Microb Ecol (2011) 62:862–867
DOI 10.1007/s00248-011-9907-7

ENVIRONMENTAL MICROBIOLOGY

Molecular Detection of Murine Herpesvirus 68 in Ticks Feeding on Free-living Reptiles

Martina Ficová · Tatiana Betáková · Peter Pančík · Radovan Václav · Pavol Prokop · Zuzana Halásová · Marcela Kúdelová

Received: 11 April 2011 / Accepted: 21 June 2011 / Published online: 6 July 2011

Abstract The MHV-68 (designed as *Murid herpesvirus 4* (MuHV 4) strain 68) isolated from two rodents, *Myodes glareolus* and *Apodemus flavicollis*, is considered as a natural pathogen of free-living murid rodents. Recently, the detection of MHV antibodies in the blood of animals living in the same biotope as MHV-infected mice has suggested that ticks may have a role in the transmission of this pathogen. *Ixodes ricinus* is one the most abundant tick species in Europe known to transmit multiple pathogens causing human and animal diseases. In this study, nymphs and larvae feeding on 116 individuals of a temperate lizard species—the green lizard *Lacerta viridis* captured in the Slovak Karst National Park, were examined for MHV-68. The specific sequence of virion glycoprotein 150 was amplified in DNA individually isolated from *I. ricinus* ticks using single-copy sensitive nested polymerase chain reaction. MHV-68 was detected in ten of 649 nymphs and in five of 150 larvae, respectively. We found that 9.6% of green lizards fed at least one MHV-68-infected immature tick. Occurrence of MHV-68 within all ticks tested was 1.8%. This study is first to show that immature *I. ricinus* ticks feeding on free-living lizards in a Central European region could be infected with gammaherpesvirus (MHV-68), naturally infecting free-living murid rodents. Our results provide evidence supporting the hypothesis that ticks may play a mediating role in circulation of MHV-68 in nature.

M. Ficová · T. Betáková · P. Pančík · Z. Halásová · M. Kúdelová (✉)
Institute of Virology, Slovak Academy of Science,
845 05 Bratislava, Slovak Republic
e-mail: virukude@savba.sk

R. Václav · P. Prokop
Institute of Zoology, Slovak Academy of Science,
Bratislava, Slovak Republic

Introduction

Ticks are ectoparasites of wild and domestic animals and humans that most notably impact global health by transmitting disease-causing pathogens. In many regions of the world, ticks are the most important vectors of life-threatening diseases of man and animals [8]. In Europe, the *Ixodes* ticks (Acari: Ixodidae) act both as important arthropod vectors and reservoirs for a series of wildlife zoonotic pathogens [14]. They transmit to vertebrates a wide array of pathogens including viruses, bacteria, protozoa, and helminthes. In Europe, *Ixodes* ticks are the most important arthropod disease vector, notorious for transmitting the tick-borne encephalitis virus and the Lyme disease spirochetes of the genus *Borrelia*. They transmit important human and animal diseases such as tick-borne encephalitis, Lyme disease, anaplasmosis, and babesiosis [23, 29]. Their vectorial capacity is due to their long-term co-evolution with the pathogens that they transmit, extended lifespan (up to years), long-lasting blood feeding by all parasitic life stages on different hosts, and other aspects of tick biology. Tick-borne pathogens are believed to be responsible for more than 100,000 cases of illnesses in humans throughout the world [8]. Rodents 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 [4, 41]. Thus, the rodents play a role in the enzootic cycles of nonviral pathogens (*Babesia* spp., *Borrelia* spp., *Rickettsia* spp., *Ehrlichia* spp., *Francisella tularensis*, and *Coxiella burnetii*) but also of viruses. Tick-borne viruses are found in six different virus families (Asfarviridae, Orthomyxoviridae, Rhabdoviridae, Reoviridae, Bunyaviridae, and Flaviviridae) [22]. The most extensively characterized viruses that have 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 Murine gammaherpesvirus 68 (abbreviated to MHV-68 or γHV68; species Murid herpesvirus 4 (MuHV4)), classified in the genus *Rhadinovirus* of the subfamily Gammaherpesvirinae [10, 20, 37]. MuHV4 was originally isolated from bank voles (*Myodes glareolus*) and yellow-necked field mice (*Apodemus flavicollis*) in Slovakia [3]. An epidemiological survey of MuHV4 infection in free-living rodents in the UK showed that MuHV4 is present in yellow-necked field and wood mice (*Apodemus sylvaticus*) [2]. A PCR-based study of mice trapped in Germany indicated that MuHV4 is present predominantly in yellow-necked field mice (*A. flavicollis*) [11]. Recently, in studies on the abundance of herpesviruses in natural populations of wood mice in Cheshire (UK), two novel gammaherpesviruses Brest herpesvirus and wood mouse herpesvirus, were isolated, one from a field vole (*Microtus agrestis*) and the other from wood mice [13]. In the population of rodents, MHV-68 is transmitted via mainly intranasal route but also through different body fluids such as saliva, urine, tears, and breast milk [26, 27]. Following infection of mice, an acute respiratory infection in the lung develops and is cleared, followed by the establishment of latency. MHV-68 infects macrophages, B-lymphocytes, lung alveolar as well as endothelial cells. From the lungs, the virus spreads via the bloodstream to the spleen and bone marrow and via the lymphatics to the mediastinal lymph nodes. Similarly to other gammaherpesviruses, MHV-68 is characterized by life-long latency maintained in host B-lymphocytes and macrophages and chronic infection is related to lymphoproliferative disorders and neoplasma [25, 32, 33]. The previous findings of neutralizing antibodies to murine herpesvirus detected in the serum of about 20.7% rodents individuals but also in that of fallow deer (*Dama dama*), wild boar (*Sus scrofa*), and red deer (*Cervus elaphus*) [19] gave rise a hypothesis that the virus could be transmitted from rodents to other animals living in the same biotope via ticks. In addition to the animal species mentioned above, reptiles also serve as a vertebrate host for hard ticks. Reptiles, including lizards, represent an excellent vertebrate model for studies on epidemiology of various pathogens because of their long life span, site faithfulness, seasonal nature of immunity, and distinct modes of reproduction and immune responses [30, 36]. We studied green lizards *Lacerta viridis*, which represent locally important hosts for the early life stages of hard ticks [5, 35]. An increasing number of recent studies suggest that lizards can be competent reservoirs not only of *Borrelia* spirochetes but also of *Ehrlichia* sp. and *Rickettsia* sp. [1, 9, 15, 17, 40]. Recently, *Anaplasma* sp. were detected in the *Ixodes ricinus* nymphs and larvae feeding on lizards achieving 13.1% and 8.7% prevalence, respectively [35].

However, little is known about the prevalence and ecology of other tick-borne pathogens associated with reptile hosts. Here, we examined the prevalence of Murine herpesvirus 68 in nature conditions using a previously unexamined host–vector–pathogen system—green lizards *L. viridis*, ticks *I. ricinus*, and murine herpesvirus. Based on molecular analyses, this study describes the presence of MHV-68, naturally infecting wild murid rodents, in ticks infesting green lizards.

Materials and Methods

Study Site and Animals

The European Green Lizard (*L. viridis*) is a relatively large lizard (up to 40 cm in the length) distributed across warm and dry areas of mid-European latitudes from the north of the Iberia to as far east as Ukraine. It has a relatively long lifespan (>9 years) and is often seen basking on rocks or lawns, or sheltering amongst bushes. Lizards, including green lizards *L. viridis* serve as a vertebrate host for ticks, the opportunistic hematophagous ectoparasites. The study site (48°57′ N, 20°44′ E, ~200 to 400 m above sea level) in the Slovak Karst National Park was ~10 ha large, characterized by warm temperate climate, open habitat and broad-leaved scrub vegetation.

Sample Collection

In April 2007, we captured 116 free-living green lizards by slipnoosing. All lizards were marked with a marker pen in the field and stored in individual containers. Immediately after capture, the lizards were examined for feeding ticks. Ticks were removed from lizards using forceps and further identified by species and life stage. In total, 799 ticks, including 649 nymphs, 150 larvae, and no adult were determined as *I. ricinus* species using microscope and standard identification keys [7]. Ticks were stored individually in microcentrifuge tubes at 2°C to 8°C prior to DNA extraction. All lizards were released exactly at the point of capture the morning after their capture.

DNA Isolation from Ticks

DNA from individual ticks was isolated using anion chelating resin Chelex 100® Resin (Bio-Rad Laboratories, CA, USA) [24] according to the manufacturer's protocol. Briefly, tick was transferred into 0.5-ml microcentrifuge tube containing 100 μl of distilled water, 0.005 g Chelex 100 resin, and proteinase K (10 μg/ml). The mixture was homogenized and vortexed 2 min at room temperature. The homogenized sample was then centrifuged at 13,000×*g* for

1 min, incubated at 96°C for 35 min, and vortexed. The mixture was then centrifuged at 13,000×*g* for 1 min, incubated at 96°C for 15 min, and vortexed. After following centrifugation at 13,000×*g* for 3 min, 1–2 μl of the supernatant was used as a template in PCR assay. The amount of DNA isolated was measured by using spectrophotometer. Each sample tested in PCR contains 200–240 ng of DNA.

Detection of MHV-68 DNA by Nested PCR and Sequencing

Nested PCR to detect gp150 gene of MHV-68 was developed to have a sensitivity of one copy of MHV-68 DNA. The sequences of the outer PCR primers employed were gpF7: 5′ GAAACAACCACCCCTTCCCAA3′ and gpR: 5′ CTGTGGGTGCCCAGCGGAGG3 which amplified a 1,011 bp product. The sequences of the inner PCR primers employed were gpF4: 5′TCCCAAACAAGAGGATG3′ and gpR2: 5′-TGCTGGTTGAGTGGTGGT3 which amplified a 640 bp product. Each primary PCR mixture used 25 to 50 ng of DNA extracted from individual ticks and 0.5 mmol/l solution of each primer in a total volume of 10 μl of Taq DNA polymerase Master Mix (Fermentas). Cycling conditions involved an initial 5 min denaturation at 95°C, 35 cycles of 60 s at 95°C, 60 s at 55°C, and 60 s at 72°C and a final extension of 5 min at 72°C. The reaction mixture for the nested amplifications used 1–5 μl of the first round PCR product as the template and 0.5 mmol/l solution of each primer in a total volume of 10 μl. The nested cycling conditions involved 40 cycles of 60 s at 95°C, 60 s at 57°C, and 45 s at 72°C and a final extension of 5 min at 72°C. DNA isolated from purified virions of MHV-68 [28] was used as a positive control. PCRs were performed on a Mastercycler Personal (Eppendorf). Second-round PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Plasmid pTargetgp150 containing gp150 of MHV-68 prepared by Mačáková et al. [16] was used to determine the sensitivity of the nested PCR for detection of MHV-68 DNA. pTargetgp150 was spectrophotometrically quantified and diluted in TE buffer containing tRNA (0.5 mg/ml); 100 ng of total nucleic acid in serial 10-fold dilutions of pTargetgp150 in tRNA was analyzed by nested PCR in a series of control PCR. Amplicons of all MHV-68 positive ticks were purified by Wizard DNA Clean-up System (Promega) and sequenced using primers gpF4 and/or gpR2 using a commercial sequencing service (BITCET).

Results

We collected 799 ticks feeding on 116 green lizards *L. viridis*. All ticks were identified as *I. ricinus* species in early life stages and included 649 nymphs and 150 larvae. Adult ticks were not found on the lizards. To identify the presence of MHV-68 in ticks a single-copy-sensitive nested PCR specific for gp150 gene of MHV-68 was developed by limiting dilution of cloned target DNA (Fig.1). The size of gp150 gene nested PCR products of MHV-68 was 640 bp. DNA of MHV-68 was detected in 15 ticks (about 1.8%) feeding on 12 green lizards (about 10%) (Table 1). MHV-68 DNA was detected in ticks in both life stages, namely ten nymphs (Fig. 2a: lanes 5 and 13; Fig. 2b: lanes 3, 7, 8, and 11; Fig. 2c: lanes 1, 4, 10, and 11) and five larvae (Fig. 2a: lane 10; Fig. 2b: lane 16; Fig. 2c: lanes 5, 8, and 15). As shown in Table 1, eight of twelve lizards with at least one MHV-68 carrying tick carried both nymphs and larvae on their body. Both life stages of ticks were MHV-68 positive only in one of the lizards (lizard No9) (Fig. 2c: lanes 4 and 5). The prevalence of MHV-68 DNA in ticks feeding on lizards was about 1.5% (ten of 649) in nymphs and about 3.3% (5 of 150) for larvae. Thus, MHV-68 prevalence for larvae was more than two times higher than that for nymphs. The specificity of PCR products amplified from all positive ticks was confirmed by sequencing. Sequencing results revealed 99–100% identity of all PCR products with gp150 gene of MHV-68 (Acc No AF105037).

Discussion

Our study is first to show that immature stages of ticks (nymphs and larvae) feeding on free-living green lizards can carry MHV-68. The sequences of amplicons yielded by MHV-68-positive ticks were all nearly fully homologous with glycoprotein 150 of MHV-68. At present, ticks infesting a free-living temperate lizard species in the same Central European region were found to be infected with multiple pathogens with co-infection rate of ticks with *Borrelia* spirochetes, *Anaplasma* sp., and *Rickettsia* sp. being higher than expected based on single infection rates

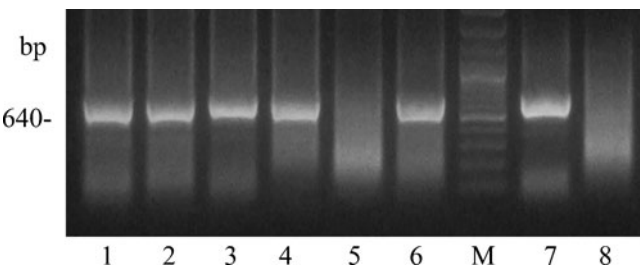

Figure 1 A single-copy-sensitive nested PCR assay specific for gp150 gene of MHV-68. *Lanes 1–5*, serial 10-fold dilutions of pTarget gp150 (from 10^3 to 0.1 copies); *6*, MHV-68 DNA (positive control); *M*, 100 bp ladder (Fermentas); *7*, primary PCR with nested primers on MHV-68 DNA (positive control); *8*, nested PCR without template (negative control)

Table 1 Prevalence of Murine herpesvirus 68 (MHV-68) for *Ixodes ricinus* nymphs and larvae feeding on green lizards *Lacerta viridis* detected by single-copysensitive nested PCR

Lizard (no)	Number of ticks feeding on lizard		Number of MHV-68-positive ticks	
	Nymphs	Larvae	Nymphs	Larvae
1	7	0	1	0
2	3	0	1	0
3	9	1	1	0
4	17	0	1	0
5	0	1	0	1
6	26	4	1	0
7	12	4	2	0
8	4	3	0	1
9	12	2	1	1
10	6	2	0	1
11	13	9	2	0
12	19	9	0	1

[21, 35]. Our result that 9.6% of green lizards *L. viridis* carried at least one tick carrying MHV-68 not only widens the knowledge on the diversity of pathogens detected in ticks from lizard hosts, but it also stimulates new questions about the role of lizards and ticks in viral transmission.

Our finding indicates that the ticks that fed on green lizards tested in this study fed on animal infected with murine herpesvirus. This virus is considered as a natural pathogen of free-living murid rodents [19, 20]. The previous findings of virus neutralizing antibodies against murine herpesvirus in the blood of about 20% of rodent individuals but also of fallow deer, red deer, wild boars, and other animals living in the same area [18] gave rise to a hypothesis that in nature murine herpesvirus could be transmitted from infected animals (most likely murid rodents) to uninfected animal via ticks. This is plausible because ticks could serve as a potential vector also for other gammaherpesviruses infecting free-living small animals, including the Wood mouse herpesvirus (*A. sylvaticus*) [13], a novel rodent gammaherpesvirus (*Mus musculus*) [11], and seven gammaherpesviruses detected in bats [39].

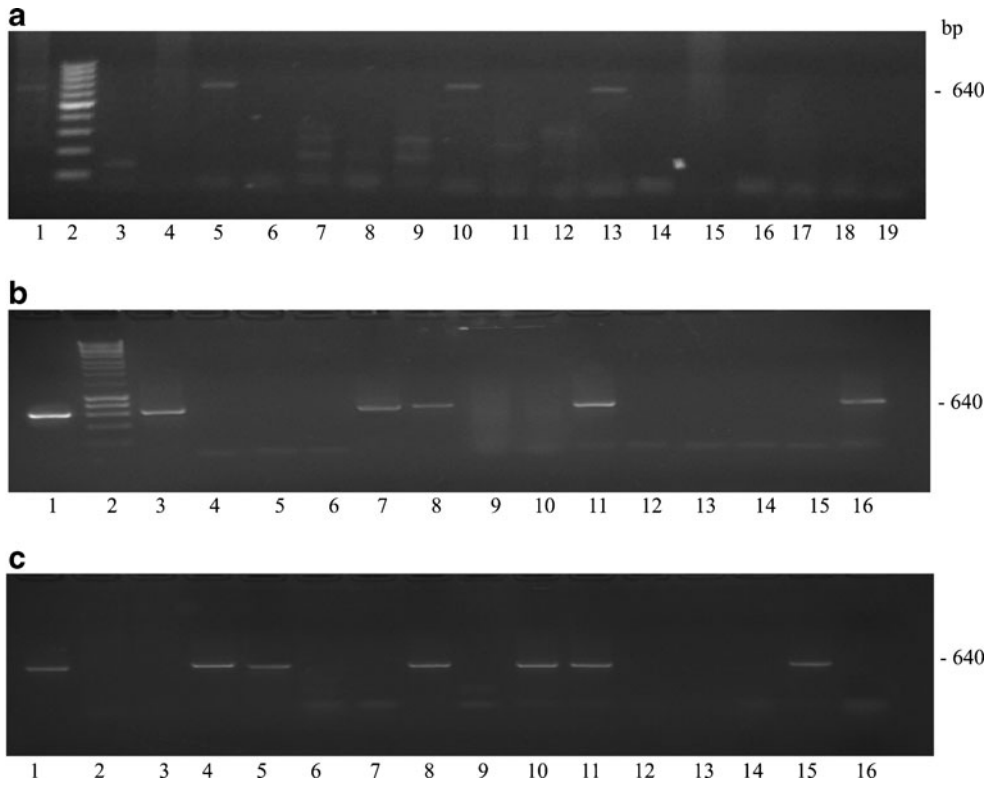

Figure 2 Detection of MHV-68 in larvae and nymphs feeding on free-living green lizards *Lacerta viridis* by single-copy-sensitive nested PCR. **a** *Lanes 1*, MHV-68 DNA; *2*, 100 bp ladder (Fermentas); *3–9*, nymphs 1–7 infesting lizard no 1; *10*, larva infesting lizard no 5; *11–17*, nymphs 3, 2, 1, and 4–7 infesting lizard no 7; *18*, nested PCR without template (negative control); *19*, primary PCR with nested primers without template (negative control). **b** *Lanes 1*, MHV-68 DNA; *2*, HyperLadderI (Bioline); *3–4*, nymphs 1, 2 infesting lizard no 2; *5–7*, nymphs 1–3 infesting lizard no 4; *8–10*, nymph and larvae 1, 2 infesting lizard no 6; *11–13*, nymph 8 and larvae 1, 2 infesting lizard no 7; *14–16*, nymph and larvae 1, 2 infesting lizard no 8. **c** *Lanes 1–2*, nymph and larva infesting lizard no 3; *3–6*, nymphs 1, 2 and larvae 1, 2 infesting lizard no 9; *7–9*, nymph and larvae 1, 2 infesting lizard no 10; *10–13*, nymphs 1–3 and larva infesting lizard no 11; *14–15*, nymph and larva infesting lizard no 12; *16*, nested PCR without template (negative control)

In this study, a group of 116 lizards and 799 ticks feeding on them were tested. Our study is first to show that ticks feeding on about 10% of lizards carried MHV-68. A single-copy sensitive nested PCR developed in this study allowed detect virus presence in immature ticks in individual assays. Thereby, we showed that both immature tick life stages—nymphs and larvae—can carry MHV-68. Moreover, one lizard was simultaneously infested by larva and nymph both carrying MHV-68. Previous studies on the mouse model showed that MHV-68 could be detected in the blood but also in the breast milk of experimentally infected mothers by using molecular methods during acute and latent infection [27, 38]. The identification of MHV-68 DNA at least in the blood of lizards could support earlier findings of antibodies against this gammaherpesvirus in others than natural hosts. Neutralizing antibodies against murine herpesvirus were detected in the blood of animals living in the same site as infected mice [18]. A relatively high prevalence of MHV-68 antibodies was detected even in the sera of persons coming professionally in contact with wild rodents and also in the sera of general human population [12]. Unfortunately, the design of this study did not permit to examine MHV-68 presence in the specific tick organs (e.g., salivary glands) or in the blood of lizards. It is interesting that MHV-68 prevalence for larvae was more than two times higher than that for nymphs. Because this type of tick remains on the first host during both the larval and nymphal life stages [8, 23], it is possible that green lizards infected ticks. 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 missing, the routes of natural infection of murid rodents with this virus (intrasanal or via body fluids), and relatively extreme stability of murine herpesvirus at wide range of pH and temperature [31] make contact infection with MHV-68 very probable. Another possible source of MHV-68 infection of lizards represents feeding of infected hard ticks in past (in this study no hard ticks were found on lizards). However, detailed studies are needed to obtain the evidence that the murine herpesvirus 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. The following studies are ongoing in our laboratory 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. Nevertheless, due to the nature of transmission of gammaherpesvirus, it is not essential for the epidemiology of MHV-68 if ticks or green lizards are infected with MHV-68 or they merely carry the virus on their surfaces. It is possible, for example, that green lizards can infect by the gammaherpesvirus their vertebrate predators such as Red fox (*Vulpes vulpes*) [34], Eurasian kestrel (*Falco tinnunculus*) [6], or Common buzzard (*Buteo buteo*) during prey handling and processing. However, data whether the virus may be transmitted from host to host, omitting the vector (ticks), lacking.

To conclude, we show that immature ticks feeding on temperate lizard species in the Central European region were infected with gammaherpesvirus naturally infecting free-living murid rodents (MHV-68). However, further laboratory studies are needed to demonstrate virus transmission between ticks and lizard hosts, as well as within individual ticks between successive life stages to confirm a role of tick as a mediator of MHV-68 circulation in nature.

Acknowledgments This work was supported by the joint grant agency of Slovak Ministry of Education and Slovak Academy of Sciences VEGA (#2/0126/10, #2/0040/09) and by the Slovak Research and Development Agency (#APVV-51-005005).

References

1. Amore G, Tomassone L, Grego E, Ragagli C, Bertolot ti L, Nebbia P, Rosati S, Mannelli A (2007) Borrelia lusitaniae in immature *Ixodes ricinus* (Acari: Ixodidae) feeding on common wall lizards in Tuscany, Central Italy. J Med Entomol 44:303–307
2. Blasdell K, McCracken C, Morris A, Nash AA, Begon M, Bennett M, Stewart JP (2003) The wood mouse is a natural host for Murid herpesvirus 4. J Gen Virol 84:111–113
3. Blaškovič D, Stančeková M, Svobodová J, Mistríková J (1980) Isolation of five strains of herpesviruses from two species of free-living small rodents. Acta Virol 24:468
4. Bown KJ, Begon M, Bennett M, Birtles RJ, Burthe S, Lambin X, Telfer S, Woldehiwet Z, Ogden NH (2006) Sympatric *Ixodes trianguliceps* and *Ixodes ricinus* ticks feeding on field voles (*Microtus agrestis*): potential for increased risk of *Anaplasma phagocytophilum* in the United Kingdom? Vector Borne Zoonotic Dis 6(4):404–410
5. Casher LE, Lane RS, Barrett RH, Eisen L (2002) Relative importance of lizards and mammals as hosts for ixodid ticks in northern California. Exp Appl Acarol 26:127–143
6. Costantini D, Bruner E, Fanfani A, Dell'Omo G (2007) Male-biased predation of western green lizards by Eurasian kestrels. Naturwissenschaften 94(12):1015–1020
7. Daniel M, Černý V (1971) Klíč zvířeny ČSSR. Československá Akademie Vied, Praha
8. de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE (2008) Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci 13:6938–6946
9. Dsouli N, Younsi-Kabachii H, Postic D, Nouira S, Gern L, Bouattour A (2006) Reservoir role of lizard *Psammodromus algirus* in transmission cycle of *Borrelia burgdorferi sensu lato* (Spirochaetaceae) in Tunisia. J Med Entomol 43:737–742
10. Efstathiou S, Ho M, Hall S, Styles CJ, Scott SD, Gompels UA (1990) Murine herpesvirus 68 is genetically related to the gammaherpesviruses Epstein–Barr virus and *Herpesvirus saimiri*. J Gen Virol 71:1365–1372
11. Ehlers B, Kuchler J, Yasmum N, Dural D, Voigt S, Schmidt-Chanasit J, Jäkel T, Matuschka FR, Richter D, Essbauer S, Hughes DJ, Summers C, Bennett M, Stewart JP, Ulrich RG (2007) Identification of novel rodent herpesviruses, including the first gammaherpesvirus of *Mus musculus*. J Virol 81:8091–8100

12. Hricová M, Mistríková J (2007) Murine gammaherpesvirus 68 serum antibodies in general human population. Acta Virol 51(4):283–287
13. Hughes DJ, Kipar A, Milligan SG, Cunningham C, Sanders M, Quail MA, Rajandream M-A, Stewart JP (2010) Characterization of a novel wood mouse virus related to murid herpesvirus 4. J Gen Virol 9:867–879
14. Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:S3–S14
15. Kuo MM, Lane RS, Giclas PC (2000) A comparative study of mammalian and reptilian alternative pathway of complementmediated killing of the Lyme disease spirochete (*Borrelia burgdorferi*). J Parasitol 86:1223–1228
16. Mačáková K, Matis J, Režuchová I, Kúdela O, Rašlová H, Kúdelová M (2003) Murine gammaherpesvirus M7 gene encoding gp 150: difference in the sequence between 72 and 68 strains. Virus Genes 26:89–95
17. Majláthová V, Majláth I, Derdáková M, Víchová B, Peťko B (2006) Borrelia lusitaniae and green lizards (*Lacerta viridis*), Karst Region, Slovakia. Emerg Infect Dis 12:1895–1901
18. Mistríková J, Kožuch O, Klempa B, Kontseková E, Labuda M, Mrmusová M (2000) New findings on the ecology and epidemiology of Murine herpesvirus isolated in Slovakia. Bratisl Lek Listy 101(3):157–162
19. Mistríková J, Rašlová H, Mrmusová M, Kúdelová M (2000) Murine herpesvirus. Acta Virol 44:211–226
20. Nash AA, Dutia BM, Stewart JP, Davison AJ (2001) Natural history of Murine gamma-herpesvirus infection. Philos Trans R Soc Lond B Biol Sci 356(1408):569–579
21. Nowak M, Cienuch S, Stańczak J, Siuda K (2010) Detection of *Anaplasma phagocytophilum* in *Amblyomma flavomaculatum* ticks (Acari: Ixodidae) collected from lizard *Varanus exanthematicus* imported to Poland. Exp Appl Acarol 51:363–371
22. Nuttall PA (2009) Molecular characterization of tick-virus interactions. Front Biosci 14:2466–2483
23. Pagel Van Zee J, Geraci NS, Guerrero FD, Wikel SK, Stuart JJ, Nene VM, Hill CA (2007) Tick genomics: the *Ixodes* genome project and beyond. Int J Parasitol 37:1297–1305
24. Penchenier L, Simo G, Grébaut P, Nkinin S, Laveissière C, Herder S (2000) Diagnosis of human trypanosomiasis, due to *Trypanosoma brucei* gambiense in central Africa, by the polymerase chain reaction. Trans R Soc Trop Med Hyg 94:392–394
25. Rajčáni J, Blaškovič D, Svobodová J, Čiampor F, Hučková D, Staneková D (1985) Pathogenesis of acute and persistent Murine herpesvirus infection in mice. Acta Virol 29:51–60
26. Rajčáni J, Kúdelová M (2007) Murid herpesvirus 4 (MHV 4): an animal model for human gammaherpesvirus research. In: Minarovits J, Gonczol E, Valyi-Nagy T (eds) Latency strategies of herpesviruses. Chapt V. Springer, Berlin, pp 102–136
27. Rašlová H, Berebbi M, Rajčáni J, Sarasin A, Matis J, Kúdelová M (2001) Susceptibility of mouse mammary glands to Murine gammaherpesvirus 72 (MHV-72) infection: evidence of MHV-72 transmission via breast milk. Microbial Pathogenesis 31:47–58
28. Rašlová H, Matis J, Režuchová I, Mačáková K, Berebbi M, Kúdelová M (2000) The bystander effect mediated by the new Murine gammaherpesvirus 72-thymidine kinase/5′-fluoro-2′-deoxyuridine (MHV72-TK/5-FUdR) system in vitro. Antivir Chem Chemother 11:273–282
29. Richter D, Matuschka FR (2006) Perpetuation of the Lyme disease spirochete *Borrelia lusitaniae* by lizards. Appl Environ Microbiol 72:4627–4632
30. Rooney AA (2005) Reptiles: the research potential of an overlooked taxon in immunotoxicology. In: Tryphonas H, Fournier M, Blakley BR, Smits J, Brousseau P (eds) Investigative immunotoxicology. CRC Press, Boca Raton, pp 107–128
31. Stančeková M, Golais F, Leššo J (1992) Some physicochemical properties of murin e herpes virus. Acta Virol 36(2):201–203
32. Sunil-Chandra NP, Arno J, Fazakerley J, Nash AA (1994) Lymphoproliferative disease in mice infected with Murine gammaherpesvirus 68. Am J Pathol 145:818–826
33. Sunil-Chandra NP, Efstathiou S, Arno J, Nash AA (1992) Virological and pathological features of mice with Murine gammaherpesvirus. J Gen Virol 73:2347–2356
34. Triggs B, Brunner H, Cullen JM (1984) The food of fox, dog and cat in Croajingalong National Park, south-eastern Victoria. Aust Wildl Res 11:491–499. doi:10.1071/WR9840491
35. Václav R, Ficová M, Prokop P, Betáková T (2010) Associations between coinfection prevalence of *Borrelia lusitaniae*, *Anaplasma* sp., and *Rickettsia* sp. in hard ticks feeding on reptile hosts. Microb Ecol. doi:10.1007/s00248-010-9736-0
36. Václav R, Prokop P, Fekiač V (2007) Expression of breeding coloration in European Green Lizards (*Lacerta viridis*): variation with morphology and tick infestation. Can J Zool 85:1199–1206
37. Virgin HW IV, Latreille P, Wamsley P, Hallsworth K, Weck KE, Dal Canto AJ, Speck SH (1997) Complete sequence and genomic analysis of Murine gammaherpesvirus 68. J Virol 71:5894–5904
38. Weck KE, Kim SS, VirginIV HW, Speck SH (1999) Macrophages are the major reservoir of latent Murine gammaherpesvirus 68 in peritoneal cells. J Virol 73(4):3273–3283
39. Wibbelt G, Kurth A, Yasmum N, Bannert M, Nagel S, Nitsche A, Ehlers B (2007) Discovery of herpesviruses in bats. J Gen Virol 88:2651–2655
40. Wielinga PR, Gaasenbeek C, Fonville M, de Boer A, de Vries A, Dimmers W, Akkerhuis OP, Jagers G, Schouls LM, Borgsteede F, van der Giessen JW (2006) Longitudinal analysis of tick densities and *Borrelia*, *Anaplasma*, and *Ehrlichia* infections of *Ixodes ricinus* ticks in different habitat areas in The Netherlands. Appl Environ Microbiol 72:7594–7601
41. Zeidner NS, Burkot TR, Massung R, Nicholson DMC, Rutherford JS, Biggerstaff BJ, Maupin GO (2000) Transmission of the agent of human granulocytic ehrlichiosis by *Ixodes spinipalpis* ticks: evidence of an enzootic cycle of dual infection with *Borrelia burgdorferi* in Northern Colorado. J Infect Dis 182 (2):616–619