašlová H, Berebbi M, Rajčáni J, Sarasin A, Matis J, Kúdelová M. Susceptibility of mouse mammary glands to Murine gammaherpesvirus 72 (MHV-72) infection: Evidence of MHV-72 transmission via breast milk. Microbial Pathogenesis. 2001;**31**:47-58

[26] Stančeková M, Golais F, Leššo J. Some physicochemical properties of murine herpesvirus. Acta Virologica. 1992;**36**(2):201-20342

[27] Burri C, Schumann O, Schumann C, Gern L. Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi*, *Candidatus Neoehrlichia mikurensis*, *Rickettsia helvetica*, *R. monacensis* and *Anaplasma phagocytophilum*? Ticks Tick-Borne Diseases. 2014;**5**:245-251

[28] Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: A One Health perspective. Trends in Parasitology. 2012;**26**(10): 437-446

[29] Estrada-Peña A, Gray JS, Kahl O, Lane RS, Nidhoj A. Research on the ecology of ticks and tick-borne pathogens-methodological principles and caveats. Frontiers in Cellular and Infection Microbiology. 2013;**3**:29

[30] Martello E, Selmi M, Ragagli C, Ambrogi C, Stella MC, Mannelli A, et al. *Rickettsia slovaca* in immature *Dermacentor marginatus* and tissues

from *Apodemus* spp. in the northern Apennines, Italy. Ticks Ticks-Borne Diseases. 2013:518-521

[31] Brackney DE, Armstrong PM. Transmission and evolution of tick-borne viruses. Current Opinion in Virology. 2016;**21**:67-74. DOI: 10.1016/j.coviro.2016.08.005

[32] Horak IG, Camicas JL, Keirans JE. The *Argasidae, Ixodidae* and *Nuttalliellidae* (Acari: Ixodida): A world list of valid tick names. Experimental & Applied Acarology. 2002;**28**:27-54

[33] Nuttall PA. Tick-borne viruses. In: Sonenshine DE, Roe RM, editors. Biology of Ticks. Vol. 2. New York, USA: Oxford University Press; 2014. pp. 180-210

[34] Labuda M, Nuttall PA. Viruses transmitted by ticks. In: Bowman AS, Nuttall PA, editors. Ticks: Biology, Disease and Control. Cambridge, UK: Cambridge University Press; 2008. pp. 253-280

[35] Hubálek Z, Rudolf I. Tick-borne viruses in Europe. Parasitology Research. 2012;**111**:9-36

[36] Baneth G. Tick-borne infections of animals and humans: A common ground. International Journal for Parasitology. 2014;**44**(9):591-598. DOI: 10.1016/j.ijpara.2014.03.011

[37] Estrada-Peña A, Gray JS, Kahl O, Lane RS, Nijhof AM. Research on the ecology of ticks and tick-borne pathogens—methodological principles and caveats. Frontiers in Cellular and Infection Microbiology. 2013b;**3**(29). DOI: 10.3389/fcimb.2013.00029

[38] Reye AL, Stegniy V, Mishaeva NP, Velhin S, Hübschen JM, Ignatyev G, et al. Prevalence of tick-borne pathogens in *Ixodes ricinus* and *Dermacentor reticulatus* ticks from different geographical locations in Belarus. PLoS One. 2013;**8**(1):e54476. DOI: 10.1371/journal.pone.0054476

[39] Estrada-Peña A, de la Fuente J. The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral Research. 2014;**108**:104-112. DOI: 10.1016/j.antiviral.2014.05.016

[40] Rubel F, Brugger K, Pfeffer M, Chitimia-Dobler L, Didyk YM, Leverenz S, et al. Geographical distribution of *Dermacentor marginatus* and *Dermacentor reticulatus* in Europe. Ticks Tick Borne Diseases. 2016;**7**:224-233

[41] Stockman S. Louping-ill. The Journal of Comparative Pathology and Therapeutics. 1918;**31**:137-193. DOI: 10.1016/S0368-1742(18)80019-4

[42] Kazimírová M, Bartíková P, Štibrániová I. Tick-borne viruses and host skin interface. In: Boulanger N, editor. Skin and Arthropod Vectors. Academic press. Chap. 10; 2017. pp. 325-383

[43] Nuttall PA. Molecular characterization of tick-virus interactions. Frontiers in Bioscience. 2009;**14**:2466-2483

[44] Shi J, Zhihong Hu Z, Fei Deng F, Shen S. Tick-borne viruses. Virologica Sinica. 2018;**33**:21-43. DOI: 10.1007/s12250-018-0019-0 www.virosin.org

[45] Kazimírová M, Thangamani S, Bartíková P, Hermance M, Holíková V, Štibrániová I, et al. Tick-borne viruses and biological processes at the tick-host-virus interface. Frontiers in Cellular and Infection Microbiology. 2017b;**7**:339. DOI: 10.3389/fcimb.2017.00339

[46] Kosoy OI, Lambert AJ, Hawkinson DJ, Pastula DM, Goldsmith CS, Hunt DC, et al. Novel Thogotovirus associated with febrile illness and death, United States, 2014. Emerging Infectious Diseases. 2015;**21**:760-764

[47] Dixon LK, Alonso C, Escribano JM, Martins C, Revilla Y, Salas ML, et al. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: Classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Inc.; 2011. pp. 153-162. DOI: 10.4149/av_2017_403

[48] Jori F, Vial L, Penrith ML, Pérez-Sánchez R, Etter E, Albina E, et al. Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian ocean. Virus Research. 2013; **173**(1):212-227

[49] Zakaryan H, Revilla Y. African swine fever virus: Current state and future perspectives in vaccine and antiviral research. Veterinary Microbiology. 2016;**185**:15-19. DOI: 10.1016/j.vetmic.2016.01.016

[50] de Carvalho Ferreira HC, Tudela Zúquete S, Wijnveld M, Weesendorp E, Jongejan F, Stegeman A, et al. No evidence of African swine fever virus replication in hard ticks. Ticks Tick Borne Diseases. 2014;**5**:582-589. DOI: 10.1016/j.ttbdis.2013.12.012

[51] Tuppurainen ESM, Stoltsz WH, Troskie M, Wallace DB, Oura CAL, Mellor PS, et al. A potential role for ixodid (hard) tick vectors in the transmission of Lumpy Skin Disease Virus in cattle. Transboundary and Emerging Diseases. 2011;**58**:93-104

[52] Tuppurainen ESM, Lubinga JC, Stoltsz WH, Troskie M, Carpenter ST, Coetzer JAW, et al. Evidence of vertical transmission of lumpy skin disease virus in *Rhipicephalus decoloratus* ticks. Ticks and Tick Borne Diseases. 2013;**4**:329-333

[53] Lubinga JC, Tuppurainen ESM, Coetzer JAW, Stoltsz WH, Venter EH. Transovarial passage and transmission of LSDV by *Amblyomma hebraeum, Rhipicephalus appendiculatus* and *Rhipicephalus decoloratus*. Experimental & Applied Acarology. 2014;**62**:67-75

[54] Lubinga JC, Tuppurainen ESM, Mahlare R, Coetzer JAW, Stoltsz WH, Venter EH. Evidence of transstadial and mechanical transmission of lumpy skin disease virus by *Amblyomma hebraeum* ticks. Transboundary and Emerging Diseases. 2015;**62**:174-182

[55] Ficová M, Betáková T, Pančík P, Václav R, Prokop P, Halásová Z, et al. Molecular detection of murine herpesvirus 68 in ticks feeding on free-living reptiles. *Microbial Ecology*. 2011; **62**:862-867. DOI: 10.1007/s00248-011-9907-7

[56] Kazimírová M, Hamšíková Z, Kocianová E, Marini G, Mojšová M, Mahríková L, et al. Relative density of host-seeking ticks in different habitat types of south-western Slovakia. *Experimental & Applied Acarology*. 2016; **69**:205-224. DOI: 10.1007/ s10493-016-0025-6

[57] Minichová L, Hamšíková Z, Mahríková L, Slovák M, Kocianová E, Kazimírová M, et al. Molecular evidence of *Rickettsia* spp. in ixodid ticks and rodents in suburban, natural and rural habitats in Slovakia. *Parasites & Vectors*. 2017;**10**:158

[58] Pangrácová L, Derdáková M, Pekárik L, Hviščová I, Víchová B, Stanko M, et al. Ixodes ricinus abundance and its infection with the tick-borne pathogens in urban and suburban areas of Eastern Slovakia. *Parasites & Vectors*. 2013;**6**:238

[59] Špitalská E, Boldiš V, Košťanová Z, Kocianová E, Štefanidesová K. Incidence of various tick-borne microorganisms in rodents and ticks of central Slovakia. Acta Virologica. 2008; **52**:175-179

[60] Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E. Evidence for an

increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Rickettsia* sp. RpA4. International Journal of Medical Microbiology. 2006;**296**(Supp. 40): 149-156

[61] Široký P, Kubelová M, Bednár M, Modry D, Hubálek Z, Tkadlec E. Thedistribution and spreading pattern of *Dermacentor reticulatus* over its threshold area in the Czech Republic - How much is range of this vector expanding. Veterinary Parasitology. 2011;**183**:130-135

[62] Nosek J. The ecology and public health importance of *Dermacentor marginatus* and *D. reticulatus* ticks in Central Europe. Folia Parasitologica. 1972;**19**:93-102

[63] Bullova E, Lukan M, Stanko M, Pet'ko B. Spatial distribution of *Dermacentor reticulatus* tick in Slovakia in the beginning of the 21st century. Veterinary Parasitology. 2009;**165**: 357-360

[64] Bartíková P, Holíková V, Kazimirova M, Štibrániová I Tick-borne viruses**.** Acta Virologica. 2017;**61**(04): 413-427

[65] Buczek A, Bartosik K, Wiśniowski Ł, Tomasiewicz K. Changes in population abundance of adult *Dermacentor reticulatus* (Acari: *Amblyommidae*) in long-term investigations in eastern Poland. Annals of Agricultural and Environmental Medicine. 2013;**20**:269-272

[66] de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Frontiers in Bioscience. 2008; (13):6938-6946

[67] Nosek J, Korolev MB, Chunikhin SP, Kožuch O, Čiampor F. The replication and eclipse-phase of the tick-borne encephalitis virus in *Dermacentor reticulatus*. Folia Parasitol (Praha). 1984; **31**:187-189

[68] Wójcik-Fatla A, Cisak E, Zając V, Zwoliński J, Dutkiewicz J. Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). Ticks Tick Borne Diseases. 2011;**2**(1):16-19

[69] Frey S, Essbauer S, Zoeller G, Klempa B, Dobler G, Pfeffer M. Full genome sequences and preliminary molecular characterization of three tick-borne encephalitis virus strains isolated from ticks and a bank vole in Slovak Republic. Virus Genes. 2014;**48**:184-188

[70] Hubálek Z, Sixl W, Halouzka J. *Francisella tularensis* in *Dermacentor reticulatus* ticks from the Czech Republic and Austria. Wiener Klinische Wochenschrift. 1998;**110**(24):909-910

[71] Špitalská E, Kocianová E. Detection of *Coxiella burnetii* in ticks collected in Slovakia and Hungary. European Journal of Epidemiology. 2003;**18**(3): 263-266

[72] Wójcik-Fatla A, Bartosik K, Buczek A, Dutkiewicz J. *Babesia microti* in adult *Dermacentor reticulatus* ticks from Eastern Poland. Vector Borne and Zoonotic Diseases. 2012;**12**(10):841-843

[73] Růžek D, Yakimenko VV, Karan LS, Tkachev SE. Omsk haemorrhagic fever. Lancet. 2010;**376**:2104-2113

[74] Biernat B, Karbowiak G, Werszko J, Stanczak J. Prevalence of tick-borne encephalitis virus (TBEV) RNA in *Dermacentor reticulatus* ticks from natural and urban environment. Poland. Experimental & Applied Acarology. 2014;**64**:543-551

[75] Dedkov VG, Markelov ML, Gridneva KA, Bekova MV, Gmyl AP, Kozlovskaya LI, et al. Prevalence of

Kemerovo virus in ixodid ticks from the Russian Federation. Ticks Tick Borne Diseases. 2014;**5**:651-655. DOI: 10.1016/j.ttbdis.2014.04.017

[76] Kúdelová M, Belvončíková P, Vrbová M, Koval'ová A, Štibrániová I, Kocáková P, et al. Detection of Murine Herpesvirus 68 (MHV-68) in *Dermacentor reticulatus* ticks. Microbial Ecology. 2015;**70**:785-794

[77] Margolis TP, Elfman FL, Leib D, Pakpour N, Apakupakul K, Imai Y, et al. Spontaneous reactivation of herpes simplex virus typ. 1 in latently infected murine sensory ganglia. Journal of Virology. 2007;**80**(20):11069-11074

[78] Rajčáni J, Čiampor F, Sabo A. Experimental latent herpesvirus infection in rabbits, mice and hamsters: Ultrastructure of virus activation in the explanted Gasserian ganglion. Acta Virologica. 1975;**19**:19-28

[79] Kúdelová M, Slovák M, Kabát P, Derka T, Števove B, Bohuš M, et al. A survey on murine gammaherpesvirus 68 in ticks collected in Slovakia. Acta Virologica. 2018a;**62**:64-69. DOI: 10.4149/av_2018_112

[80] Kúdelová M, Jánošová M, Vrbová M, Matúšková R, Slovák M, Belvončíková P. Detection of transcripts and an infectious dose of Murine Gammaherpesvirus 68 in *Dermacentor reticulatus* ticks. Journal of Infectious Diseases and Therapy. 2017;**5**(4):330. DOI: 10.4172/2332-0877.1000330. ISSN 2310-9386

[81] Rubel F, Brugger K, Walter M, Janna R, Vogelgesang J, Didyk YD YM, et al. Geographical distribution, climate adaptation and vector competence of the Eurasian hard tick. Haemaphysalis concinna Ticks and Tick-Borne Disease. 2018. DOI: 10.1016/j.ttbdis.2018.04.002

[82] Švehlová A, Berthová L, Sallay B, Boldiš V, Sparagano OAE, Špitalská E. Sympatric occurrence of Ixodes ricinus, *Dermacentor reticulatus* and *Haemaphysalis concinna* ticks and their pathogens *Rickettsia* and *Babesia* species in Slovakia. Ticks Tick Borne Diseases. 2014;**5**:600-605

[83] Lvov D, Alkhovsky S, Shchelkanov M, Shchetinin A, Deryabin P, Gitelman A, et al. Taxonomic status of the Burana virus (BURV) (*Bunyaviridae*, Nairovirus, Tamdy group) isolated from the ticks *Haemaphysalis punctata* Canestrini et Fanzago, 1877 and *Haem. concinna* Koch, 1844 (*Ixodidae, Haemaphysalinae*) in Kyrgyzstan (in Russian). Voprosy Virusologii. 2014;**59**: 10-15

[84] Riedl H, Kožuch O, Sixl W, Schmeller E, Nosek J. Isolation of the tick-borne encephalitis virus (TBE-virus) from the tick *Haemaphysalis concinna* Koch. Archiv für Hygiene und Bakteriologie. 1971;**154**:610-611

[85] Vrbová M, Belvončíková P, Kovaľová A, Matúšková R, Slovák M, Kúdelová M. Molecular detection of MHV-68 in *Haemaphysalis concinna* ticks collected in Slovakia. Acta Virologica. 2016;**60**(04):426-428. DOI: 10.4149/av_2016_04_426

[86] Brugger K, Boehnke D, Petney T, Dobler G, Pfeffer M, Silaghi C, et al. A density map of the tick-borne encephalitis and *Lyme borreliosis* vector *Ixodes ricinus* (Acari: Ixodidae) for Germany. Journal of Medical Entomology. 2016;**53**:1292-1302

[87] Hubálek Z, Halouzka J. Prevalence rates of *Borrelia burgdorferi* sensu lato in host-seeking *Ixodes ricinus* ticks in Europe. Parasitology Research. 1998;**84**: 167-172

[88] Mansfield KL, Johnson N, Phipps LP, Stepheson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus—A review of an emerging zoonosis. The

Journal of General Virology. 2009;**90**: 1781-1794

[89] Pagel Van Zee J, Geraci NS, Guerrero FD, Wikel SK, Stuart JJ, Nene VM, et al. Tick genomics: The Ixodes genome project and beyond. International Journal for Parasitology. 2007;**37**(12):1297-1305. DOI: 10.1016/j.ijpara.2007.05.011

[90] Černý V. The tick fauna of Czechoslovakia. Folia Parasitologica. 1972;**19**:87-92

[91] Špitalská E, Sparagano O, Stanko M, Schwarzová K, Špitalský Z, Škultéty Ľ, et al. Diversity of *Coxiella-like* and *Francisella-like* endosymbionts, and *Rickettsia* spp., *Coxiella burnetii* as pathogens in the tick populations of Slovakia, Central Europe. Ticks and Tick-borne Disease. 2018. DOI: 10.1016/j.ttbdis.2018.05.002

[92] Mansfield KL, Jizhou L, Phipps LP, Johnson N. Emerging tick-borne viruses in the twenty-first century. Frontiers in Cellular and Infection Microbiology. 2017;**7**:298. DOI: 10.3389/fcimb.2017.00298

[93] Kúdelová M, Jánošová M, Belvončíková P. First detection of murine herpesvirus 68 in adult *Ixodes ricinus* ticks. Folia Microbiologica. 2018b. DOI: 10.1007/s12223-018-0586-3

[94] Reháček J. Transovarial transmission of tick-borne encephalitis virus by ticks. Acta Virologica. 1962;**6**: 220-226

[95] Rennie L, Wilkinson PJ, Mellor PS. Transovarial transmission of African swine fever virus in the argasid tick *Ornithodoros moubata*. Medical and Veterinary Entomology. 2001;**15**: 140-146. DOI: 10.1046/j.1365-2915.2001.00282.x

[96] Hajnická V, Kúdelová M, Štibrániová I, Slovák M, Bartíková P, Halásová Z, et al. Tick-borne transmission of Murine Gammaherpesvirus. Frontiers in Cellular and Infection Microbiology. 2017;**68**. DOI: 10.3389/fcimb.2017.00458

[97] King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. San Diego, CA: Elsevier Inc; 2011