University of Veterinary Medicine, Budapest

Doctoral School of Veterinary Sciences,
Aladár Aujeszky Doctoral Program of Theoretical Veterinary Sciences

0

The role of urban and wild-living small mammals in the epidemiology of ticks and tick-borne pathogens

PhD thesis

Sándor Szekeres

2017

Supervisor and consultants:

Gábor Földvári, PhD

UVM, Department of Parasitology and Zoology

Supervisor

Gábor Majoros, DVM, PhD

UVM, Department of Parasitology and Zoology

consultant

Miklós Gyuranecz, DVM, PhD

Institute for Veterinary Medical Research
Centre for Agricultural Research
Hungarian Academy of Sciences

consultant

Made in 8 copies. This is the ….th copy.

.....

Sándor Szekeres

Table of contents

List of Figures

List of Tables

Abbreviations

bp	base pair
Can.	Candidatus
LB	Lyme borreliosis
LNA	Locked Nucleic Acid
RF	relapsing fever
rRNA	ribosomal ribonucleic acid
PCR	polymerase chain reaction
qPCR	quantitative real-time PCR
s.l.	*sensu lato*
s.s.	*sensu stricto*
sp.	species (singular)
spp.	species (plural)

Abbreviations of primers used:

CRYPTO	whole 18S rRNA gene
flaB	flagellin gene
gltA	citrate synthase gene
GroEL	heat shock protein gene
IGS	inter genic spacer region gene
ompB	outer membrane protein B gene
ospA	outer surface protein A gene
msp2	major surface protein gene
RLB	V4 region of the 18S rRNA gene

1. Summary

Small mammals are abundant in urban and natural habitats of Hungary and are serving as important feeding source for non-adult stages of ticks. Tick-borne pathogens have veterinary and public health importance as well. Examination of the eco-epidemiology of tick-borne diseases is difficult, the different tick and host species have different role in the pathogen life-cycle.

In the natural study site (Gemenc) we collected ticks with flagging and small mammals with modified Sherman-traps. We euthanized the small mammals and collected tissue samples for further examination. We collected five tick (161 with flagging and 181 from small mammals) and three flea species (131 individuals from small mammals). In these arthropods, DNA of eight different pathogens were amplified with real-time and conventional PCR. Altogether 525 rodents were caught from six species, we collected and examined 348 tissue samples from them. We found five different pathogens in the collected skin and spleen samples from the natural habitat.

Apodemus flavicollis mice were found infected with *Borrelia miyamotoi*, thus species could be a new candidate reservoir for this spirochete. Among the *Ixodes acuminatus* samples we found one nymph and two larvae pools infected with *Borrelia afzelii.* This pathogen was reported from *I. acuminatus* females before, thus we suggest, the endophilic *I. acuminatus* may indicate important role in the LB pathogen cycle in the nest. *Anaplasma phagocytophilum* and *Can*. Neoehrlichia mikurensis was also found in tissue and tick samples from Gemenc. Human pathogenic rickettsiae were also found in the field collected tick samples, so all aforementioned pathogens are real risk factors for humans in natural habitats of Hungary.

We found morphological and molecular evidence of *Hepatozoon* spp. just in bank voles but other rodents and tick samples were negative, thus we examined the collected flea samples. There were positive flea samples, therefore we suggest this protozoon is the long not reported *Hepatozoon erhardovae*. We deposited the first sequence about this parasite to the NCBI database.

In the urban habitat, we collected tick samples with flagging and removing ectoparasites from road-hit carcasses. We also collected ear tissue samples from wild hedgehogs from the Margaret Island and several tissue samples from the road-killed carcasses. On the Margaret Island hedgehogs (n=88) we found *A. phagocytophilum* and *Can* N. mikurensis. In the questing ticks (n=538) from urban habitat we find two *Rickettsia* species. In the road-killed carcasses we found six; from the removed ticks we found four ectoparasite-borne pathogens.

With this dissertation, I would like to try to guide the reader in the maze of the multileveled complex relations between tick-borne pathogens, ticks and host species in two different habitats, and especially research the contribution of different host species in this system.

2. Introduction

Ticks are ectoparasitic mites feeding on blood of several vertebrate hosts. These arthropods have important vector role in the epidemiology of several causative agents with major economic losses (in case of livestock) and causing severe symptoms, even death of the host (including humans and pets). The epidemiology of tick-borne diseases is more complex and divergent than the direct dispersal of some other pathogens. The different stages and species of tick vectors and also the host species have marked effect on this process.

In the natural habitats, the pathogens have a so called “sylvatic cycle” involving many different host species. Additionally, some of these vertebrates have reservoir potential which means they do not just spread the agents, but can also maintain pathogens (which means the pathogens can multiply within the host) (Földvári, 2016; Szekeres et al., 2016b). In rural habitats, the several tick and host species could indicate higher diversity in tick borne pathogens. In urban habitats ticks and also vertebrate hosts occur, but with only few dominant species. For example, in Budapest, the capital and the biggest city of Hungary, forty-eight different mammal species from bats to wild boars have been recorded, since 1990 (Tóth-Ronkay et al., 2015). Hedgehogs and squirrels found a niche with many resources, thus they can live in higher densities in cities compared to the forests (Reeve, 1994; Tóth-Ronkay et al., 2015). This multi levelled host-vector-pathogen-environment system is the most fascinating part to investigate and also gave several paths in this complex labyrinth.

In this part of my thesis I only focus on the most important features of ticks. I wanted to help the understanding of the origin, the mechanism of feeding and reproduction of ticks as well as host–vector-pathogen interaction of small mammals, ticks and tick-borne diseases in nature and also in our close proximity, in the cities.

2.1. Biology of ticks

Ticks are land living mites belonging to phylum Arthropoda, subphylum Chelicerata and class Arachnida. Arachnids are characterised mainly by tracheal respiration and a division of the body part, consisting of one prosoma and an opisthosoma. Arachnids have six pairs of body appendages, one pair of chelicerae, one pair of pedipalps (or palps) and four pairs of legs. Members of this class do not have wings and antennae.

Ticks belong to mites (Acari) and are further classified into the superorder of Parasitiformes. Parasitiformes could further be divided into the order Ixodida (=Metastigmata), characterized by being obligatory temporary blood-sucking ectoparasites. In this group, the size of the adult body is highly dependent on the feeding status, could vary from 1mm in an unfed status up to 3 cm when completely engorged. Additionally, a toothed hypostome is present at the mouthpart that is usually visible from above. There are three families of ticks: Argasidae, Nuttaliellidae and Ixodidae (Bowmann and Nuttall, 2008).

The Ixodidae family or hard ticks, with approximately 700 species, is the dominant taxon in the order with major veterinary and public health importance. The Ixodidae are further classified into two major groups, the Prostriata and Metastriata, consisting of 5 subfamilies and 13 genera. Prostriata ticks have the anal groove anterior to the anus, however Metastriata have it posterior.(Hillyard, 1996)

Argasidae or soft ticks include approximately 190 species. The most significant soft ticks belong to two genera; *Ornithodoros* (approximately 100 species) and *Argas* (56 species).

The third family is the Nuttaliellidae with only one species, *Nuttalliella namaqua*. This tick species can be found in the semiarid area of Namaqualand, Cape Province, Republic of South Africa (Oliver, 1989) (Figure 1.).

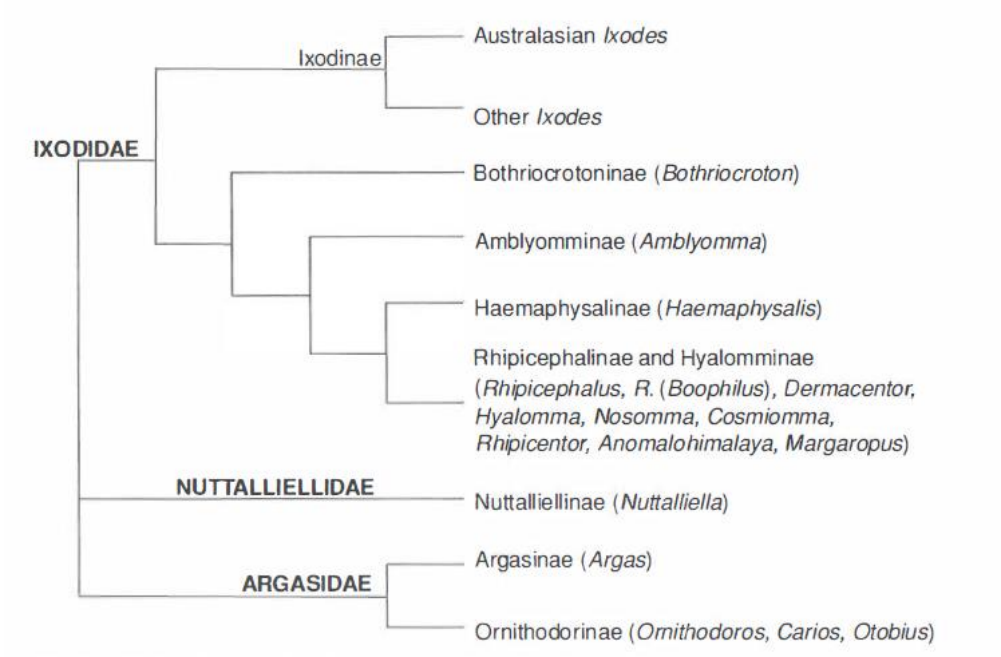

Figure 1.: Diagram of the systematic classification of Ixodidae.
(Barker and Murrel, 2004)

As mentioned before, all tick species are obligatory temporary blood sucking ectoparasites. Tick saliva contains anti-inflammatory, anti-haemostatic and anti-immune (immunosuppressive) molecules. These ingredients are bioactive proteins what control histamine, bind immunoglobulins, and inhibit the alternative complement cascade. The effect of these molecules is providing a unique site (or place) in the tick-host interface. Here, Borreliae and other tick-borne pathogens can hide from the host immune system (Nuttall et al., 2000). Ticks use their highly sensitive organs to find vertebrate hosts. The most important organ that helps in this process is the Haller's organ. This complex of sensory pits and bristle-like sensilla is located on the dorsal surface of the tarsus of the first pair legs. When this first pair of legs are waved in the air (during questing) this organ receives many external stimuli for example temperature, humidity, CO_2 concentration, ammonia, aromatic chemicals and even pheromones (intraspecific communication) and air vibration. Some tick species also have paired simple eyes located on the lateral margins of the scutum. These eyes are broadly similar to the simple eyes of many other arthropods, no evidence of true rhabdoms and screening pigment has been reported in them. In general, ticks respond to shadows and variations in light intensity, and some species, especially those that employ the "hunter" host-finding strategy (actively searching for host), are believed to be capable of discriminating shapes.(Sonenshine and Roe, 2014)

Ticks have altogether four developmental stages. The first egg stage and further three parasitic stages such as the larva, nymph and sexual dimorph adult stage (Sonenshine and Roe, 2014). The life cycle of hard ticks is similar in the whole family. Larvae emerging from eggs have only three pairsof legs, while the further stages have four pairs. After the first blood

meal, these larvae search for a shelter. Unlike other mites Ixodid ticks have only a single nymphal stage. Nymphs and adults pose the highest risk for humans to become infected, but it is known that also the larvae have epidemiological role via transovarial transmission of various pathogens (Földvári et al., 2016; Socolovschi et al., 2009)

Figure 2.: Female *Ixodes ricinus* and their laid eggs in a glass container
(photo by Sándor Szekeres)

The size of the feeding ticks could become much bigger when feeding on the appropriate host, for example female ticks can even become 100 times heavier of their original body size. Compared to the females, males only feed shortly and multiple times (Hillyard, 1996). Prostriate ticks are facultative blood feeders. Mating in prostriate ticks could occur on the host during the feeding or before feeding on the vegetation. For male ticks, except for those belonging to the genus *Ixodes*, a blood meal is required for initiation of the gonotrophic cycle. In contrast to the prostriate ticks, which mating may occur either on the host or in the environment, the metastriate ticks exclusively mate on the host. After finishing the blood meal, the female falls off the host and searches (with limited motility) for a shelter with an optimal microclimate and starts the oviposition. After a short preoviposition period females start to lay thousands of eggs (Figure 2.). However, some species can have a morphogenetic diapause between the blood meal and the oviposition, and egg laying will not occur immediately

afterwards. The oviposition lasts approximately 10-20 days. Most of the egg mass is laid within one or two weeks, however for a smaller amount of eggs 5-10 additional days are required, which is finally followed by the death of the female. In total, more than the half of the engorged female body weight is converted into eggs; this is the highest profitability amongst all arthropods (Sonenshine and Roe, 2014).

The six-legged larvae emerge from the eggs approximately 22 days after the oviposition. Larvae immediately start to seek for potential hosts or may enter to a diapause. Diapause mainly occurs prior to overwintering, rarely also observed during the summer months when the environmental conditions are not ideal. The feeding procedure and engorgement takes several days which is highly dependent on the tick species as well as on the host. Following the detachment it finally moults into a nymph. The same cycle of host contact (attachment, feeding, engorgement and detachment) is repeated and the fully fed nymph undergoes a second moulting to an unfed male or female. Adult ticks start to crawl upwards to find a place (usually on a tip of grass or underneath of a leaf or on a small branch) where they can find a suitable host.

Hard ticks can be divided to groups based on many factors:

- where they quest, moult and lay eggs
- how many host species they feed on
- how many host they need to fulfill a whole cycle
- how do they search for host.

Ticks have two groups based on the locations in which they quest for their hosts, moult, and lay eggs. There are nidicolous or endophilic (nest or burrow living) and non-nidicolous or exophilic (so-called pasture) ticks. However, it should be noted that in many cases, there is no clear border between these two types. For example, *Dermacentor reticulatus* in the larval and nymphal stages lives in the host's nest and after developing into adult tick, they change to exophilic life style. Endophilic ticks, like subadult stages of *D. reticulatus* or all three stages of *Ixodes trianguliceps* are more specialised regarding their hosts by living in their nests or in their close environment thus may provide stable local niche cycles in rodents' nest for pathogens such as *Anaplasma phagocytophilum* (Bown et al., 2006).

Based on the number of species they feed on a tick can be host specific, moderate specific and opportunistic. Species in the strict group only feed on one species, for example *Ixodes lividus* feed on sand martin (*Riparia riparia*) in their nesting burrows. This species lives the whole life in the sand martin nests. Unfed larvae feed on adult sand martins that have recently arrived from their overwintering sites (larvae overwintered in the nest). These host specific species could almost never be found out of the nest or burrow.

Moderately specific species for example bat parasitizing tick species use just some species that live together in caves. The most common species are the opportunistic ticks like *I. ricinus*, they feed on any available host species including humans as well.

Ticks can be divided also to different groups based on how many different vertebrate host species are needed to complete a whole developmental cycle. Ticks could rarely feed on one or two host species; the majority of hard ticks need to feed on three hosts to fulfil their cycle.

In case of one-host life cycle ticks all stages feed on the same host, and they do not need to leave the host, they moult on the host. This mechanism provides a protected environment and almost always available food source

In case of the two-host tick species the larvae and nymphs stay and feed on the same host. The engorged larvae undergo ecdysis on the host, moult into an unfed nymph and feed. After dropping off from the first host, they moult and start to seek for a potential second host (where the adults can feed) to complete their life cycle. (Hillyard, 1996)

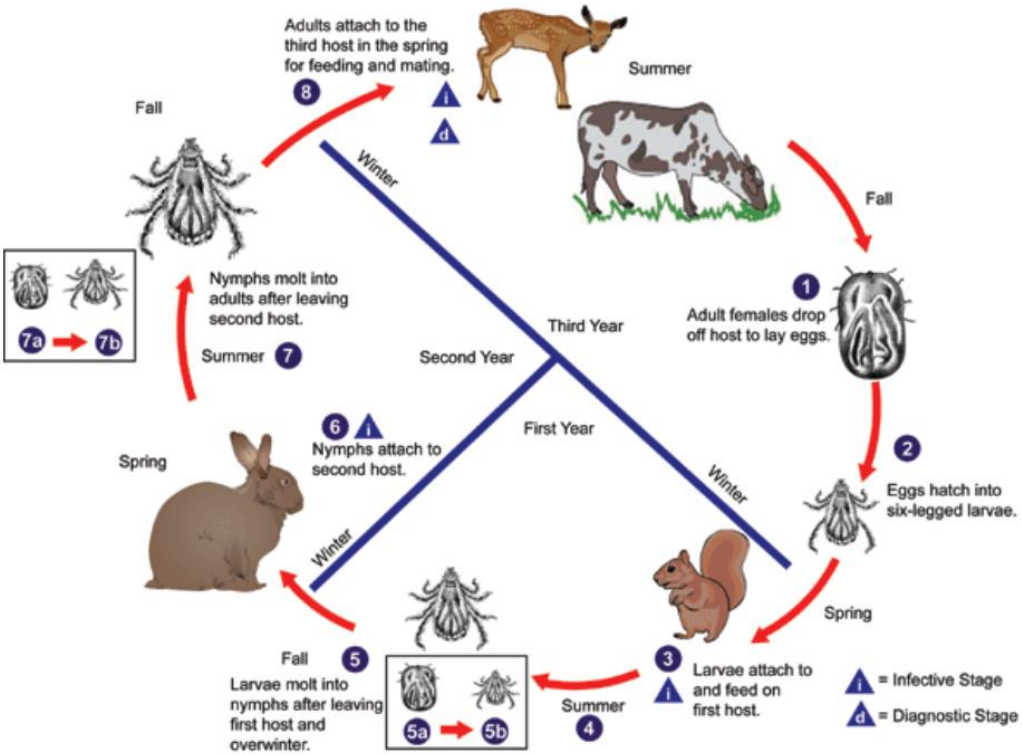

Figure 3.: Life cycle of a three-host tick

(http://www.cdc.gov/dpdx/ticks/, Download: 26.09.2017)

The three-host life cycle is the most common way of development. The whole tick cycle can be finished in one year. However, this is highly dependent on climate factors and diapause that could delay either the further development of the ticks or their host seeking behaviour as well as oviposition. Thus, the time to complete the life cycle might be extended to as much as four-five years with the maximum eight years in case of *I. ricinus* (Földvári, 2016) (Figure 3.).

Nearly all members of the genera *Amblyomma*, *Bothriocroton*, *Haemaphysalis* and *Ixodes* and the majority of *Rhipicephalus* and *Dermacentor* species are obligate three host ticks. Regarding *Hyalomma* it is usually a one or two host species, however facultatively sometimes might also undergo the three host life cycle (Sonenshine and Roe, 2014)

As mentioned before, some tick species can actively search for hosts ("hunter ticks" e.g. *Hyalomma* spp.), but most of the ticks use an "ambush" strategy (e.g. *I ricinus*); they are waiting on an optimal hiding place for a passing host.

2.2. Ticks as vectors: tick-borne pathogens in natural habitats

The emergence of Lyme-borreliosis and other tick-borne diseases with veterinary and medical importance and their association with leisure activities has brought the subject of ticks as vectors of pathogens and methods how to avoid tick bite, to general attention. The number of tick-borne pathogens are the greatest among any other arthropods. Several viruses, bacteria, fungi and protozoa are transmitted via tick bite or contamination with secretion, faeces or crushed bodies of ticks. Ticks can acquire pathogens directly from the host (during the blood meal) or vertically from the female tick (from the ovary to the eggs) and also pathogen transmission between feeding individuals via feeding pool without infesting the host (called co-feeding)(Bowmann and Nuttall, 2008; Hillyard, 1996).

For the domestic animals, ticks are one of the most important vectors of diseases worldwide. From the public health view their importance as vectors of pathogens approaches that of mosquitoes. The epidemiologically important ticks usually accept a wide range of hosts (including humans). The ability to acquire, maintain and transmit pathogens among hosts is called vector competence (Hillyard, 1996).

In the wild ticks, tick-transmitted organisms and their host live in natural balance called enzootic cycle. These hosts usually do not show any sign of infection unless they are in stressed conditions or with low immunity.

In case of host species there are many types of hosts with different functions in the life of ticks according to Kahl et al. (Kahl et al., 2002). Reservoir hosts are suitable to maintain and transmit pathogens to vectors. It is therefore common to all reservoir hosts that increase the number of infected ticks in a particular area and thereby exert a positive ecological effect on the pathogens (Figure 4.).

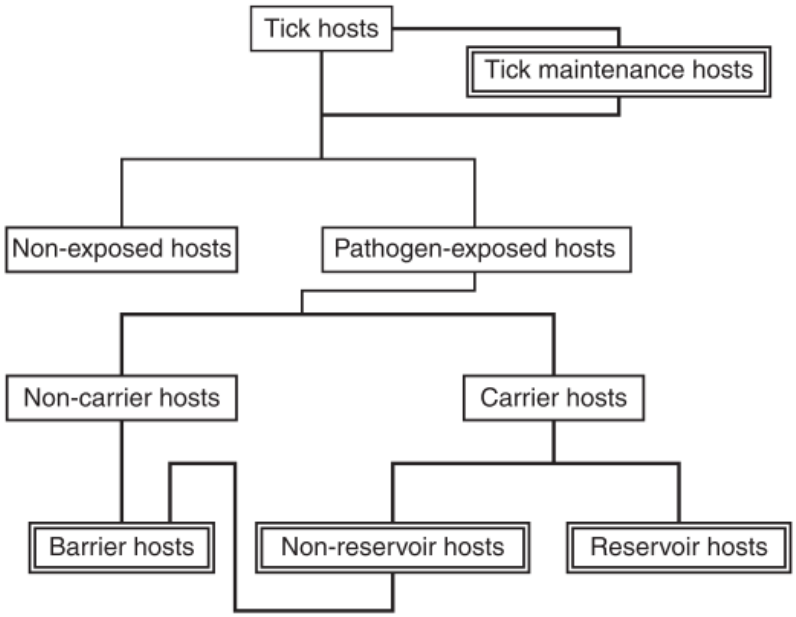

Figure 4.: Host individuals divided by the functional role in the life of ticks according to Kahl et al. 2002. Host with double frame have important ecological role.
(Kahl et al., 2002)

Carrier hosts are those animals that are not suitable to be called reservoir hosts, they harbour pathogens via tick bite at least temporarily. Reproductive or tick maintenance host can be also a reservoir and also non-reservoir species, the important is to serve as a feeding source for ticks. Barrier or dilution host are exposed to the pathogens but they are able to effect pathogens negatively (via immune response) or vectors (effective grooming).(Kahl et al., 2002)

One of the most important tick-transmitted virus is the tick-borne encephalitis (TBEV). This virus belongs to the Flaviviridae family. The general symptoms include headache, fever, coma or paralysis. TBEV can be divided into three subtypes: European (TBEV-Eu), Siberian (TBEV-Sib) and Far Eastern (TBEV-Fe). TBEV is transmitted by 11 tick species, but only two species are the most important vectors: *Ixodes ricinus* for TBEV-Eu and *Ixodes persulcatus* for TBEV-Sib and TBEV-Fe. Several animal species act as major food source of ticks. TBEV can be transmitted by feeding/co-feeding on the same host, transovarial and transstadial (transmission from stage to another) routes. Horizontal transmission between ticks and their vertebrate reservoir host is crucial for virus survival.

In majority of cases, human infections are caused by an infected tick's bite. Another important route of virus transmission is through the consumption of unpasteurized dairy products from viremic livestock, mainly goat milk.(Zöldi et al., 2013)

There are several bacteria transmitted by tick bite e.g. *Coxiella burnetti, Francisella tularensis*, *Borrelia burgdorferi* s.l., *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Can*. Neoehrlichia mikurensis and several *Rickettsia* species.

Human pathogenic members of the genus *Borrelia* consist of two main groups of spirochetes. The first group consists the causative agents of Lyme borreliosis (LB), which is widespread throughout the Northern Hemisphere and transmitted by members of the *Ixodes ricinus* complex. While the second group, causing relapsing fever (RF) in humans, is transmitted by soft ticks, hard ticks (Platonov et al., 2010) and lice (Raoult et al., 1999).

Lyme borreliosis is the most abundant human tick-borne disease in the Northern Hemisphere caused by spirochetes of the *Borrelia burgdorferi* genospecies complex (s.l.). In Europe the main vectors are the *Ixodes ricinus* ticks. In Eastern-Europe *I. ricinus* has overlapping area with *I. persulcatus*, the main vector of LB in Asia (Gray et al., 2002). *Ixodes hexagonus* has also proven role in the cycle of these pathogens (Gern et al., 1991). Due to suitable wild hosts such as hedgehogs, foxes getting prevalent and cats and dogs living in urban areas *I. hexagonus* has the opportunity to contribute more often to the transmission of LB.

The disease has first been described in the mid 1970's in Old Lyme in Connecticut, USA. *Borrelia burgdorferi* s.l. infection then was referred to as Lyme arthritis because several cases of rheumatoid arthritis have been described, especially in very young children, after being exposed to a tick bite.

Borrelia burgdorferi s.l. bacteria cause unspecific flu-like symptoms like fever, headache and muscle pain. Erythema migrans, as an early dermatological sign can appear after few days on the skin where the tick was attached. This bacterium can cause symptoms such as Lyme meningitis, Lyme carditis, borrelial lymphocytoma, Lyme arthritis, neuroborreliosis, paralysis and acrodermatitis chronica atrophicans on skin. Both in Europe as well as in North America, clinical symptoms of the disease are quite similar.

LB became compulsory notifiable in certain European countries such as Slovenia, United Kingdom, Ireland and also in Hungary. Thus, comparable data are available nowadays that have shown that there is an increasing incidence of LB cases from the western to the eastern parts of Europe (Stanek et al., 2011). Pathogenic members of *B. burgdorferi* s.l. - *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., *B. bavariensis* and *B. spielmanii* - are the causative agents of Lyme borreliosis, which is the most prevalent vector-borne disease in the temperate zone of the Northern Hemisphere. A further three species of the *B. burgdorferi* s.l. complex (*B. bissettii, B. lusitaniae* and *B. valaisiana*) have only occasionally been detected in patients (Stanek et al.,

2012). These bacteria can cause various serious dermatological, rheumatological and neurological symptoms. In Hungary, 947–1811 patients are reported yearly to suffer from LB (Zöldi et al., 2013). Considering other European and North-American data the estimated LB incidence may be ten times higher in Hungary (Lakos, 2009). All outdoor activities like hiking, mushroom picking, jogging and also some outdoor maintenance works (mowing, clearing the bushes, collecting fallen leaf litter in fall); outdoor workers with increased contact possibility with ticks, such as forestry workers, game keepers, hunters, rangers and military service personnel in field have higher chance to acquire LB infection via tick bite. Ownership of dogs and cats are also risk factors, because engorged females will detach in home or in a garden and their offspring could hatch and survive.

Several vertebrate species such as rodents, hedgehogs, shrews, hares and also birds and lizards are important host species for this bacterium. Some of these hosts are also proven reservoir of this pathogen (Table 1.). Twenty-one different genotypes of B. burgdorferi s.l. complex have been described so far and nine of these have been reported to occur in Europe including the following genotypes: *Borrelia burgdorferi* s.s., *B. garinii*, *B. spielmanii*, *B. bavariensis*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii*. For the latter genotype the disease in humans has not been confirmed so far (Briciu et al., 2014; Stanek et al., 2011).

Table 1.: Reservoir and candidate mammal species of *Borrelia burgdorferi* sensu lato in Europe

Species	**Pathogen**	**Reference**
Apodemus flavicollis	*Borrelia afzelii* *Borrelia burgdorferi s.s.* *Borrelia spielmanii*	(Bowmann and Nuttall, 2008; Richter et al., 2011)
A. sylvaticus	*Borrelia afzelii* *Borrelia burgdorferi s.s.* *Borrelia spielmanii*	(Bowmann and Nuttall, 2008; Richter et al., 2011)
A. agrarius	*Borrelia afzelii*	(Bowmann and Nuttall, 2008)
Mus musculus	*Borrelia spielmanii*	(Richter et al., 2011)
Myodes glareolus	*Borrelia afzelii* *Borrelia burgdorferi s.s.*	(Bowmann and Nuttall, 2008)
Rattus norvegicus	*Borrelia afzelii* *Borrelia spielmanii*	(Matuschka et al., 1997; Richter et al., 2011)
Eliomys quercinus	*Borrelia spielmanii*	(Richter et al., 2011)
Muscardinus avellanarius	*Borrelia spielmanii*	(Richter et al., 2011)
Sciurus caroliensis	*Borrelia afzelii*	(Bowmann and Nuttall, 2008)

Small rodents (mice and dormice) are considered to be the main reservoir host for LB across Europe. In urban habitats rats (*Rattus rattus* and *Rattus norvegicus*), house mice, hedgehogs, squirrels and mustelid species may have important role to maintain *Borrelia* spp. (Humair and Gern, 1998; Matuschka et al., 1997; Skuballa et al., 2012). Ground-foraging birds such as robins (*Erythacus rubecula*), black birds (*Turdus merula*), song thrushes (*Turdus philomelos*) and pheasants (*Phasianus colchicus*) are not only involving the LB cycle but they can transfer pathogens between far habitats (Dubska et al., 2009; Humair et al., 1993; Kurtenbach et al., 1998a; Taragelová et al., 2008).

Again, the popular opinion that *Borrelia burgdorferi* s.l. infection is only associated with outdoor activities such as hiking and mushroom picking, several studies show the presence of infection risk near to our home (e.g. gardening, dog walking) (Rizzoli et al., 2014)

Borrelia miyamotoi, belonging to the relapsing fever group, is transmitted by the same *Ixodes* species that also transmit LB spirochetes and is the only known agent causing relapsing fever transmitted by hard ticks. *Borrelia miyamotoi* was isolated for the first time in Japan in 1995 from *Ixodes persulcatus* ticks as well as from *Apodemus argenteus* mice (Fukunaga et al., 1995; Fukunaga and Koreki, 1995) and, over the last decade, it has also been detected in *I. ricinus* ticks throughout Europe (Cochez et al., 2015; Geller et al., 2012; Kiewra et al., 2014; Michelet et al., 2014; Richter et al., 2003). Its ability to cause disease was unknown until the first human cases of *B. miyamotoi* infection were reported in Russia in 2011 (Platonov et al., 2011) and, more recently, in the USA, in the Netherlands and in Germany (Boden et al., 2016; Hovius et al., 2013; Krause et al., 2013)].

Based on the high seroprevalence of *B. miyamotoi* in forestry workers reported in the Netherlands (Jahfari et al., 2014) and the relatively common occurrence of the relapsing fever spirochetes in questing ticks in Europe (Cosson et al., 2014; Crowder et al., 2014), *B. miyamotoi* infection probably also occurs in Hungary. However, the currently used diagnostic methods for patients are not suitable for detecting these spirochetes. The above mentioned seroepidemiological study in the Netherlands showed that forestry workers and patients suspected for human granulocytic anaplasmosis have significantly higher seroprevalence of *B. miyamotoi* compared to the average population (Jahfari et al., 2014). They suggest that some LB patients might also have *B. miyamotoi* infection (either undiagnosed, misdiagnosed or asymptomatic).

We also have sporadic information about the natural cycle of *B. miyamotoi*. It has so far been detected only from *Apodemus argenteus* (small Japanese field mouse) from Japan (Fukunaga and Koreki, 1995), *Peromyscus leucopus* (white- footed mouse) from USA (Scoles

et al., 2001)] and *Myodes glareolus* (bank vole) from France (Cosson et al., 2014). Based on xenodiagnostic experiments of Burri et al. (2014), *Myodes glareolus* and *Apodemus flavicollis* (yellow-necked field mouse) are proven reservoirs of *B. miyamotoi* (Burri et al., 2014), and *A. argenteus* and *P. leucopus* are candidate reservoir species. Up to date, no other eco-epidemiological studies focusing on the natural cycle of *B. miyamotoi* in Europe were performed.

Anaplasma phagocytophilum is an obligate Gram-negative intracellular bacterium. It has been a well-known pathogen among the domestic ruminants causing “tick-borne fever” but it is a generalist pathogen and can infect several other land-living vertebrate species (including humans) on the Northern hemisphere where ticks of the *I. ricinus* complex are endemic. Fatal infection cases were reported in sheep, horse, roe deer, dogs and humans. This bacterium infects and colonizes the neutrophils, thus the pathogen decreases the number of the useful immune cells often leading to immunodeficiency (Stuen et al., 2013).

Wild ruminants and probably small mammals (rodents and insectivores) play the most important role in the life cycle of *A. phagocytophilum*. Other animals (bear, wild boar, foxes, horses, hedgehogs and reptiles) can also serve as hosts or possible reservoirs (Overzier et al., 2013; Stuen et al., 2013; Vichová et al., 2014, 2010). In the USA the white-footed mouse (*Peromyscus leucopus*) is considered the major reservoir of this pathogen (Stuen et al., 2013). The bank vole (*My. glareolus*), the yellow-necked mouse (*A. flavicollis*) and the field vole (*Microtus arvalis*) are the candidate rodent reservoirs in Europe (Stuen et al., 2013), but in a xenodiagnostic study the *Apodemus* spp. mice and *My. glareolus* did not infect larvae that had fed on them (Burri et al., 2014). Thus, the exact role of European rodent species in the circulation and maintenance of bacteria is unclear and prevalence rate of *A. phagocytophilum* DNA is low in this group of animals (Stuen et al., 2013). *Anaplasma phagocytophilum* can also be transmitted by ticks to a wide range of domestic ruminants e.g. bovines (cattle, yak), camelids (llama, alpaca), sheep and goats.

In a recent study, based on groEL heat-shock protein sequences (extracted from tissue and tick samples) and the vertebrate host range differences, four distinct *A. phagocytophilum* ecotypes was separated by a large-scale study (Jahfari et al., 2014). The first ecotype associated with human cases are also found in domestic animals, red deer, wild boar and hedgehogs; the second ecotype affected roe deer and some rodent species, the third one is associated with rodents and the last ecotype belonging to birds.

In Europe, the increasing geographic range of *I. ricinus* as well as the expansion to higher altitudes opened new regions and heights to this pathogen (Jaenson et al., 2012; Medlock et al., 2013)..

Candidatus Neoehrlichia mikurensis is a coccoid Gram-negative pathogen belonging to the family Anaplasmataceae (Kawahara et al., 2004). It was first detected in the late 1990's in *I. ricinus* in the Netherlands and Italy and later it was also found in China in a wild Norway rat (*Rattus norvegicus*). It was initially called Ehrlichia-like due to a diverging 16S rRNA gene sequence (Schouls et al., 1999). Further findings of the microorganism in rats and *Ixodes ovatus* ticks in Japan and the passaging of the agent in laboratory rats led to its description as the new species *Candidatus* Neoehrlichia mikurensis in 2004 (Kawahara et al., 2004). This emerging zoonotic intracellular tick-borne pathogen forms a separate cluster in the family Anaplasmataceae together with the North American *Candidatus* Neoehrlichia lotoris, which has been detected in raccoons (*Procyon lotor*)(Yabsley et al., 2008). In Switzerland, Sweden, Germany, Czech Republic and in China *Candidatus* N. mikurensis was shown to be a human and in Germany as a canine pathogen (Grankvist et al., 2014; Jahfari et al., 2012; Li et al., 2012; Pekova et al., 2011; Silaghi et al., 2012; Tijsse-Klasen et al., 2014). Most of the human patients were immunocompromised due to splenectomy or immunosuppressive therapy and the reported manifestations of neoehrlichiosis were severe. In China, however, *Candidatus* N. mikurensis infection was also reported in immuno-competent patients (Li et al., 2012). *Ixodes ricinus* is most likely the vector in Europe, but the range of reservoir hosts is not fully known. Some studies suggested rodents as potential reservoirs (Jahfari et al., 2012) and recently the reservoir role of *Apodemus* mice (*A. flavicollis, A. sylvaticus*) and bank voles (*Myodes glareolus*) has unambiguously been proven in a xenodiagnostic study (Burri et al., 2014).

Several studies have identified DNA of *Candidatus* N. mikurensis in questing or host-attached *I. ricinus* in Europe including Hungary (Derdáková et al., 2014; Hornok et al., 2013; Jahfari et al., 2012). However, potential rodent reservoir hosts have thus far not been examined in Hungary.

Tick-borne rickettsioses, caused by obligate intracellular bacteria within the genus *Rickettsia*, mainly transmitted by arthropods caused by spotted fever group rickettsiae and cause an expanding spectrum of clinical signs. Until recently, Mediterranean spotted fever caused by *Rickettsia conorii* was considered the only tick-borne rickettsiosis in Europe (Oteo and Portillo, 2012). In the last decade, many other species and subspecies of *Rickettsia* have been discovered and implicated as human pathogens, and new rickettsial syndromes have been described. For instance, other subspecies such as *R. conorii caspia* and *R. conorii israelensis* have been discovered as MSF causative agents. Dermacentor-borne necrosis

erythema and lymphadenopathy/tick-borne lymphadenopathy (DEBONEL/TIBOLA) cases caused by *Rickettsia slovaca* and *Rickettsia raoultii* been described in several countries where *Dermacentor marginatus* and *D. reticulatus* ticks (the mainly implicated vector) are endemic (Földvári et al., 2013). *Rickettsia helvetica* has also been involved as a human pathogen in cases of fever with and without rash and in patients with meningitis and carditis (Fournier et al., 2000). Other rickettsial diseases such as lymphangitis-associated rickettsioses (LAR), caused by *Rickettsia sibirica mongolitimonae*, have been diagnosed in different European countries (e.g. France, Spain, Portugal)(Aguirrebengoa et al., 2008; Edouard et al., 2013; Ramos et al., 2013). *Rickettsia massiliae* is considered an etiological agent of MSF-like illness in the Mediterranean basin. Furthermore, *Rickettsia monacensis* that is distributed all along Europe has been isolated from patients with MSF-like illness in Spain (Jado et al., 2007). Although *Rickettsia aeschlimannii* has been associated with MSF-like disease in Africa and is distributed in the Mediterranean area, no autochthonous human cases have been reported for Europe.

Eukaryotic haemoparasites belonging to genus *Hepatozoon* (Apicomplexa: Hepatozoidae) have been described from a wide range of animals (from dogs to snakes). These intracellular parasites have heteroxenous life-cycle. It includes the vertebrate intermediate host and a haematophagous invertebrate definitive host, which also serves as a vector. Asexual reproduction (schizogony) can occur in different organs of mammalian hosts and gamonts are found in blood cells. Sexual reproduction (sporogony) takes place in the hemocoel of the invertebrate definitive host. As there are no observed occurrences of the migration of *Hepatozoon* sporozoits to the salivary gland of the arthropod host, it is assumed that the ingestion of the definitive host containing the sporulated oocysts is required for transmission (Craig, 2001a; Laakkonen et al., 2001a; Smith, 1996).

In the last 50 years, *Hepatozoon* infection of small mammals was found in several studies, in different parts of Europe. The differentiation of these species –when it was even attempted– was based on the vertebrate host, the geographical region where the samples were collected and the morphology of the bloodstream developmental forms (Criado-Fornelio et al., 2003; Karbowiak et al., 2005; Laakkonen et al., 2001b). The life cycle and host range of most of these species is still unknown.

Besides the previously mentioned pathogens small mammals are exceptional hosts for other vector-borne (e.g. flea-borne) pathogens e.g. *Bartonella* species. In the recent years there are many records of *Bartonella* spp. found in several hard tick species around the word, for example *Dermacentor* and *Ixodes* spp as well (Angelakis et al., 2010). Thirteen *Bartonella*

species and subspecies have been associated with an increasing spectrum of clinical syndromes in humans, from cat-scratch disease and chronic bacteraemia to myocarditis.

2.3. Tick-borne pathogens in urban habitats

People living in urban areas love to be in “green” for leisure activities or just to enjoy the calmness of nature, therefore, cities and houses are designed with some kind of green areas; like alleys, smaller or bigger city parks and nicely cared front or back gardens. These green areas could serve as suitable habitat for some urban animal species. For example in Budapest, the capital and the biggest city of Hungary, forty-eight different mammal species from bats (Chiroptera) to wild boars (*Sus scrofa*) have been recorded, since 1990 (Tóth-Ronkay et al., 2015). Some of these urbanised mammal species, such as hedgehogs (*Erinaceus* spp.) and squirrels (*Sciurus* spp.), can even reach higher densities in urban/suburban habitats than usually in rural environments (Reeve, 1994; Tóth-Ronkay et al., 2015) (Figure 5.).

The main blood meal source in urbanised habitats for the non-adult tick stages are rodents like mice (Muridea), voles (Arvicolinae) and dormice (Gliridae), lizards and birds living in urban

Figure 5.:Urban red squirrel (Sciurus vulgaris) Margaret Island, Pet Zoo (photo by Sándor Szekeres)

and periurban habitats. Adult ticks usually feed on larger mammals like dogs (*Canis lupus familiaris*), red foxes (*Vulpes vulpes*), wild and domestic herbivores and occasionally also on humans. In urban areas, the diversity of host species is not as high as in rural habitats (e.g. forest), but in contrast, the few species present are abundant and they serve as hosts for a stable and large tick population increasing the risk of acquiring tick-borne pathogens (Rizzoli et al., 2014).

Reservoir hosts are proven natural hosts of vector ticks, and ticks may become infected while feeding on these animals (Kahl et al., 2002). In case of LB distinct genospecies of *B.*

burgdorferi s.l. are associated with different reservoir hosts (Hanincová et al., 2003a, 2003b, Humair et al., 1999, 1998, 1995, Humair and Gern, 2000, 1998, Kurtenbach et al., 1998b, 1998c). According to individual groups of reservoir hosts, specific maintenance cycles are distinguished. In this section, I would like to introduce additional important but often neglected hosts in urban habitats the medium-sized mammals, for example squirrels, hedgehogs and mustelids.

European red squirrels (*Sciurus vulgaris*) are common rodent species living in natural forests and city parks in Eurasia. This squirrel species, like most tree squirrels, has sharp, curved claws that help to climb on broad tree trunks and thin branches. The long tail helps the squirrel to balance, when jumping with its strong hind legs from tree to tree and running along branches. The coat of the red squirrels varies from red to greyish or blackish red, the ventral part is always white. These tree squirrels are omnivorous, solitary animals being active during daylight. The size of the territory of the species depends on the “nesting” and food source trees and also on the sex of the squirrel. The red squirrel is found in both coniferous forest and temperate broadleaf woodlands. Squirrels build dreys out of twigs in a branch-fork, forming a domed structure or use a tree hole or a forsaken woodpecker hole as shelter lined with moss, grass and leaves. In western and southern Europe, they are found in broad-leaved woods where the mixture of tree and shrub species provides a better year-round food source. The main food sources are hazelnuts (*Coryllus avellana*), walnuts (*Juglans* spp.), beechnuts (*Fagus sylvatica*), acorns (*Quercus* spp.) and younger cones and nuts of pine trees (Pinaceae); the seeds of these plants are rich in vitamins and nutrients. Squirrels supplement their diet with young shoots, leaf and flower buds, tree flowers, bark-growing fungi and insects (Grönwall and Pehrson, 1984; Gurnell, 1987; Moller, 1983; Wauters et al., 1992; Wauters and Dhondt, 1987). Rarely, red squirrels may eat bird eggs or nestlings (Fontaine and Martin, 2006). For the harsh winter times these arboreal rodents store excess food in tree holes, underground holes or other proper storage places.

The Eastern grey squirrel (*S. carolinensis*) has predominantly grey fur, but it can have a brownish colour and a usual white underside. This invasive species competes with the native red squirrel for resources, such as food and habitat. It was introduced from North America to several locations like South Africa, Australia and also Europe. In Europe, the Eastern grey squirrel was introduced several occasions from the late XIX. Century to the British Isles and Italy.

In the last century, they have colonised Great Britain except the northern parts of Scotland, and also big territories in Ireland and Italy. In addition, in Great Britain, the abundant grey squirrels are considered as pest because of bark stripping and ring barking of trees, and conservationist, foresters and hunters are trying to decrease the numbers of these rodents. According to data from the literature and personal communication with Mária Ronkayné-Tóth grey squirrels are not presented in the Hungarian fauna. But, with the constant area expansion of this invasive mammal it could occur in the future in Hungary.

Natural predators of the red squirrel are wild cats (*Felis silvestris*), pine and stone martens (*Martes martes* and *M. foina*) (Tóth Apáthy, 1998), red foxes, stray dogs and cats and also bird of prey like northern goshawks (*Accipiter gentilis*) and common buzzards (*Buteo buteo*) (Bősze, 2007). Squirrels forage most of the day after food on the ground when they can “collect” ticks from the leaf litter.

The first report about *Borrelia* infection related with European red squirrel was in 1998 by Humair and Gern from Switzerland. They found *B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *Borrelia* sp. single infection and *B. burgdorferi* s.s. and *B. afzelii* co-infection in *I. ricinus* from a road-killed carcass (Humair and Gern, 1998). In red squirrel tissue samples all the aforementioned species were present and even single infection of *B. valaisiana* (Morán Cadenas et al., 2007), co-infection of *B. burgdorferi* s.s. and *B. garinii* and triple infection of *B. burgdorferi* s.s., *B. afzelii* and *B. garinii* (Pisanu et al., 2014) (Table 2.).

In tissue samples of grey squirrel, *B. burgdorferi* s.l. was found. In a xenodiagnostic experiment, Eastern grey squirrel was proved to serve as a reservoir for LB spirochetes. In a pool from three nymphs from an experimentally used squirrel Craine et al (1997) found *B. afzelii* (Table 2.)

Table 2.: *Borrelia burgdorferi* s.l. in squirrels in Europe

Source			Pathogen	Prevalence (positive/tested)	Country	Reference
Eastern grey squirrel (*Sciurus carolinensis*)						
	tissue		*B. burgdorferi* s.l.	14.15% (15/106)	United Kingdom	(Craine et al., 1997)
	removed tick	*I. ricinus*	*B. burgdorferi* s.l.	32% (8/25)*	United Kingdom	(Craine et al., 1997)
				16.14% (31/192)*	United Kingdom	(Craine et al., 1997)
			B. afzelii	3 nymph in a pool**	United Kingdom	(Craine et al., 1997)
European red squirrel *(S. vulgaris)*						
	tissue		*B. burgdorferi* s.s.	33.33% (2/6)	Switzerland	(Humair and Gern, 1998)
				8.1% (11/135)***	Switzerland	(Morán Cadenas et al., 2007)
				11% (30/273)	France	(Pisanu et al., 2014)
			B. afzelii	5.5% (15/273)	France	(Pisanu et al., 2014)
				6.7% (9/135)***	Switzerland	(Morán Cadenas et al., 2007)
			B. garinii	16.66% (1/6)****	Switzerland	(Humair and Gern, 1998)
				0.74% (1/135)***	Switzerland	(Morán Cadenas et al., 2007)
				1.8% (5/273)	France	(Pisanu et al., 2014)
			B. valaisiana	0.74% (1/135)***	Switzerland	(Morán Cadenas et al., 2007)
			B. burgdorferi s.l.	1.48% (2/135)***	Switzerland	(Morán Cadenas et al., 2007)
			B. burgdorferi s.s. + *B. afzelii*	33.33% (2/6)	Switzerland	(Humair and Gern, 1998)
				4.4% (12/273)	France	(Pisanu et al., 2014)
			B. burgdorferi s.s. + *B. garinii*	0.74% (2/273)	France	(Pisanu et al., 2014)
			B. burgdorferi s.s. + *B. garinii* + *B. afzelii*	0.37% (1/273)	France	(Pisanu et al., 2014)
	removed tick	*I. ricinus*	*B. burgdorferi* s.s.	13.6% (31/227)	Switzerland	(Humair and Gern, 1998)
			B. afzelii	19% (43/227)	Switzerland	(Humair and Gern, 1998)
			B. garinii	1.76% (4/227)	Switzerland	(Humair and Gern, 1998)
			B. burgdorferi s.s. + *B. afzelii*	4.4% (10/227)	Switzerland	(Humair and Gern, 1998)
			Borrelia sp.	2.2% (2/227)	Switzerland	(Humair and Gern, 1998)

* xenodiagnostic ticks analysed with PCR (32%) and with IFAT (16.14%)
** xenodiagnistic nymph pool (3 individuals) from grey squirrel (code: C)
*** based on blood meal analysis of questing ticks
****not confirmed: The mentioned data is in an unpublished report

Hedgehogs are common insectivores in Europe. They feed on annelids, insects (larvae, pupae and imagoes as well), snails and slugs, small vertebrates (amphibians, lizards and occasionally young rodents), chicks and eggs of birds (Jackson and Green, 2000) and even some berries and fruits (Jones and Norbury, 2010; Yalden, 1976).

In urban habitat, motorized vehicles and dogs pose a large risk to hedgehogs. The majority of the run overs happen in the mating period when the males search intensively for females. Some dogs (including strays) are known to prey upon them when the opportunity arises.

Three hedgehog species live in Europe. The European hedgehog (*Erinaceus europaeus*) occurs in Western Europe, Scandinavia and the Baltic region. The Northern white-breasted hedgehog (*E. roumanicus*) inhabits from the Eastern part of Europe to the European part of Russia and the Ponto-Mediterranean region. The third species, the Southern white-breasted hedgehog (*E. concolor*), is found in Asia Minor and Eastern-Mediterranean. Among the European and Northern white-breasted hedgehogs, there are hybridization zones; one in north-south direction from Poland to Italy and another in west-east direction in the Baltic-Russian border of the two areas. For the Northern and the Southern white-breasted hedgehog, the Caucasus and the two straits of the Sea of Marmara (Bolfíková and Hulva, 2012) form natural barriers. After the last glacial period the ancestors of these hedgehog species recolonised the thawing Europe from Mediterranean refuges (Bolfíková and Hulva, 2012) (Figure 6.).

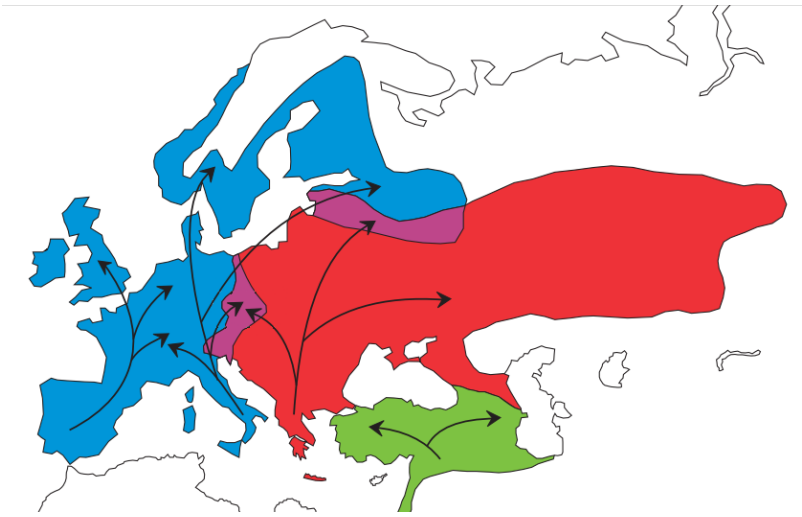

Figure 6.: Distribution of the three hedgehog species (*Erinaceus europaeus* (blue), *E. roumanicus* (red), *E. concolor* (green), hybridisation zones (purple), and main colonisation routes from the refuges after the last ice age in Europe based on Bolfíková and Hulva (2012).

Hedgehogs are appropriate and attractive hosts for several ecto- and endoparasites (Figure 7.). First of all, they feed on the typical intermediate host species (e.g. slugs, snails, earthworms, beetles) of different endoparasitic helminths such as roundworms, tapeworms and acanthocephalans. Second, the undergrowth and dry leaf litter dwelling lifestyle is ideal for collecting and maintaining ectoparasites such as ticks and fleas, which are often vectors of several viruses, bacteria and protozoa. *Ixodes hexagonus* the hedgehog tick, *I. ricinus* (Földvári et al., 2011; Pfäffle et al., 2011) and *Archaeopsylla erinacei*, the hedgehog flea (Földvári et al., 2011; Gilles et al., 2008; Hornok et al., 2014; Marié et al., 2011; Visser et al., 2001) are common ectoparasites of hedgehogs in Europe. *Ixodes acuminatus* Neumann and *Hyalomma marginatum* nymphs were also reported from Northern white-breasted hedgehog from a city park of Budapest (Földvári et al., 2011). High tick burden can exert negative effect on the hedgehog's health. Tick burden can cause tick-induced regenerative anaemia in European hedgehogs by blood loss (Pfäffle et al., 2009). The energy, which is invested into immune responses and regeneration combined with suboptimal environmental factors could lead to secondary infections. Moreover, the spiny armour is ideal for maintaining ectoparasites, because it limits antiparasitic behaviour of hedgehogs.

Figure 7.: Ectoparasites (fleas and ticks) from a single road-hit Northern white-breasted hedgehog (photo by Sándor Szekeres).

The summer and winter shelter (hibernaculum) of the hedgehogs play important role in the life cycle of the nidicolous hedgehog ectoparasites. Eggs and larvae of the hedgehog flea (*A. erinacei*) develop in the bedding of the nest. Moreover, the non-adult stages of some tick species also live in the nest (e.g. *Dermacentor* spp.) and there are some species of which all the developmental stages live in the nest (e.g. *I. hexagonus*) (Morris, 1973). The occurrence of *I. hexagonus* in the urban environment is due to the presence of suitable hosts such as hedgehogs, cats and dogs in gardens and public parks (Gern et al., 1997, 1991).
European hedgehogs are reservoir hosts for *B. burgdorferi* s.l., and take part in the maintenance of several *Borrelia* species in an enzootic cycle (Gern et al., 1997; Skuballa et al., 2007).

In tissue samples of European hedgehogs from Germany, Switzerland and Czech Republic *B. afzelii, B. spielmanii, B. bavariensis, B. garinii* and *B. burgdorferi* s.s. have been found (Table 3.). In a recent paper *B. afzelii*, *B. spielmanii*, *B. garinii*, and *B. burgdorferi* s.s. were detected in both tick species commonly found on European hedgehog (Krawczyk et al., 2015).

The eastern relative of the aforementioned hedgehog species, the Northern white-breasted hedgehog, had been studied only in the previous decade. Tissue samples were collected from naturally died specimens (n=4) from an Austrian rehabilitation centre not far from the Hungarian border and *B. afzelii* and *B. bavariensis* infection was detected (Skuballa et al., 2012). In addition, in *I. ricinus* ticks removed from anesthetized Northern white-breasted hedgehogs, *B. afzelii* was found. European hedgehogs might also serve as reservoir hosts for another tick-borne pathogen, *A. phagocytophilum* (Silaghi et al., 2011), which causes granulocytic anaplasmosis in humans (Dumler et al., 2005).

Unfortunately, we do not have any data about *Borrelia* infection of the third European hedgehog species. Nevertheless, the area of *I. ricinus* and *E. concolor* is overlapping in Turkey, suggesting that this hedgehog species could possibly serve as a suitable host for *Borrelia* spirochetes.

Table 3.: *Borrelia burgdorferi* s.l. in hedgehogs in Europe

Source		Pathogen	Prevalence (positive/tested)	Country	Reference
European hedgehog (*Erinaceus europeaus*)					
tissue		*B. spielmanii*	1.4% (3/211)	Germany	(Skuballa et al., 2012)
		B. afzelii	5.68% (12/211)	Germany	(Skuballa et al., 2012)
			25% (4/16)	Czech Republic	(Skuballa et al., 2012)
			14.3% (1/7)	Switzerland	(Gern et al., 1997)
		B. bavariensis	0.94% (2/211)	Germany	(Skuballa et al., 2012)
		B. garinii	42.9% (3/7)	Switzerland	(Gern et al., 1997)
		B. afzelii +	2.37% (5/211)	Germany	(Skuballa et al., 2012)
		B. bavariensis	12.5% (2/16)	Czech Republic	(Skuballa et al., 2012)
		B. afzelii + *B. spielmanii*	0.94% (2/211)	Germany	(Skuballa et al., 2012)
		B. bavariensis + *B. spielmanii*	0.94% (2/211)	Germany	(Skuballa et al., 2012)
		B. burgdorferi s.s. + *B. garinii*	14.3% (1/7)	Switzerland	(Gern et al., 1997)
		B. afzelii + *B. bavariensis* + *B. spielmanii*	0.47% (1/211)	Germany	(Skuballa et al., 2012)
		Borrelia sp.	0.94% (2/211)	Germany	(Skuballa et al., 2012)
removed tick	*I. hexagonus*	*B. burgdorferi* s.l.	14% (60/435)	the Netherlands	(Krawczyk et al., 2015)
		B. afzelii	76% (37/49)	the Netherlands	(Krawczyk et al., 2015)
		B. bavariensis	6% (3/49)	the Netherlands	(Krawczyk et al., 2015)
		B. spielmanii	14% (7/49)	the Netherlands	(Krawczyk et al., 2015)
		B. burgdorferi s.s.	4% (2/49)	the Netherlands	(Krawczyk et al., 2015)
	I. ricinus	*B. burgdorferi* s.l.	28% (7/25)	the Netherlands	(Krawczyk et al., 2015)
serum		*B. burgdorferi* s.l. #	-	France	(Doby et al., 1991)
Northern white-breasted hedgehog (*E. roumanicus*)					
tissue		*B. afzelii*	25% (1/4)	Austria	(Skuballa et al., 2012)
		B. bavariensis	25% (1/4)	Austria	(Skuballa et al., 2012)
removed tick	*I. ricinus*	*B. afzelii*	0.4% (4/959)	Romania	(Dumitrache et al., 2013)

serological evidence from one individual: hedgehog titer 1/100

In addition to the easily noticeable urban mammals such as hedgehogs and squirrels, mustelid species form another group of urbanised medium-sized mammals with a more hidden, nocturnal nature. Mesocarnivores, like mustelids are generally rather successful in highly fragmented and urbanised landscapes (Crooks, 2002). In general, mustelids are carnivores, but some species for example stone martens *(Martes foina*) and European badgers *(Meles meles)* have considerable amount of fruits in their diet.

Stone martens, *Martes foina* is the most abundant mustelid in urban areas, use lofts and abandoned garrets in downtowns, and outbuildings and sheds in suburban regions as hiding places (Figure 8.). In central Europe, it is generally regarded as a synanthropic species (Tóth-Ronkay et al., 2015). The spectrum of food sources of this species is very broad from arthropods, fishes, reptiles and amphibians, small mammals, birds and eggs to fruits and seeds (Lanszki, 2003; Lanszki et al., 1999; Tóth-Ronkay et al., 2015). In urban environment, they supplement their diet with garbage and leftover dog and cat food (Tóth et al., 2011).

Figure 8.: Urban stone marten (*Martes foina*) (photo by Mária Tóth-Ronkay)

In addition to stone martens, three other mustelids are sporadically reported in urban habitats. The smallest of these species is the least weasel (*M. nivalis*), the medium is the stoat and the biggest is the European badger. In Budapest, there are few sightings of the least weasel in gardens and bushy forest edges in the suburban parts of the city (Tóth-Ronkay et al., 2015). Least weasel has been found in three out of twelve trapping areas with various habitat characteristics (e. g. scrubs, orchards or long grass areas) in built-up areas of Oxford (Dickman, 1986). European badgers are also commonly reported in the rural areas near to the cities, where the human disturbance such as noise pollution, vehicles and dogs are not frequently presented (Tóth-Ronkay et al., 2015).

Our knowledge about *Borrelia* infection in mustelid species is scarce, thus we tried to collect all data about *Borrelia* infection in these animals (Table 4.). The main tick species associated with mustelid species is *I. hexagonus* (Jaenson et al., 2012; Lorusso et al., 2011), but there are reports about *I. ricinus* ticks as well (Lorusso et al., 2011). There are no data about *Borrelia* infection in stone martens. In an article about pathogens and diseases in mustelid species, *Borrelia burgdorferi* s.l. infection was mentioned from British stoats (McDonald and Lariviere, 2001). There is one serological report of *B. burgdorferi* s.l. infection in one least weasel (Doby et al., 1991). In European badgers, *B. afzelii* (Gern and Sell, 2009; Morán Cadenas et al., 2007) and *B. afzelii* and *B. valaisiana* coinfection was found (Gern and Sell, 2009).

In other not urbanised mustelid species, like marbled polecat (*Vormela peregusna* Güldenstädt), European mink (*M. lutreola*) and European polecat (*M. putorius*), *Borrelia* infections were reported. *Borrelia burgdorferi* s.s. was found in marbled polecat and in European mink in Romania (Gherman et al., 2012). In Switzerland, analysis of host blood remnants in field collected ticks showed that the European polecat had been the previous host of ticks that were found infected with *Borrelia burgdorferi* s.s (Morán Cadenas et al., 2007).
Some mustelids live in close proximity around human dwellings. In conclusion, in urban environment these species can serve as host for *B. burgdorferi* s.l., especially the highly adaptive and synanthropic stone martens, but the role of these medium-sized mammals in *B. burgdorferi* s.l. cycle needs further examination.
In contrast to *I. ricinus*, *I. hexagonus* is an endophilic (or nidicolous) tick species living in the nest of the vertebrate host. Therefore, the host range of *I. hexagonus* is more restricted than that of *I. ricinus.* It feeds primarily on carnivores such as foxes and mustelids, and on hedgehogs, but also, less frequently on other species such as rodents, hares and rabbits (Arthur, 1953; Hornok et al., 2017; Toutoungi et al., 1991). *Ixodes hexagonus* has occasionally been collected from Eurasian magpie (*Pica pica*), common kestrel (*Falco tinnunculus*) and Eurasian roe deer (*Capreolus capreolus*) (Hubbard et al., 1998; Toutoungi et al., 1991). Domestic animals such as cats, dogs, horses, goats and cows have also been found to be infested (Arthur, 1968; Bernasconi et al., 1997; Földvári and Farkas, 2005; Toutoungi et al., 1991). Although less frequently than *I. ricinus; Ixodes hexagonus* apparently also bite humans (Arthur, 1953; Hubbard et al., 1998; Liebisch et al., 1998), thus its epidemiological role in transmitting LB spirochetes deserves further investigations.

Table 4.: *Borrelia burgdorferi* s.l. in mustelids in Europe

Source	Pathogen	Prevalence (positive/tested)	Country	Reference
European polecat (*Mustela putorius*)				
tissue	*B. burgdorferi* s.s.	1.48% (2/135)***	Switzerland	(Morán Cadenas et al., 2007)
European mink (*M. lutreola*)				
tissue	*B. burgdorferi* s.s.	66.6% (2/3)	Romania	(Gherman et al., 2012)
Marbled polecat (*Vormella peregusna*)				
tissue	*B. burgdorferi* s.s.	50% (1/2)	Romania	(Gherman et al., 2012)
Stoat (*M. erminia*)				
tissue	*B. burgdorferi* s.l.	22.2% (10/45) ****	United Kingdom	(McDonald and Lariviere, 2001)
Least weasel (*M. nivalis*)				
serum	*B. burgdorferi* s.l. #	-	France	(Doby et al., 1991)
European badger (*Meles meles*)				
tissue	*B. afzelii*	24% (2/8)	Switzerland	(Gern and Sell, 2009)
	B. afzelii	0.74% (1/135)***	Switzerland	(Morán Cadenas et al., 2007)
	B afzelii + *B valaisiana*	12.5% (1/8)	Switzerland	(Gern and Sell, 2009)

*** based on blood meal analysis of questing ticks
****not confirmed: The mentioned data is in an unpublished report
serological evidence from one individual, least weasel titer 1/50

3. Aims of the study

The aim of this study was to investigate the occurrence of tick-borne human pathogens in small mammals and ticks from a natural habitat in Southern Hungary, where forestry works, hunting and recreational activities are intensive; and from accidentally killed urbanised, city dwelling mammals and ticks removed from them. With the gained data we wanted to shed light on some interesting parts of some well-known and some new pathogens in our natural study site and also the less investigated researched side of the tick-borne pathogens within cities.

I had the following aims:

- assess the tick fauna parasitizing rodents in a natural floodplain forest and hedgehogs in an urban habitat.
- find rodent and ectoparasite species that carry *B. burgdorferi* s.l., *B. miyamotoi*, *A. phagocytophilum*, *Can*. N. mikurenis, *Rickettsia* spp., *Hepatozoon* spp. and *Bartonella* spp. and might be involved in the epidemiology of these pathogens
- find *B. burgdorferi* s.l., *B. miyamotoi*, *A. phagocytophilum*, *Can*. N. mikurenis, *Rickettsia* spp. and *Bartonella* spp. in road hit or accidentally died small and medium sized mammals and their ectoparasites in urban areas
- asses the contribution of Northern white-breasted hedgehogs in the cycle of tick-borne pathogens on Margaret Island.

4. Materials and methods

4.1. Sample collection

4.1.1. Natural habitat

Between July 2010 and May 2013, small mammals were live-trapped with 100 modified Sherman-traps (17×7×8 cm) within the Gemenc area which is a forest covered floodplain near the Danube River, in Southern Hungary (Figure 9.). On this study site the sample was started by my colleges from the Department of Parasitology and Zoology, UVM, Budapest; I joined to this process in 2012. The total number of trap nights (the sum of the total number of nights each trap was used) was 2200. Traps were set at sunset and checked early the following morning. The species and sex of trapped rodents was identified (Aulagnier et al., 2009) and animals belonging to protected species were then released. All the other rodents were euthanized. The carcasses were checked for ticks and other ectoparasites and samples from spleen and skin were collected. The spleen and skin samples in this study did not originate from the same individuals. During the trapping in May 2012, ticks were collected with flagging from the vegetation in several different locations within the Gemenc area. Ectoparasites were stored in 70% ethanol, and were later identified using standard identification keys (Hillyard, 1996; Nosek and Sixl, 1972; Rosický, 1957; Szabó, 1975).

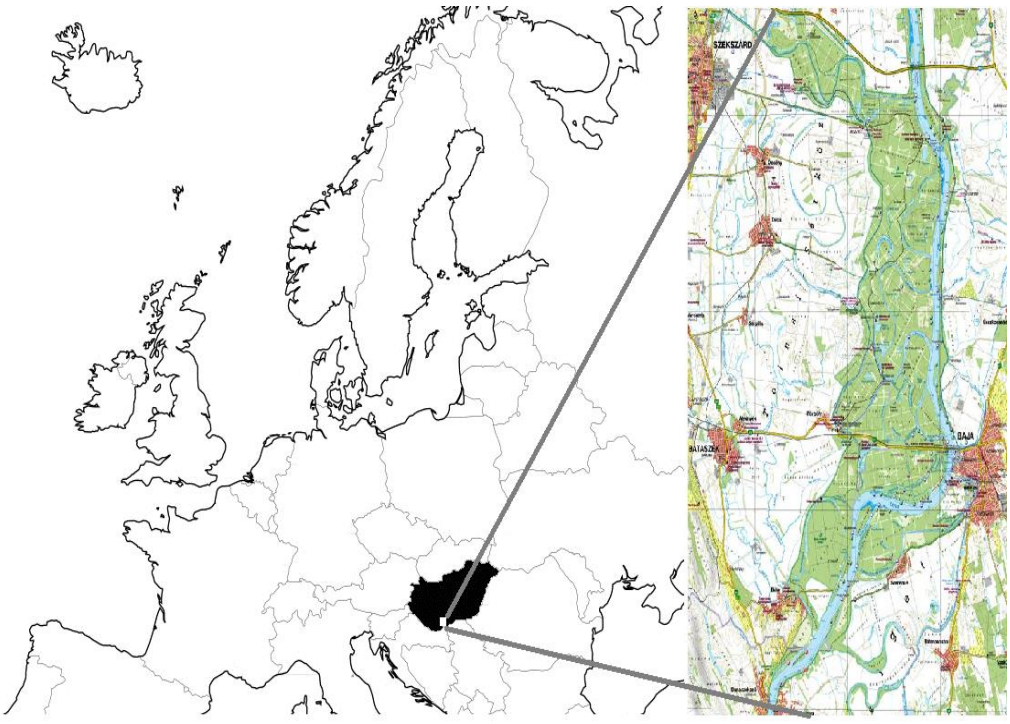

Figure 9.: Location of the natural study site in Southern Hungary (Gemenc).

4.1.2. Urban habitat

Questing ticks were collected with flagging in 2011–2012 on Margaret-Island (Budapest). The collection was done by my supervisor and his former PhD student an I joined the systematic flagging in 2012. Ear tissue samples were obtained from hedgehogs anesthetized with intramuscular ketamine (5 mg/kg) and dexmedetomidine (50 µg/kg) in 2011.

Between April and August of 2015 we collected road-hit hedgehogs with the help of volunteers mainly from Budapest and some other locations around Hungary (Figure 10). In addition, we also collected some animals died for other reasons (e.g. caught by cats). We collected samples from all the possible identifiable tissues (minimum: skin, maximum: five different tissues). The species, date of collection, location and the degree of degradation were recorded. Before dissection, we collected all the ectoparasites and stored in 70% ethanol at 4°C until the molecular analysis. The ticks were identified using standard identification keys (Hillyard, 1996; Nosek and Sixl, 1972). The carcasses and the collected tissue samples were stored at -20°C.

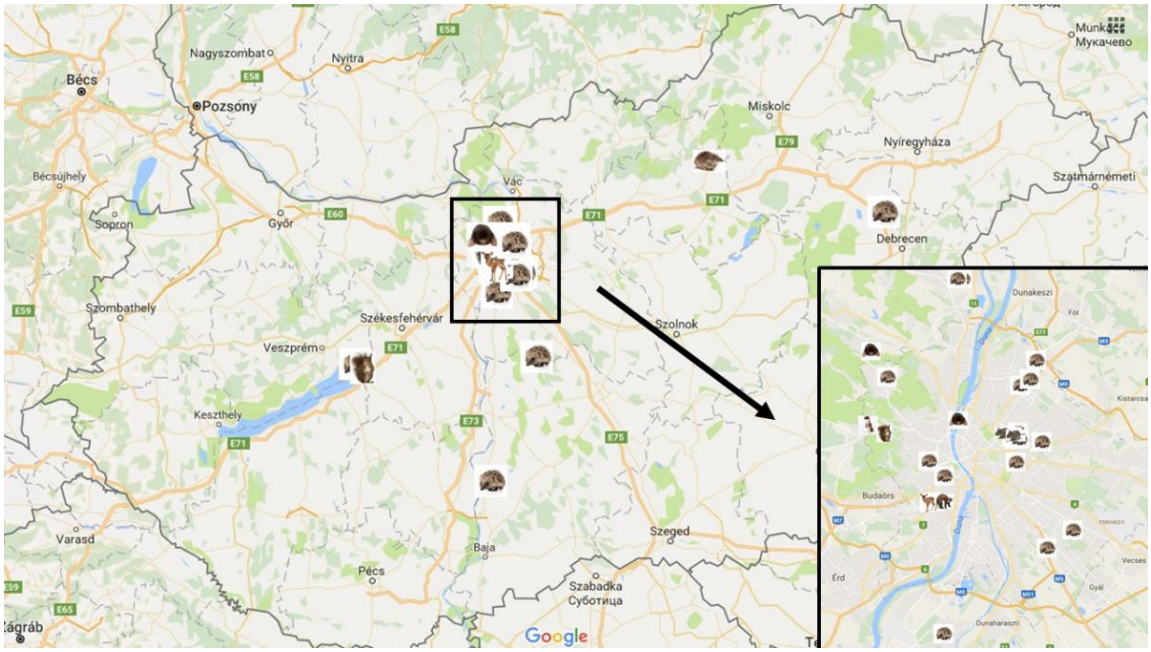

Figure 10.: Locations of the studied road-killed urban mammals in Hungary.
An online version of the map is available at: https://goo.gl/9eeZm7

4.2. Molecular methods

4.2.1. DNA extraction from ticks and tissue samples

The different extraction methods previously were compared with DNA concentration measurement, test conventional PCR and sequencing as well. The DNA concentration were checked after extraction every.

Tick samples

DNA was extracted from ticks by alkaline hydrolysis (Guy and Stanek, 1991) from both habitats. The cleaned ticks were boiled in NH_4OH for 30 minutes with closed lid and 30 minutes with opened lid. Pool samples were prepared from each 10 larvae removed from the same host. Adult ticks were processed individually from both habitats. All nymphs collected from the natural habitat were examined individually, but the nymphs removed from urban road-hit or accidentally died animals were pooled by 5 and nymphs from the same host in this study.

Tissue samples

DNA was isolated from tissue samples of the natural habitat with a modified Miniprep Express Matrix protocol (MP Biomedicals, Santa Ana, USA). DNA was extracted from the hedgehog ear samples by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) or the Miniprep Express Matrix protocol (MP Biomedicals, Santa Ana, USA). We used ISOLATE II Genomic DNA Kit (Bioline Reagents Ltd, London, UK) to isolate the nucleic acid from the urban road-killed tissue samples.

Sample storage

We stored extracted DNA in 1.5 ml, 2 ml microcentrifuge tube or 2ml screwcapped and rubberband sealed microtube at −20°C in the freezer for further analyses.

4.2.2. PCR analysis

By the analysis of qPCR results we selected the positive samples by two criteria, the shape of curves (compared to positive controls) and CT (threshold cycle) values. After the conventional PCR, all the samples were visualized with UV light and ethidium-bromide stained agarose gel. All used primer and probe sequences are presented in the Table 5. In the PCR assay we used negative controls to verify and exclude any contaminations.

4.2.2.1. *Borrelia burgdorferi* s.l. real-time and conventional PCR

To determine whether samples contained *B. burgdorferi* s.l. we used a qPCR targeting a part of the flagellin B (flaB) gene. For *B. burgdorferi* s.l. we used forward primer B-FlaB-F and reverse primers B-FlaB-Rc and B- FlaB-Rt, with the probe B-FlaB-P (Heylen et al., 2013). Samples were considered positive with CT values below 41 cycles for *B. burgdorferi* s.l. All qPCR-positive samples were examined by conventional PCR and sequencing. We amplified the intergenic spacer region (IGS) of *B. burgdorferi* s.l. with forward primer B5Sborseq and reverse primer B23Sborseq (Hansford et al., 2015).

4.2.2.2. *Borrelia miyamotoi* real-time and conventional PCR

For *B. miyamotoi* we used forward primer FlabBm.motoiF reverse primer FlabB.m.motoiR, with the probe labBm.motoiPro (Hovius et al., 2013). Samples were considered positive with CT values below 38 cycles for *B. miyamotoi*. All qPCR-positive samples were examined by conventional PCR and sequencing. We targeted the glycerophosphodiester phosphodiesterase gene (glpQ) of *B. miyamotoi* with forward primer glpQ-BM-F2 and reverse primer glpQ-BM-R1 (Hovius et al., 2013).

4.2.2.3. *Anaplasma phagocytophilum* real-time and conventional PCR

For *A. phagocytophilum*, we targeted the major surface protein 2 gene with the forward primer apMSP2F, reverse primer apMSP2R and probe apMSP2P (Courtney et al., 2004), resulting in a 77 bp long product. Conventional PCRs were used to amplify the GroEL gene of *A. phagocytophilum* with forward primer EphplgroEL(569)F and reverse primer EphgroEL(1142)R (Alberti et al., 2005).

4.2.2.4. *Can*. Neoehrlichia mikurensis real-time and conventional PCR

For *Candidatus* N. mikurensis we targeted GroEL heat shock protein gene, the product length was 102 bp, with forward primer groEL-F2a. We used two reverse primers groEL-R2a and groEL-R2, with the probe groEL-P2a (Jahfari et al., 2012).

4.2.2.5. *Rickettsia* sp. real-time and conventional PCR

GltA (citrate synthase) gene of *Rickettsia* spp. was targeted with forward primer CS-F, reverse primer CS-R and the probe CS-P (Stenos et al., 2005). We used conventional PCR according to Choi et al. 2005 to amplify a part of the outer surface protein B (ompB) gene with forward primer rompB OF and reverse primer rompB OR (Choi et al., 2005).

4.2.2.6. *Rickettsia helvetica* real-time PCR

To investigate the presence of *R. helvetica* we used a species specific qPRC with forward primer Rick_HelvgltA_F2, reverse primer Rick_HelvgltA_R2 and probe Rick_HelvgltA_pr3 targeting the gltA gene (de Bruin et al., 2015).

4.2.2.7. *Hepatozoon* sp. conventional PCR

To determine which samples contained *Hepatozoon* DNA first, forward and reverse primer RLB-F and RLB-R were used targeting an ~500 bps length fragment of the V4 region of the 18S rRNA gene (Gubbels et al., 1999). The positive samples was also tested for the presence of the complete 18S rRNA gene with a second pair of primers (CRYPTO F and CRYPTO R) (Herwaldt et al., 2003).

4.2.2.8. *Bartonella* sp. conventional PCR

For detection of *Bartonella* spp. a conventional PCR assay was used, which targets a part of the citrate synthase gene (gltA) with forward and reverse primer BhCS.781p and BhCS.1137n (de Bruin et al., 2015; De Sousa et al., 2006; Norman et al., 1995).

4.2.3. Statistical and phylogenetical analysis

For statistical analysis, R (The R Development Core Team, 2010) and Quantitative Parasitology 3.0 (Rózsa et al., 2000) statistical programs were used. Results with p-values under 0.05 were considered significant.

4.2.4. Sequence analysis

All samples that were positive by conventional PCR have been submitted to sequencing.

The phylogenetic tree was created using selected complete (and near complete) 18S rDNA *Hepatozoon* sequences originating from different mammals. The multiple sequence alignment was generated using MUSCLE (Edgar, 2004). Conserved blocks from the alignment were selected with Gblocks (Castresana, 2000). The phylogenetic tree was created using a maximum likelihood approach with PhyML (Guindon et al., 2010). The Hasegawa-Kishino-Yano 85 (HKY85) nucleotide substitution model was selected for the analysis. Branch support was calculated by running 500 non-parametric bootstrap steps.

Table 5.: Sequences of the primers used in the real-time and conventional PCR

Pathogen		Primer	Sequence
***B. burgdorferi* s.l.**	real-time	B-FlaB-F	CAGAIAGAGGTTCTATACAITTGAIATAGA
		B-FlaB-Rc	GTGCATTTG GTTAIATTGCGC
	probe	B-FlaB-P	CAACTIACAGAIGAAAXTAAIAGAATTGCTGAICA
	conventional	B5Sborseq	GAGTTCGCGGGAGAGTAGGTTATTGCC
		B23Sborseq	TCAGGGTACTTAGATGGTTCACTTCC
B. miyamotoi	real-time	FlabBm.motoiF	AGAAGGTGCTCAAGCAG
		FlabB.m.motoiR	TCGATCTTTGAAAGTGACATAT
	probe	FlabBm.motoiPro	AGCACAACAGGAGGGAGTTCAAGC
	conventional	glpQ-BM-F2	ATGGGTTCAAACAAAAAGTCACC
		glpQ-BM-R1	CCAGGGTCCAATTCCATCAGAATATTGTGCAAC
A. phagocyto-philum	real-time	apMSP2F	ATGGAAGGTAGTGTTGGTTATGGTATT
		apMSP2R	TTGGTCTTGAAGCGCTCGTA
	probe	apMSP2P	TGGTGCCAGGGTGAGCTTGAGATTG
	conventional	EphplgroEL(569)F	ATGGTATGCAGTTTGATCGC
		EphgroEL(1142)R	TTG AGTACAGCAACACCACCGGAA
***Can.* N. mikurensis**	real-time	groEL-F2a	CCTTGAAAATATAGCAAGATCAGGTAG
		groEL-R2a	CCACCACGTAACTTATTTAGCACTAAAG
		groEL-R2b	CCACCACGTAACTTATTTAGTACTAAAG
	probe	groEL-P2a	CCTCTACTAATTATTGCTGAAGATGTAGAAGGTG AAGC
R. helvetica	real-time	Rick_HelvgltA_F2	ATGATCCGTTTAGGTTAATAGGCTTCGGTC
		Rick_HelvgltA_R2	TTGTAAGAGCGGATTGTTTTCTAGCTGTC
	probe	Rick_HelvgltA_pr3	CGATC+C+ACG+TG+CCGCAGT-X-3'
Rickettsia spp.	real-time	CS-F	TCGCAAATGTTCACGGTACTTT
		CS-R	TCGTGCATTTCTTTCCATTGTG
	probe	CS-P	TGCAATAGCAAGAACCGTAGGCTGGATG
	conventional	rompB OF	GTAACCGGAAGTAATCGTTTCGTAA
		rompB OR	GCTTTATAACCAGCTAAACCACC
***Hepatozoon* spp.**	conventional	RLB-F	GAGGTAGTGACAAGAAATAACAATA
		RLB-R	TCTTCGATCCCCTAACTTTC
		CRYPTO F	AACCTGGTTGATCCTGCCAGT
		CRYPTO R	GCTTGATCCTTCTGCAG-GTTCACCTAC
***Bartonella* spp.**	conventional	BhCS.781p	GGGGACCAGCTCATGGTGG
		BhCS.1137n	AATGCAAAAAGAACAGTAAACA

X= black hole quencher
+ = LNA

5. Results

5.1. Rodents end ectoparasites collected at the natural habitat

We trapped altogether 525 rodents in the study sites. Tissue samples of six species were analysed: *A. flavicollis* (yellow-necked filed mouse; skin: 102, spleen: 67), *A. agrarius* (striped filed mouse; skin: 202, spleen: 92), *Myodes glareolus* (bank vole; skin: 29, spleen: 11), *Microtus arvalis* (common vole; skin: 7, spleen: 4), *Micromys minutus* (harvest mouse; skin: 3), *Mus musculus* (house mouse; skin: 5, spleen: 3) (Table 6.).

Table 6.: Removed ticks from small mammals in the natural habitat and *Can*. Neoerlichia mikurensis and *Anaplasma phagocytophilum* prevalence with qPCR in skin and spleen samples

Rodent species	**Tick species**				***Can.* N. mikurensis**		***A. phagocytophilum***	
					(+/tested/%)			
	I. ricinus	*I. acu-minatus*	*D. mar-ginatus*	*H. concinna*	skin	spleen	skin	spleen
A. flavicollis	34	54	46	15	3/102/2.9	3/67/4.5	14/102/13.7	3/67/4.5
A. agrarius	2	2	11	-	3/202/1.5	3/92/3.3	8/202/4	2/92/2.2
My. glareolus	4	-	5	-	0 /29/-	0 /11/-	1/29/3.5	2/11/18.2
Mi. arvalis	1	-	4	3	0 /7/-	0 /4/-	0 /7/-	1/4/25
M. minutus	-	-	-	-	0 /3/-	-	0 /3/-	-
Mu. musculus	-	-	-	-	0 /5/-	0 /3/-	0 /5/-	1/3/33.3
sum	**41**	**56**	**66**	**18**	**6/348/2.3**	**6/177/3.4**	**23/348/7.2**	**8/177/4.5**

Altogether 343 ticks belonging to five species were found with flagging (n=162) and on rodents (n = 181). *Haemaphysalis concinna* and *I. ricinus* occurred on both the rodents and the vegetation. Endophilic *I. acuminatus* ticks were found only on rodents. Adult *D. reticulatus* and *D. marginatus* were collected only from the vegetation (Table 6. And 7.) (Szekeres et al., 2015a).

One hundred and thirty-one fleas belonging to three different species (*Ctenophthalmus agyrtes, Ctenophthalmus assimilis* and *Megabothris turbidus*) were collected from 81 small mammals (Table 17) (Rigó et al., 2016).

Table 7.: Number of collected ticks in the natural habitat from small mammals and vegetation.

Species	ticks from rodents	questing ticks
	larva/nymph/female/male	
I. ricinus	36/5/0/0	0 /21/5/8
I. acuminatus	52/1/3/0	0 /0/0/0
H. concinna	15/3/0/0	33/10/11/8
D. reticulatus	0/0/0/0	0/0/41/23
D. marginatus	61/5/0/0	0/0/2/0
sum	**181**	**162**

5.2. Small and medium size mammals and ectoparasites collected at the urban habitat

From the Margaret Island 88 Northern white-breasted hedgehogs were caught and ear biopsy was taken under veterinary supervision and anaesthesia.(Földvári et al., 2014)

Twenty-three road-killed hedgehogs (*E. roumanicus*) and twelve other collected mammals from seven different species (e.g. European red squirrel and European mole) were included into the study. We collected carcasses of accidentally killed animals (struck and killed by motor vehicles on highways or e.g. killed by cat) from urbanised habitats, mainly from Budapest, Hungary (Figure 10).

From the carcasses, we collected 90 tissue samples for molecular analysis (52 from hedgehogs and 38 from the other species) (Table 20.). The degree of degradation of the carcasses was different; some specimens were in “perfect” condition with no sign of degradation (degree of degradation 1) and some were dry and heavily damaged by vehicles (degree of degradation 5). The explanation of these categories is in the legend of the Table 21.

From the 417 removed ticks (363 *I. ricinus* and 53 *Ixodes hexagonus*) 124 samples were created (111 *I. ricinus* and 13 *I. hexagonus*) using adults individually, nymphs pooled by five and larvae pooled by 10 per host. All the removed ticks were from nine hedgehogs. The maximum number of ticks/host was 219 and were removed from the same hedgehog (code: H4) (Table 8.).

Table 8.: Number of removed *Ixodes ricinus* and *Ixodes hexagonus* ticks from road-killed or accidentally killed urban hedgehogs (*E. roumanicus*) in Hungary.

Host code	*Ixodes ricinus*				*Ixodes hexagonus*				Tick/Host
	larva	nymph	female	male	larva	nymph	female	male	
H1	-	1	-	-	-	-	-	-	1
H4	35	156	11	5	7	5	-	-	219
H7	-	87	10	4	-	-	-	-	101
H9	-	-	1	1	-	-	-	-	2
H11	2	4	2	3	-	-	-	-	11
H12	-	10	1	1	-	-	-	-	12
H15	-	5	4	-	-	-	-	-	9
H16	-	15	1	1	-	41	2	-	60
H21	-	-	1	1	-	-	-	-	2
Sum	**37**	**278**	**31**	**16**	**7**	**46**	**2**	**-**	**417**

5.3. Pathogens in the natural habitats

5.3.1. *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi*

The prevalence of *B. burdorferi* s.l. in rodent tissue samples was 6.6% in skins and 2.3% in spleens. *Borrelia miyamotoi* was found in 0.3% of skin and 0.5% of spleen samples removed from the captured small mammals (Table 9). *Borrelia burgdorferi* s.l. was found in *A. flavicollis*, *Apodemus agrarius* and *My. glareolus* samples. *Borrelia miyamotoi* was detected in two *A. flavicollis* males.

Table 9.: Occurrence of *B. miyamotoi* and *B. burgdorferi* s.l. in rodent tissue samples from Southern Hungary

Rodent species	*B. miyamotoi*		*B. burgdorferi s.l.*	
	(+/tested/prevalence)			
	skin	spleen	skin	spleen
A. flavicollis	1/102/0.9%	1/67/1.5%	6/102/5.8%	3/67/4.5%
A. agrarius	0/202/-	0/92/-	16/202/7.9%	1/92/1%
My. glareolus	0 /29/-	0 /11/-	1/29/3.5%	0/11/-
Mi. arvalis	0 /7/-	0 /4/-	0 /7/-	0/4/-
M. minutus	0 /3/-	-	0 /3/-	-
Mu. musculus	0 /5/-	0 /3/-	0 /5/-	0/3/-
Sum	**1/348/0.3%**	**1/177/0.5%**	**23/348/6.6%**	**4/177/2.3%**

In the tested questing *Ixodes ricinus* ticks (21 nymphs and 13 adults). *Borrelia burgdorferi* s.l. was detected in three nymphs and five adults and *B. miyamotoi* was detected in one nymph (Table 10). In the four tick species removed from rodents, *B. miyamotoi* was detected in engorged *I. ricinus* larvae and *B. burgdorferi* s.l. was detected in engorged *I. ricinus* larvae and a nymph, *I. acuminatus* larvae and a nymph, and *D. marginatus* larvae (Table 11).

Table 10.: Prevalence of *B. miyamotoi* and *B. burgdorferi* s.l. in questing ticks collected in the natural habitat

Tick species	***B. miyamotoi***	***B. burgdorferi* s.l.**
	(+/tested/prevalence)	
I. ricinus	1/34/2.9%	8/34/23.5%
D. reticulatus	0/64/-	0/64/-
D. marginatus	0/2/-	0/2/-
H. concinna	0/62/-	0/62/-
Sum	**1/162/0.6%**	**8/162/4.9%**

Table 11.: Minimum prevalence of *B. miyamotoi* and *B. burgdorferi* s.l. in engorged ticks from rodents in the natural habitat

Tick species	***B. miyamotoi***	***B. burgdorferi* s.l.**
	(+/tested/minimum prevalence)	
I. ricinus	2/41/4.9%	4/41/9.7%
I. acuminatus	0/56/-	5/56/8.9%
D. marginatus	0/66/-	3/66/4.5%
H. concinna	0/18/-	0/18/-
Sum	**2/181/1.1%**	**12/181/6.6%**

The two *B. miyamotoi* positive *I. ricinus* larva pools originated from two *A. flavicollis* males with unknown infectious status. Developmental stage and host infectious status for sequenced *B. burgdorferi* positive *I. ricinus* samples are shown in Table 12.. Two *I. acuminatus* larva pools originated from *A. flavicollis* hosts with unknown infectious status and one larva pool and one nymph were removed from uninfected *A. flavicollis* hosts. In the ticks removed from rodents, DNA amplification of both pathogens was successful from *I. ricinus* larvae (*B. burgdorferi* s.l. 11.1 %, *B. miyamotoi* 5.6 %) while from 2 *Ixodes acuminatus* larvae (7.7 %), and the single tested nymph only *B. burgdorferi* s.l. DNA was amplified. There was no significant difference in *B. burgdorferi* s.l. minimum infection prevalence between *I. ricinus* and *I. acuminatus* larvae

(p>0.05). Three *D. marginatus* larva samples (two pools and one single; 4.5% minimum infection prevalence) removed from two uninfected *A. flavicollis* and an uninfected *A. agrarius* were also *B. burgdorferi* s.l. positive.

Sequencing was successful for 18 *B. burgdorferi* s.l. positive samples: one *B. lusitaniae* was found in a questing *I. ricinus* nymph and altogether 17 *B. afzelii* were identified in questing *I. ricinus* nymphs and adults, in engorged *I. ricinus* larvae and a nymph, engorged *I. acuminatus* larvae and a nymph, and in rodent skin samples. The two *Dermacentor marginatus* engorged larva pools originating from uninfected hosts were also infected with *B. afzelii* (Table 12.). We sequenced *B. miyamotoi* amplicons from one questing *I. ricinus* nymph, one engorged *I. ricinus* larva pool and a skin sample of an *A. flavicollis* (Szekeres et al., 2015b).

Table 12.: Sequenced *B. miyamotoi* and *B. burgdorferi* s.l. samples from the natural habitat

***Borrelia* species**	**Source**	**GenBank accession number**
B. lusitaniae	questing *I. ricinus* nymph	KM657411
B. afzelii	*A. flavicollis* male skin	KM657412
B. afzelii	*A. agrarius* male skin	KM657417
B. afzelii	questing *I. ricinus* nymph	KM657413
B. afzelii	questing *I. ricinus* nymph	KM657418
B. afzelii	questing *I. ricinus* female	KM657421
B. afzelii	questing *I. ricinus* female	KM657423
B. afzelii	questing *I. ricinus* male	KM657414
B. afzelii	questing *I. ricinus* male	KM657415
B. afzelii	engorged *I. ricinus* larva from *A. flavicollis* female	KM657425
B. afzelii	engorged *I. ricinus* pool (4 larvae) from *A. flavicollis* female	KM657426
B. afzelii	engorged *I. ricinus* pool (8 larvae) from *A. flavicollis* male*	KM657416
B. afzelii	engorged *I. ricinus* nymph from *A. flavicollis* male	KM657424
B. afzelii	engorged *I. acuminatus* pool (6 larvae) from *A. flavicollis* male **	KM657427
B. afzelii	engorged *I. acuminatus* pool (10 larvae) from *A. flavicollis* male **	KM657428
B. afzelii	engorged *I. acuminatus* nymph from *A. flavicollis* male***	KM657419
B. afzelii	engorged *D. marginatus* pool (4 larvae) from *A. agrarius* male	KM657422
B. afzelii	engorged *D. marginatus* pool (8 larvae) from *A. flavicollis* male***	KM657420
B. miyamotoi	questing *I. ricinus* nymph	LC006119.1
B. miyamotoi	engorged *I. ricinus* pool (8 larvae) from *A. flavicollis* male*	LC006120.1
B. miyamotoi	*A. flavicollis* female spleen	LC006118.1

*co-infection

** from the same rodent individual

*** from the same rodent individual

5.3.2. *Anaplasma phagocytophilum* and *Can.* Neoehrlichia mikurensis

We found 23 (6.6%) and 9 (5.1%) *A. phagocytophilum* PCR positives in the skin and spleen samples of rodents (Table 13.). The prevalence of *A. phagocytophilum* in skin samples of *A. flavicollis* was significantly higher compared to the *Candidatus* N. mikurensis (Fisher test, p=0.0036). Five (3.1%) questing ticks were PCR-positive, namely one *I. ricinus* male, two *D. reticulatus* females and two *H. concinna* females (Table 14.). One *I. ricinus* nymph removed from a PCR-positive male *A. flavicollis* was infected with *A. phagocytophilum* (Table 10.). CT-values of the 38 *A. phagocytophilum* positive samples varied between 29.14 and 40.86 (average 36.78).

Table 13.: Number of ticks on the different rodent species from the natural habitat and the positivity of the tissue samples for *Can*. N. mikurensis and *A. phagocytophilum*

Rodent species	**Tick species**				***N. mikurensis***		***A. phagocytophilum***	
	I. ricinus	*I. acuminatus*	*D. marginatus*	*H. concinna*	***(+/tested/%)***			
					skin	spleen	skin	spleen
A. flavicollis	34	54	46	15	3/102/2.9	3/67/4.5	14/102/13.7	3/67/4.5
A. agrarius	2	2	11	-	3/202/1.5	3/92/3.3	8/202/4	2/92/2.2
My. glareolus	4	-	5	-	0 /29/-	0 /11/-	1/29/3.5	2/11/18.2
Mi. arvalis	1	-	4	3	0 /7/-	0 /4/-	0 /7/-	1/4/25
M. minutus	-	-	-	-	0 /3/-	-	0 /3/-	-
Mu. musculus	-	-	-	-	0 /5/-	0 /3/-	0 /5/-	1/3/33.3
sum	**41**	**56**	**66**	**18**	**6/348/2.3**	**6/177/3.4**	**23/348/7.2**	**8/177/4.5**

Table 14.: Prevalence of *Can*. N. mikurensis and *A. phagocytophilum* in questing ticks from the natural habitat

Tick species	***N. mikurensis***	***A. phagocytophilum***
	(+/tested/min. prevalence %)	
I. ricinus	3/34/8.8	1/34/2.9
D. reticulatus	0 /64/-	2/64/3.1
D. marginatus	0 /2/-	0 /2/-
H. concinna	0 /62/-	2/62/3.2
sum	**3/162/1.9**	**5/162/3.1**

Table 15.: Prevalence of *Can*. N. mikurensis and *A. phagocytophilum* in engorged ticks from the natural habitat

Tick species	***N. mikurensis***	***A. phagocytophilum***
	(+/tested/min. prevalence %)	
I. ricinus	0 /41/-	1/41/2.4
I. acuminatus	0 /56/-	0 /56/-
D. marginatus	0 /66/-	0 /66/-
H. concinna	0 /18/-	0 /18/-
sum	**0 /181/-**	**1/181/0.6**

Six (1.7%) out of 348 rodent skin samples and six (3.4%) out of 176 spleen samples were positive for *Candidatus* N. mikurensis (Table 13.). Only two (*A. flavicollis* and *A. agrarius*) out of six examined rodent species were infected with *Candidatus* N. mikurensis. Three (8.8%) out of 34 questing *I. ricinus* ticks were infected (Table 14.). The other tick species and the engorged ticks were negative for this pathogen (Table 15.). CT-values of the 15 *Candidatus* N. mikurensis positive samples varied between 25.55 and 40.03 (average 32.22) (Szekeres et al., 2015a).

Anaplasma phagocytophilum and *Can.* Neoehrlichia mikurensis conventional PCR and sequencing was not successful from these samples (data not shown).

5.3.3. Rickettsiae in field collected ticks

Rickettsiae were detected in 57.8 %of *D. reticulatus.* We identified *R. raoultii* infection with sequencing in 31 qPCR-positive *D. reticulatus* samples from the rural habitat (Table 16.) (Szekeres et al., 2016a).

Table 16.: *Rickettsia* infection in questing ticks form the two different study sites in Hungary

Tick species		**Margaret Island**		**Gemenc**	
		R. helvetica	***Rickettsia* spp.**	***R. helvetica***	***Rickettsia* spp.**
		(+/tested/prevalence)			
I. ricinus	female	78/166/44.6%	40/166/24.1%	1/5/20%	1/5/20%
	male	45/214/21%	34/214/15.9%	1/8/12.5%	3/8/37.5%
	nymph	20/150/13.3%	14/150/9.3%	7/21/33.3%	0/21/-
	larva	0/4/-	0/4/-	-	-
***I. ricinus* Sum**		**139/534/26%**	**88/534/16.5%**	**9/34/26.5%**	**4/34/11.8%**
*D. reticulatus**		-	-	0/64/-	37/64/57.8%
*D. marginatus***		-	-	0/2/-	0/2/-
*H. concinna****		-	-	0/62/-	0/62/-
Sum		**139/534/26%**	**88/534/16.5%**	**9/162/5.5%**	**41/162/25.3%**

Gender and stage of the collected ticks: * only females and males, ** only females, *** all stages presented

5.3.4. *Hepatozoon* sp. in tissue samples and ectoparasites

From 528 trapped small mammals in the early stage of the study right after the dissection spleen smear samples were made. During the examination of spleen smears with light microscopy, ellipsoidal-shaped intra- and extraerythrocytic stages (gamonts) of *Hepatozoon* parasites were observed (by Gábor Majoros) from eight of the 36 trapped bank voles (*M. glareolus*) (Figure 11.). These were also found positive with apicomplexan-specific primers. All spleen samples from other small mammal species were found negative both with morphological and molecular methods.

Thirteen fleas (including all three species) were found to be infected with *Hepatozoon* spp. (Table 17.) but none of the tick samples (data not shown). Prevalence was as follows: *C. agyrtes*, 8.97 %, *C. assimilis*, 30 % and *M. turbidus*: 9.3 %.The most similar sequences in the NCBI GenBank only showed 95–96 % similarity to our sequenced amplicons created with primers RLB-F and RLB-R. Amplicons of the whole 18S rDNA reaction (accession numbers: JX644996, JX644997, JX644998) proved to be very similar to *Hepatozoon* sp. detected in *Myodes glareolus* in Spain (accession numbers: AY600625.1, AY600626.1) (Criado-Fornelio et al., 2006) and Poland (accession numbers: KF418366 and KF418367) (Bajer et al., 2014) and also to the sequence of a *Hepatozoon ayorgbor* sample collected from *Python regius* snakes imported from Ghana (EF157822.1) (Sloboda et al., 2007).Unfortunately, 18S rDNA sequencing was not successful for any of the PCR-positive flea samples. Therefore, in this case, partial 18S sequences sequenced using primers RLB-F and RLB-R have been submitted to the NCBI GenBank (accession numbers: KJ634066 and KJ608372). These partial sequences were almost identical with the corresponding regions of the whole 18S sequences from tissue samples. Based on gamont morphology and 18S rDNA sequences (Figure 12.), the bank vole as the exclusive host and fleas (and not ticks) as probable vectors, we identified the parasite as *Hepatozoon erhardovae* (Rigó et al., 2016).

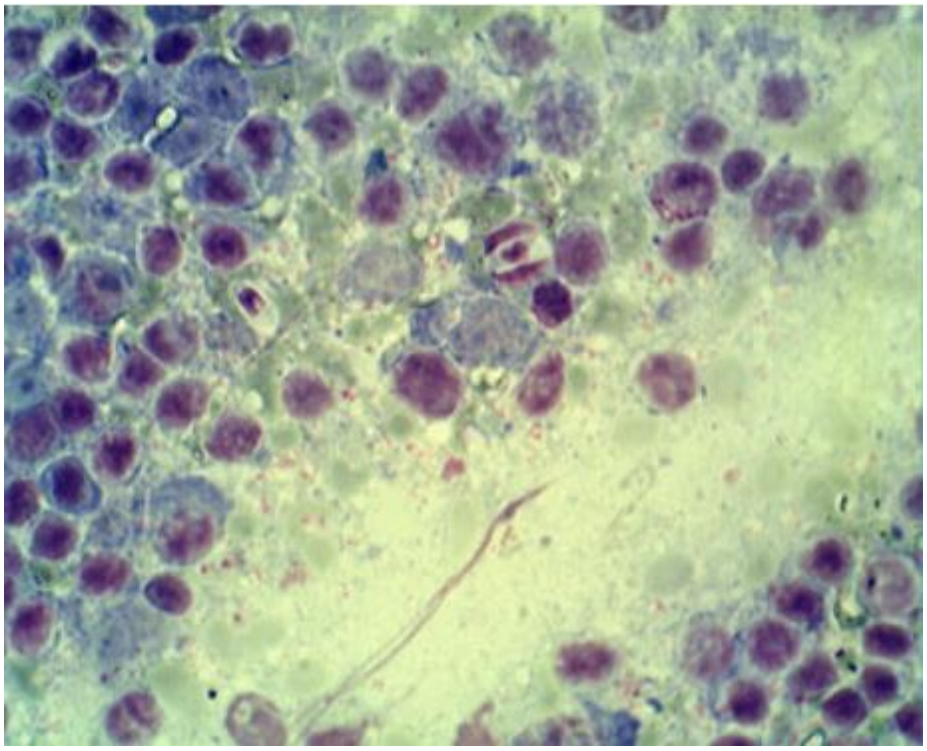

Figure 11.: Ellipsoidal-shaped intra- and extraerythrocytic stages (gamonts) in a Giemsa-stained spleen impression of a bank vole

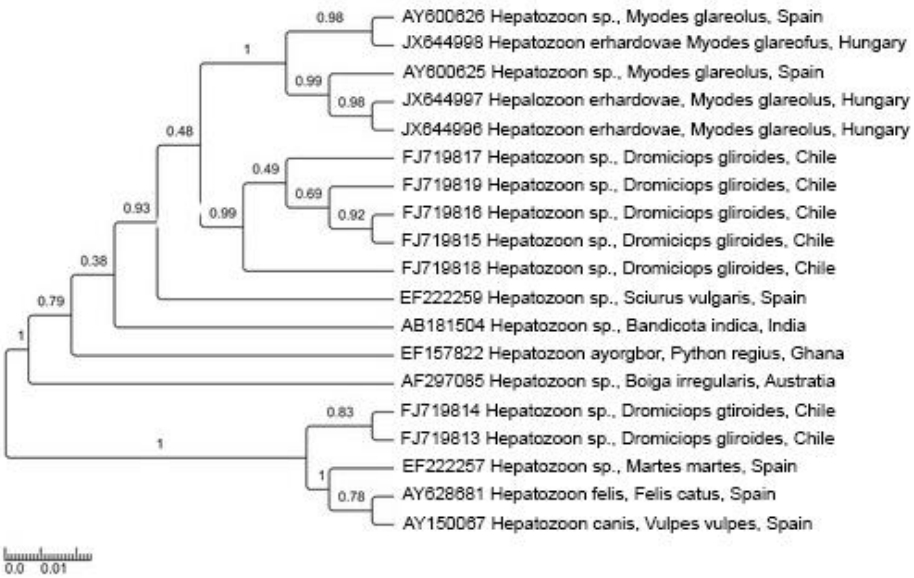

Figure 12.: Phylogenetic tree of selected (near) complete 18S rDNA sequences. Note the similarity between samples originating from geographically and/or taxonomically very distant hosts (Rigó et al. 2016)

Table 17.: Number of collected and tested fleas with Apicomplexan PCR from small mammals at the natural habitat, Hungary (2010-2013).

Species	**Infected flea individuals**	***Ctenophthalmus agyrtes***	***Ctenophthalmus assimilis***	***Megabothris turbidus***	**Total per host species**
		positive/tested **(prevalence)**			
Apodemus flavicollis	30	3/24 **(13%)**	1/4 **(25%)**	1/19 **(5%)**	5/47 **(11%)**
Apodemus agrarius	41	3/46 **(7%)**	1/2 **(50%)**	2/14 **(14%)**	6/62 **(10%)**
Myodes glareolus	8	0/5	0/0	1/10 **(10%)**	1/15 **(7%)**
Microtus arvalis	2	1/3 **(33%)**	1/4 **(25%)**	0/0	2/7 **(29%)**
Total	**81**	**7/78 (9%)**	**3/10 (30%)**	**4/43 (9%)**	**13/131 (10%)**

5.4. Pathogens in urban habitats

5.4.1. *Anaplasma phagocytophilum* and *Can*. Neoehrlichia mikurensis in urban hedgehogs

We detected *A. phagocytophilum* in 67 (76.1%) and *Candidatus* N. mikurensis in 2 (2.3%) of 88 ear tissue samples from urban hedgehogs collected on the Margaret Island (Földvári et al., 2014).

5.4.2. Pathogens in ticks removed from road-hit and accidentally died mammals

Ticks were only found on hedgehog carcasses. *Borrelia miyamotoi* and *Can*. N. mikurensis DNA were not detected in tick samples. *Borrelia burgdorferi* s.l. was detected in 16% (minimum prevalence) of ticks; all stages of *I. ricinus* (female: 32%, male: 38%, nymph minimum prevalence: 17%, larva minimum prevalence: 6%) and *I. hexagonus* nymphs (minimum prevalence: 2%). *Anaplasma phagocytophilum* was presented in all stages of both tick species. *Rickettsia helvetica* was found in *I. ricinus* females, males, nymphs and larvae and in *I. hexagonus* nymphs with an overall *R. helvetica* prevalence of 20.5% (minimum prevalence) in ticks. *Rickettsia* sp. was found in all stages of *I. ricinus* and *I. hexagonus* nymphs. Pathogen prevalence in ticks is presented in the Table 18.

Anaplasma phagocytophilum prevalence was significantly lower (Fisher-test, $p<0.00001$) in natural rodents of the present study compared to urban hedgehogs

5.4.3. Rickettsiae in field collected ticks

From the urban habitat 22 *R. monacensis* and 9 *R. helvetica* out of 534 questing *I. ricinus* were identified with the less sensitive conventional PCR and sequencing (Szekeres et al., 2016a).

Table 18.: Prevalence of vector-borne pathogens in ticks removed from hedgehogs with real-time PCR

Tick species and stage		***B. burgdorferi* s.l.**	***B. miyamotoi***	***A. phagocytophilum***	***Can.* N. mikurensis**	***R. helvetica***	***Rickettsia sp.***
		positive/tested/prevalence (%)					
Ixodes ricinus	female	10/31/**32.3 %**	0/31/**0 %**	23/31/**74.2 %**	0/31/**0 %**	19/31/**61.3 %**	26/31/**83.9 %**
	male	6/16/**37.5 %**	0/16/**0 %**	8/16/**50 %**	0/16/**0 %**	7/16/**43.7 %**	10/16/**62.5 %**
	nymph*	34/59/**17 %**	0/59/**0 %**	54/59/**44.6 %**	0/59/**0 %**	34/59/**17%**	44/59/**26.3 %**
	larva*	2/5/**6.4 %**	0/5/**0 %**	5/5/**100 %**	0/5/**0 %**	3/5/**10.4 %**	3/5/**10.4 %**
I. hexagonus	female	0/2/**0 %**	0/2/**0 %**	2/2/**100 %**	0/2/**0 %**	0/2/**0 %**	0/2/**0 %**
	nymph*	1/10/**2.3 %**	0/10/**0 %**	9/10/**53.5 %**	0/10/**0 %**	3/10/**7.2 %**	6/10/**19 %**
	larva*	0/1/**0 %**	0/1/**0 %**	1/1/**96.2 %**	0/1/**0 %**	0/1/**0 %**	0/1/**0 %**
Sum*		51/124/**16 %**	0/124/**0 %**	101/124/**55.6 %**	0/124/**0 %**	66/124/**20.5 %**	89/124/**32.6 %**

*Minimum prevalence (nymphs pooled by 5, larvae by 10 from the same host)

5.4.4. Pathogens in road-hit and accidentally died mammals

All examined pathogens occurred in the collected road-killed mammal tissue samples except *Can*. N. mikurensis. *Borrelia burgdorferi* s.l. was detected in the muscle and skin of hedgehogs (11.5%) and skin sample from a squirrel. *Borrelia miyamotoi* was only detected in squirrel spleen sample. *Anaplasma phagocytophilum* was found in muscle, skin, liver, spleen and coagulated blood from hedgehogs (52%); in a roe deer skin and a lesser shrew muscle sample. *Rickettsia helvetica* DNA was amplified in skin, spleen and muscle from hedgehogs (29%); skin of a house mouse and muscle of a stone marten. We found *Rickettsia* sp. positive skin, spleen, liver, muscle and coagulated blood of hedgehogs (37%); skin of a house mouse and a mole and a muscle sample of a lesser weasel. *Bartonella* species were detected in all tissue sample types of moles, skin samples of hedgehogs, muscle of a house mouse and coagulated blood and muscle of a lesser weasel.

Detection of ectoparasite-borne pathogens with real-time and conventional PCR in tissue samples according to the degree of degradation of the samples presented in the Table 19. All additional data to the positive samples from the small mammals are shown in the Table 21.

5.4.5. Pathogen identification in the road hit samples

The qPCR-positive samples were also amplified with specific conventional PCR assays and were sequenced if the qPCR was not species specific or it is needed to separate different subgroups within one species (ecotypes in case of *A. phagocytophilum*). *Borrelia afzelii* was found in one *I. ricinus* female, one male and seven nymph pools. *Anaplasma phagocytophilum* ecotype I was found in two females and five *I. ricinus* nymph pools and one *I. hexagonus* female. *Rickettsia monacensis* occurred in four *I. ricinus* nymph and a larva pool. *Borrelia afzelii* and *B. spielmanii* was found only in hedgehog skin and muscle. *Anaplasma phagocytophilum* was detected in liver, skin and spleen samples from hedgehogs. *Bartonella* species DNA was amplified in *E. roumanicus* (muscle, skin), house mouse (muscle), mole (coagulated blood, liver, muscle, skin, spleen) and lesser weasel (coagulated blood, spleen) tissues.

These samples were sequenced; and aligned using GenBank BLAST. The identified pathogens are shown in Table 22. The highest similarity in the case of *Bartonella* sequences was found with a *Bartonella taylorii* (98%) sequence from a plateau pika (*Ochotona curzoniae*; Tibetian plateau, China; accession numbers: KT445922; KT445921; KT445919) (Rao et al., 2015) and 97 % with a *Bartonella* sp. sequence from Yunnan red-backed vole (*Eothenomys miletus*; Yunnan, China; accession number: AF391281) (Ying et al., 2002).
All identified pathogen and accession numbers are shown in Table 22.

Table 19.: Prevalence of vector-borne pathogens in road-killed small and medium size mammal tissue samples with real-time PCR.

Mammal/Pathogen species	***B. burgdorferi* s.l.**	***B. miyamotoi***	***A. phagocyto-philum***	***Can.* N. mikurensis**	***R. helvetica***	***Rickettsia sp.***	***Bartonella* sp.**
	positive/tested/prevalence (%)						
Northern white-breasted hedgehog (*Erinaceus. roumanicus*)	6/52/**11.5 %**	0/52/**0 %**	27/52/**52 %**	0/52/**0 %**	15/52/**28.8 %**	19/52/**36.5 %**	4/52/**7.7 %**
European mole (*Talpa europea*)	0/15/**0 %**	0/15/**0 %**	0/15/**0 %**	0/15/**0 %**	0/15/**0 %**	1/15/**6.6 %**	9/15/**60 %**
House mouse (*Mus musculus*)	0/5/**0 %**	0/5/**0 %**	0/5/**0 %**	0/5/**0 %**	1/5/**20 %**	1/5/**20 %**	1/5/**20 %**
European red squirrel (*Sciurus vulgaris*)	1/6/**17 %**	1/6/**17 %**	0/6/**0 %**	0/6/**0 %**	0/6/**0 %**	0/6/**0 %**	0/6/**0 %**
Roe deer (*Capreolus capreolus*)	0/3/**0 %**	0/3/**0 %**	1/3/**33 %**	0/3/**0 %**	0/3/**0 %**	0/3/**0 %**	0/3/**0 %**
Lesser shrew (*Crocidura suaveolens*)	0/3/**0 %**	0/3/**0 %**	1/3/**33 %**	0/3/**0 %**	0/3/**0 %**	0/3/**0 %**	0/3/**0 %**
Stone marten (*Martes foina*)	0/2/**0 %**	0/2/**0 %**	0/2/**0 %**	0/2/**0 %**	1/2/**50 %**	0/2/**0 %**	0/2/**0 %**
Lesser weasle (*Mustela nivalis*)	0/4/**0 %**	0/4/**0 %**	0/4/**0 %**	0/4/**0 %**	0/4/**0 %**	1/4/**25 %**	2/4/**50 %**
Sum	7/90/**7.7%**	1/90/**1.1 %**	29/90/**32.2 %**	0/90/**0 %**	17/90/**18.8 %**	22/90/**24.4 %**	16/90/**17.8 %**

Table 20.: Specific data about location, degradation rate, sample type and real-time PCR positivity of vector-borne pathogens in tissue samples of road-killed small and medium size mammal with real-time and conventional PCR. (Explanation of degradation grades are presented in the end of this table)

Host ID (species)	Location	Degradation rate (1-5)	Sample type	*A. phagocytophilum*	*B. burgdorferi* s.l.	*B. miyamotoi*	*R. helvetica*	*Rickettsia* sp.	*Bartonella* sp.
H1 (*Erinaceus roumanicus*)	Szentendre, Pest County	4	heart	-	-	-	-	-	-
			liver	-	-	-	-	-	-
			skin	+	-	-	-	-	-
H2 (*E. roumanicus*)	Budapest, XV. district	2	liver	+	-	-	-	+	+
			skin	-	-	-	+	+	-
H3 (*E. roumanicus*)	Budapest, II. district	5	muscle	-	-	-	-	-	-
			skin	-	-	-	-	-	-
H4 (*E. roumanicus*)	Budapest XIV. district	1	muscle	+	+	-	+	+	-
			skin	+	-	-	+	+	-
			spleen	+	-	-	-	-	-
H5 (*E. roumanicus*)	Budapest X. district	3	muscle	+	-	-	+	+	-
			skin	+	-	-	+	+	-
H6 (*E. roumanicus*)	Hajdúböszörmény, Hajdú-Bihar County	3	skin	-	-	-	-	-	-
H7 (*E. roumanicus*)	Budapest, XIV. district	2	muscle	+	+	-	+	-	-
			skin	+	-	-	+	+	-
H8 (*E. roumanicus*)	Budapest, XIV. district	5	skin	+	-	-	-	+	-
H9 (*E. roumanicus*)	Kalocsa, Bács-Kiskun County	5	muscle	-	-	-	+	-	-
			skin	-	-	-	-	-	-
H10 (*E. roumanicus*)	Budapest X. district	1	blood	-	-	-	-	+	-
			muscle	-	-	-	-	-	-
			skin	+	-	-	-	-	-
			spleen	-	-	-	-	-	-
H11 (*E. roumanicus*)	Budapest X. district	1	blood	+	-	-	-	-	-
			muscle	-	-	-	-	-	-
			skin	+	+	-	-	-	-
			spleen	+	-	-	-	+	-
H12 (*E. roumanicus*)	Szigetszentmiklós, Pest County	3	muscle	-	-	-	-	-	-
			skin	+	+	-	+	+	+

Host ID (species)	Location	Degradation rate (1-5)	Sample type	*A. phagocytophilum*	*B. burgdorferi* s.l.	*B. miyamotoi*	*R. helvetica*	*Rickettsia* sp.	*Bartonella* sp.
H13 (*E. roumanicus*)	Budapest, XIV. district	3	muscle	-	-	-	-	-	-
			skin	+	+	-	+	+	-
H14 (*E. roumanicus*)	Budapest, XIV. district	1	blood	-	-	-	-	-	-
			liver	-	-	-	-	-	-
			muscle	-	-	-	-	-	-
			skin	-	-	-	-	+	+
			spleen	+	-	-	+	+	-
H15 (*E. roumanicus*)	Kunpeszér, Bács-Kiskun County	4	muscle	+	-	-	-	-	-
			skin	+	-	-	+	+	-
H16 (*E. roumanicus*)	Budapest, XI. district	3	muscle	+	-	-	-	-	-
			skin	+	+	-	+	+	-
			spleen	+	-	-	-	-	-
H17 (*E. roumanicus*)	Budapest, XIV. district	5	skin	+	-	-	-	+	-
H18 (*E. roumanicus*)	Budapest, XVIII. district	4	muscle	-	-	-	-	-	-
			skin	-	-	-	+	+	-
H19 (*E. roumanicus*)	Szentendre, Pest County	5	skin	-	-	-	-	-	-
H20 (*E. roumanicus*)	Budapest XII. district	4	muscle	-	-	-	-	-	-
			skin	-	-	-	-	-	-
H21 (*E. roumanicus*)	Budapest, XXIII. district	3	muscle	-	-	-	-	-	-
			skin	+	-	-	+	+	-
			spleen	+	-	-	-	-	-
H22 (*E. roumanicus*)	Balatonkenese, Veszprém County	4	muscle	-	-	-	-	-	-
			skin	+	-	-	-	-	-
H23 (*E. roumanicus*)	Budapest, XI. district	5	skin	+	-	-	-	-	-
D1 (*Martes foina*)	Budapest, XI. district	4	muscle	-	-	-	+	-	-
			skin	-	-	-	-	-	-
D2 (*Mus musculus*)	Budapest, XIV. district	1	muscle	-	-	-	-	-	-
			skin	-	-	-	+	+	-
D3 (*Crocidura suaveolens*)	Eger, Heves County	2	muscle	+	-	-	-	-	-
			skin	-	-	-	-	-	-
			spleen	-	-	-	-	-	-

Host ID (species)	Location	Degradation rate (1-5)	Sample type	*A. phagocytophilum*	*B. burgdorferi* s.l.	*B. miyamotoi*	*R. helvetica*	*Rickettsia* sp.	*Bartonella* sp.
D4 (*Sciurus vulgaris*)	Budapest, XII. district	1	blood	-	-	-	-	-	-
			muscle	-	-	-	-	-	-
			skin	-	-	-	-	-	-
D5 (*M. musculus*)	Budapest, XIV. district	1	muscle	-	-	-	-	-	+
			skin	-	-	-	-	-	-
			spleen	-	-	-	-	-	-
D6 (*Talpa europea*)	Solymár, Pest County	1	blood	-	-	-	-	-	+
			muscle	-	-	-	-	-	+
			skin	-	-	-	-	-	+
			spleen	-	-	-	-	-	+
D7 (*T. europea*)	Solymár, Pest County	1	blood	-	-	-	-	-	+
			liver	-	-	-	-	-	+
			muscle	-	-	-	-	-	+
			skin	-	-	-	-	-	+
			spleen	-	-	-	-	-	+
D8 (*T. europea*)	Margaret Island, Budapest	1	blood	-	-	-	-	-	-
			skin	-	-	-	-	+	-
			spleen	-	-	-	-	-	-
D9 (*Mustela nivalis*)	Budakeszi Pest County	1	blood	-	-	-	-	-	+
			muscle	-	-	-	-	+	-
			skin	-	-	-	-	-	-
			spleen	-	-	-	-	-	+
D10 (*T. europea*)	Solymár Pest County	1	muscle	-	-	-	-	-	-
			skin	-	-	-	-	-	-
			spleen	-	-	-	-	-	-
D11 (*Capreolus capreolus*)	Budapest, XI. district	1	skin	+	-	-	-	-	-
			spleen	-	-	-	-	-	-
			thymus	-	-	-	-	-	-
D12 (*S. vulgaris*)	Balatonakarattya Veszprém County	1	muscle	-	-	-	-	-	-
			skin	-	+	-	-	-	-
			spleen	-	-	+	-	-	-

*Degree of degradation: 1- intact perfect condition for inner organ tissue collection at most skull crashed; 2- crushed body with mostly intact inner organs; 3- smashed inner organs; 4- heavely smashed inner organs; 5- flat and dry carcass without recognisable organ (except skin)

Table 21.: Specification of vector-borne pathogens in tissue and tick samples from road-killed small and medium size mammals.

Sample type	Pathogen	Host/tick species	Tissue type/tick stage	Number of samples	Accession numbers
Tissue	*Borrelia afzelii*	Northern white-breasted hedgehog (*Erinaceus roumanicus*)	skin	1	MF163403
	Borrelia spielmanii	Northern white-breasted hedgehog (*E. roumanicus*)	muscle	2	MF163401, MF163402
	Anaplasma phagocytophilum ecotype I	Northern white-breasted hedgehog (*E. roumanicus*)	liver	1	MF372764
			skin	2	MF372765, MF372766
			spleen	3	MF372767-MF372769
	*Bartonella sp.**	Northern white-breasted hedgehog (*E. roumanicus*)	muscle	1	MF372778
			skin	2	MF372780, MF372781
		House mouse (*Mus musculus*)	muscle	1	MF372781
		European mole (*Talpa europea*)	blood	2	MF372782, MF372786
			liver	1	MF372787
			muscle	2	MF372785, MF372789
			skin	2	MF372784, MF372788
			spleen	1	MF372783
		Lesser weasel (*Mustela nivalis*)	blood	1	MF372790
			spleen	1	MF372791
Tick	*Borrelia afzelii*	*Ixodes ricinus*	female	1	MF163404
			male	1	MF163405
			nymph **	7	MF163406-MF163412
	Anaplasma phagocytophilum ecotype I	*Ixodes ricinus*	female	2	MF372771, MF372772
			nymph **	5	MF372773-MF372777
		Ixodes hexagonus	female	1	MF372770
	Rickettsia monacensis	*Ixodes ricinus*	nymph **	4	MF170619-MF17062
			larva #	1	MF170623

*similar to a *Bartonella* sp. sequence from China (AF391281.1)

**pooled sample from 5 nymphs

pooled sample from 10 larvae

6. Discussion

Small mammals are one of the most important sources of blood meal for the subadult stages of ticks. Rodents have high metabolic and reproduction rate with relatively large body surface compared to their body weight and these small mammals are in high densities in the natural habitats. All these features make rodents suitable hosts for ticks and also suitable reservoirs for many pathogens (Ostfeld et al., 2014). Pathogen cycles probably differ in urban and natural habitats. The gene pool of pathogens in the natural habitat compared with the “urban” pathogens is probably larger because the genetic diversity of the pathogens follows the biodiversity of host species (Cisarovsky and Schmid-Hempel, 2014). The selective pressure on the pathogen with wide range of possible host species increases its genetic diversity under natural circumstances. If in turn the number of hosts is limited the pathogen has to specialise for these which decreases its genetic diversity. These local limitations in available hosts might have also led to the evolution of different *A. phagocytophilum* ecotypes (Jahfari et al., 2014). The presence of similar processes should also be investigated in *Can*. N. mikurensis, *B. miyamotoi* and other tick-borne pathogen populations.

Because of the complexity of my thesis topic I would like to discuss the different subtopics separately such as, Pathogens in rural habitats, Pathogens in urban habitat; before drawing the conclusions.

6.1. Pathogens in the natural habitat

6.1.1 Ticks and small mammals

Gemenc is a natural open habitat with a broad range of possible host species compared with the urban habitats e.g. the Margaret-Island in Budapest, where the possible host diversity is relatively low. In city parks e.g. hedgehogs, squirrels and other urbanised mammals and also the frequent human and canine visitors serve as the main source of nourishment for ticks. In natural habitat ticks may can find broad range of host species but their chance for finding a host is lower in contrast to the urban (closed) habitats which have only a few potential host species but usually in a higher density (Földvári et al., 2011)

6.1.2. *Borrelia burgdorferi* s.l. and *Borrelia miyamotoi*

We found, altogether, 42 *B. burgdorferi* s.l. positive samples in all types of samples and from 18 of them we could also sequence the LB spirochete. Compared to Egyed et al. (2012), who found 2.5% average minimum prevalence of *B. burgdorferi* s.l. in questing *I. ricinus* in different collection sites in Hungary (Egyed et al., 2012), our study shows a much higher prevalence (23.5%). In one questing *I. ricinus* nymph we found *Borrelia lusitaniae* infection. This nymph may have fed as larva on lizards that are potential reservoirs of these spirochetes (Földvári et al., 2009; Majláthová et al., 2006). *Lacerta viridis* (green lizard), *Lacerta agilis* (sand lizard) and *Podarcis muralis* (common wall lizard) live in this region (Mayer, 1992) and *L. agilis* was observed also in the vicinity of our trapping sites (Balázs Velekei, personal communication). The presence of *B. lusitaniae* is of public health relevance, since this spirochete can also infect humans (Collares-Pereira et al., 2004).

Borrelia afzelii was the most prevalent among the sequenced LB spirochetes (17/18) in the rural study site. This is the most widespread *Borrelia* species in Europe (Stanek et al., 2012), usually maintained by rodents (Burri et al., 2014; Rigó et al., 2011). Also, this spirochete is probably the most important LB causative agent in Hungary (Földvári et al., 2005). In a sero-epidemiological study in the neighbouring Austria a nearly linear increase of LB seroprevalence with duration of hunting activity was shown among hunters (Cetin et al., 2006). Lakos et al. reported that erythema migrans occurred ten times more frequently among Hungarian forestry workers than in the average population, but the rate of seropositivity was much higher (indicating frequent asymptomatic infection) (Lakos et al., 2012). The hunters' elevated risk of tick bites is obvious (Kubo et al., 1992) and infections with other tick-borne pathogens, such as *A. phagocytophilum* in the neighbouring Slovakia (Nováková et al., 2010), *Rickettsia* spp. in Germany (Jansen et al., 2008) and tick-borne encephalitis virus in Italy (Pugliese et al., 2007), were also observed in this group. Thus, the presence of at least two pathogenic LB spirochetes in the Gemenc area can pose a risk of LB infection to the occupationally exposed persons.

Ixodes acuminatus individuals are endophilic (or nidicolous) ticks. All stages of this tick species live in rodents' nests, thus, being capable of maintaining a local cycle of pathogens similar to the natural cycle of *A. phagocytophilum* and *Babesia microti* with the endophilic *I. trianguliceps* (Bown et al., 2008, 2006). We found *B. afzelii* in one nymphal and four larval pools (4/52, minimum prevalence: 7.7%) of *I. acuminatus*. Rigó et al. (2011) detected *B. afzelii* in an adult *I. acuminatus* female and the three sequenced *I. acuminatus* samples in the present study identified the same LB spirochete. *Ixodes ricinus* ticks are the connecting link (bridge vectors) between the rodent's local (nest) infection and the "world outside the nest" i.e. other vertebrate hosts including humans. Between these two *Ixodes* species we could not find any

significant difference in *B. burgdorferi* s.l. prevalence. Thus, *I. acuminatus* may have similarly important role in the endophilic pathogen cycle as *I. ricinus* has in the exophilic pathogen cycle involving human infection (Figure 13.). This double natural cycle has also been observed in the case of *B. burgdorferi* s.l. and *I. ricinus* vs. *I. hexagonus* (Gern et al., 1997) and might be a general trait for several tick-borne pathogens. Being present in two different (endophilic and exophilic) transmission cycles is clearly an evolutionarily stable strategy increasing survival of the LB spirochetes. Both of these cycles have to be considered and monitored in order to forecast and prevent human infection risk. Furthermore, *I. acuminatus* occasionally can bite humans (Hillyard, 1996) posing a direct infection threat as well.

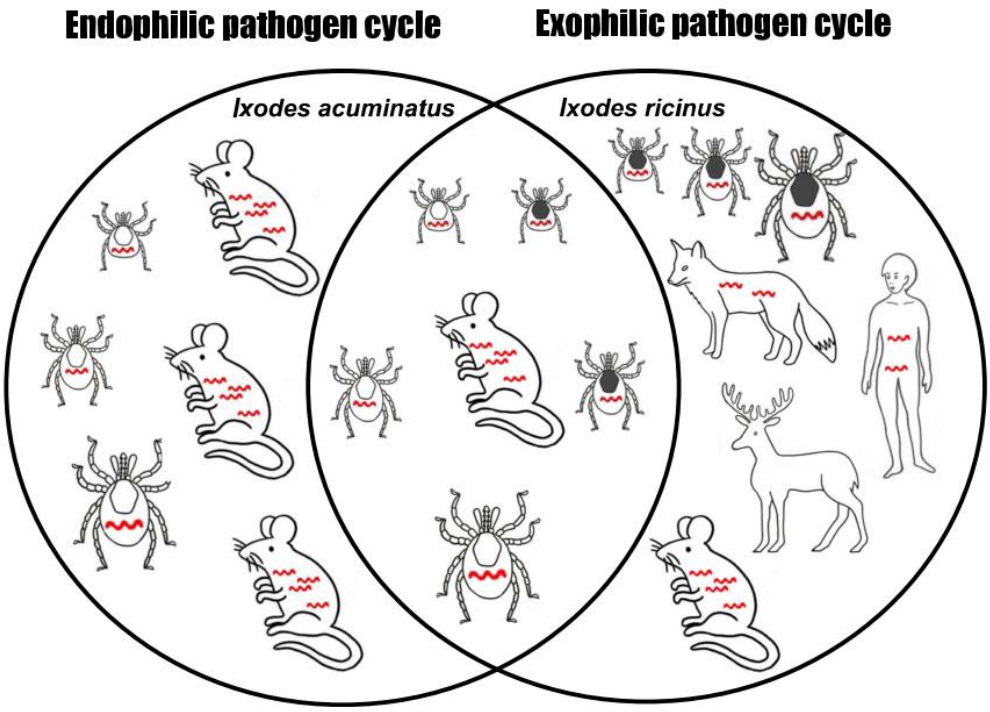

Figure 13.: The two transmission cycles involved in the natural maintenance of *Borrelia afzelii*. Scutum of larvae, nymphs and adults of the exophilic tick, *I. ricinus* are marked with dark grey and with white colour in case of the endophilic tick, *I. acuminatus*. Red spirochetes indicate ticks and hosts that can potentially be infected with *B. afzelii*. Cervids are important tick maintenance hosts, however they are not reservoirs of LB spirochetes, thus they are known to be dilution hosts. Original drawings were made by Gábor Majoros (Szekeres et al., 2015)

We also found *B. burgdorferi* s.l. infection in three engorged *D. marginatus* (two larva pools and one single larva sample). The two pools contained four and eight specimens respectively, and the bacterium identified in these samples was *B. afzelii*. One of these pools was collected from one *Borrelia*-negative *A. flavicollis* male, the other pool was removed from an *A. agrarius* male. The single engorged *B. burgdorferi* s.l.-positive *D. marginatus* larva was removed from an *A. flavicollis* female. In previous studies, American *Dermacentor* spp. were experimentally shown to be unable to transmit LB spirochetes (Gray et al., 2002) but questing adult *D.*

reticulatus ticks in Germany had 11.3% prevalence of *Borrelia* spp. (detected with indirect immunofluorescence assay) (Kahl et al., 1992). Recently, a defensin was reported to have a possible role in the clearing of *Borrelia* ingested by *D. marginatus* ticks (Chrudimská et al., 2014). As both European *Dermacentor* ticks can bite humans (Földvári et al., 2013), their potential role in the life-cycle of LB spirochetes should be further explored.

Borrelia miyamotoi spirochetes have been previously detected only in wild *A. argenteus* in Asia*, P. leucopus* in North-America and *My. glareolus* in Europe (Cosson et al., 2014; Fukunaga et al., 1995; Scoles et al., 2001). The reservoir role of *A. flavicollis* and *My. glareolus* was proven under xenodiagnostic laboratory conditions. Our study provides the first evidence for the presence of *B. miyamotoi* infection in a wild *A. flavicollis* population. Further eco-epidemiological studies in other natural habitats will shed more light on the importance of one of the most common rodents in Europe, the yellow-necked field mouse, in the cycle of *B. miyamotoi*.

Relapsing fever spirochetes' DNA was detectable in five samples with a sensitive qPCR method (Hovius et al., 2013). *Borrelia miyamotoi* DNA sequencing was successful from only three samples: one questing *I. ricinus* nymph, one pooled sample containing eight *I. ricinus* larvae from an *A. flavicollis* male and one spleen removed from an *A. flavicollis* female. All three sequences were 100% identical suggesting the circulation of the same relapsing fever spirochete genotype between natural populations of the yellow-necked field mouse and *I. ricinus*. In the case of an *A. flavicollis* male skin and one questing *I. ricinus* nymph sample the conventional PCR and sequencing were not successful, probably due to low DNA concentration.

One engorged *I. ricinus* larva pool from an unidentified rodent (n=8 larvae) had co-infection with *B. afzelii* and *B. miyamotoi*. Cosson et al. (2014) found *B. miyamotoi* co-infection with another LB spirochete, *B. garinii* in France. This indicates that *I. ricinus* might spread both pathogens even synchronously and act like a bridge vector between the most important rodent species and humans. This tick species is the key risk factor for humans acquiring most tick-borne pathogens in Europe (Rizzoli et al., 2014), especially in areas with frequent human presence as the popular hunting ground in our study site.

The present study identified *B. miyamotoi* and *B. burgdorferi* s.l. from samples of different years (2011–2012) indicating that these pathogens seem to have a stable cycle in this area even surviving rodents that usually live for less than a year (Szekeres et al., 2015b).

6.1.3. *Anaplasma phagocytophilum* and *Can*. Neoehrlichia mikurenis

Numerous studies reported the role of ticks and small mammals in the tick-borne pathogens' epidemiological cycle in Europe. Rodent species and insectivores are also important hosts of the subadult stages of the exophilic ticks and all stages of endophilic species. Within the present study we examined the presence and diversity of tick species and the occurrence of two emerging zoonotic bacteria in a small mammal community of Southern Hungary.

We found lower tick prevalence (8%) on the small mammals compared with other European studies (Khanakah et al., 2006; Kiffner et al., 2011). This may be due to the regular floods in the study area. The majority of the collected ticks (70%) were in larval and nymphal stages. *H. concinna, I. ricinus* and *Dermacentor* individuals were collected both from the vegetation and from the captured small mammals. The endophilic ticks like the subadult stages of the *Dermacentor* species and all stages of *I. acuminatus* were found on small mammals only. Interestingly, we detected only *D. marginatus* larvae and nymphs on rodents but no *D. reticulatus* subadults, although the questing adults of the latter species were present in this area.

We found *Can*. N. mikurensis and A. *phagocytophilum* with relatively low prevalence in most samples (Table 13., 14. and 15). *A. phagocytophilum* DNA was amplified from five individuals of three tick species: two *H. concinna* and two *D. reticulatus* adults that are not known to be vectors and one *I. ricinus* nymph which is the main vector of this causative agent in Europe. Other papers have also reported *A. phagocytophilum* infection in *H. concinna* and *D. reticulatus* ticks (Tomanović et al., 2013; Wirtgen et al., 2011), however, the detection of the bacterial DNA in questing ticks does not provide evidence for their vector role. To investigate this, xenodiagnostic experiments have to be performed.

Among the questing ticks we found a male, a female and a nymphal *I. ricinus* to be *Can*. N. mikurensis-positive. This is the first report about the presence of this pathogen from Gemenc since Hornok et al. (2013) collected ticks from this area in 2007 but they have not found any positive samples.

Among the 162 engorged ticks we found one *I. ricinus* with *A. phagocytophilum* infection. The PCR positivity of engorged ticks however does not prove infection of the tick itself because it contains high amount of host blood. Thus, if we have only prevalence data from engorged ticks, it is not clear whether the tick or the host was infected with the pathogen. That was the reason why we collected ticks also from the vegetation having a control group for engorged ticks. Based on the limited number of engorged specimens analysed (Table 15.), *I. acuminatus*, *D. marginatus* and *H. concinna* ticks are probably not involved in the natural cycle of *N. mikurensis* and *A. phagocytophilum*.

A. phagocytophilum has a broad host range from wild to domestic animals and humans. Some ruminants, rodents and insectivores can maintain this pathogen (Stuen et al., 2013)). Rodent species were considered the most important reservoirs (Burri et al., 2014; Jahfari et al., 2012; Silaghi et al., 2012; Stuen et al., 2013) in the natural maintenance of both *A. phagocytophilum* and *Can*. N. mikurensis. *Apodemus flavicollis*, *A. sylvaticus* and *My. glareolus* rodents have recently been shown to be reservoirs of *Can*. N. mikurensis, but not for *A. phagocytophilum* (Burri et al., 2014). Compared with other Hungarian small mammals, like urban *E. roumanicus* hedgehogs (*A. phagocytophilum* 76.1%, *Can.* N. mikurensis 2.3%) here we found lower *A. phagocytophilum* (7.2%) and similar *Can*. N. mikurensis (2.3%) prevalence using skin samples (Földvári et al., 2014). The significantly lower *A. phagocytophilum* prevalence (Fisher-test, $p<0.00001$) in natural rodents of the present study compared to urban hedgehogs was obtained with the same molecular methods. Thus, we can conclude that the studied natural rodent population seems to contribute to the epidemiology of the pathogen in a smaller extent compared to hedgehogs in the urban situation. In case of *Can*. N. mikurensis the spleen sample (containing higher amount of possibly infected blood cells) were reported to be significantly more positive than ear biopsy samples (Silaghi et al., 2012). In the present study, we did not observe this difference in *Can*. N. mikurensis prevalence between skin and spleen samples, however, the two samples of the same individuals were not analysed (Szekeres et al., 2015a).

6.1.4. Rickettsiae in field collected ticks

Rickettsiae were detected in 57.8 % of *D. reticulatus* which is much higher compared to the prevalence of 15.5 % reported previously in questing ticks of the same species collected throughout the country (Hornok et al., 2010). Both female (53.7 %) and male (65.2 %) *D. reticulatus* ticks had high prevalence of *Rickettsia* spp. After sequencing and using NCBI Nucleotide BLAST we found this pathogen is *Rickettsia raoultii*. This corroborates with previous findings about the equal role of both tick sexes and both *Dermacentor* spp. in TIBOLA epidemiology (Földvári et al., 2013). The accession numbers of the sequenated pathogens are: LC060664 and LC060713 to LC060722 (Szekeres et al., 2016a).

6.1.5. *Hepatozoon* sp. in rodents and ectoparasites

Only tissue samples from *My. glareolus* were found to be infected with *Hepatozoon erhardovae*. The prevalence of the infection was relatively high (17.02%), (especially compared to the latest report by Šebek (Sebek, 1978) of *Hepatozoon* from *My. glareolus* in Hungary, which was ~7%), but even higher values have been reported previously: 18-57% in Northern Europe (Laakkonen et al., 2001a) and 18.9-64.2 % in Poland (Bajer et al., 2014; Karbowiak et al., 2005). Bank voles seem to have a unique role in maintaining *Hepatozoon*

species in Europe. Not only they had been found to be infected with these parasites in several different geographical locations and often at a high prevalence. Unlike most other examined mammal species, not only one, but multiple *Hepatozoon* species have been reported from this host (e.g. *Hepatozoon griseisciuri*, *Hepatozoon sylvaticus* and *H. erhardovae*) (Craig, 2001b; Krampitz, 1981; Krampitz and Wongchari, 1980). Unfortunately, there are very few available DNA sequences from any of these species in the NCBI GenBank and our is the first entry for *H. erhardovae*.

The similarity of 18S genes of *Hepatozoon* spp. detected in *My. glareolus* in Europe and in a *Python regius* originating from Ghana has already been pointed out by Sloboda et al. (Sloboda et al., 2007). The occurence of these apicomplexans, which seem to be genetically very similar, but found in taxonomically and geographically very diverse hosts, raises a question about the host and vector specificity of these parasites. The published data about this issue are controversial. Apparently, in some cases, genetically very similar parasites can infect a range of different hosts and vectors (Criado-Fornelio et al., 2006; Sloboda et al., 2007). On the other hand, infection experiments using closely related host or vector species often fail for some *Hepatozoon* parasites but not for others (Harkness et al., 2010). Some genetically diverse *Hepatozoon* species infecting the same host species has also been reported (Harris et al., 2011). Although information deriving from single gene analyses have to be treated with caution, the results of our phylogenetic analysis seems to support the theory, that genetically very similar *Hepatozoon* spp. can occur in geographically diverse hosts (see in Figure 12.). To clarify the phylogenetic relationship between these parasites detected in different hosts at different locations, a multi-gene, multi-species phylogenetic study is needed.

Hepatozoon species were found in all three collected flea species. These have been reported previously as vectors of *H. erhardovae* (Krampitz and Wongchari, 1980) and likely play an important part in the maintenance of this apicomplexan parasite. As most of the members of this parasitic insect order, these three species have a wide host range among rodents and even insectivores and have been reported in Hungary before (Rigó et al., 2011; Szabó, 1975). The lack of *H. erhardovae* infection in the large number of other rodent species analysed and the positivity of all three flea species removed from them supports the known host specificity of this blood parasite.

Identification of *Hepatozoon* species of mammals proves to be a challenge, as most of the species have been described in the 1920's to 1980's, and the description was based solely on morphological features of a single developmental stage (Craig, 2001b; Smith, 1996). Although, in the last few years, a number of studies were published, that used DNA based methods to characterize and compare *Hepatozoon* samples collected from different intermediate and

definitive hosts, in most cases the species remain unidentified and unnamed (Aydin et al., 2015; Criado-Fornelio et al., 2006; de Azevedo Gomes et al., 2017; Hamšíková et al., 2016; Pinto et al., 2013; Sloboda et al., 2007). A combination of morphological examination of multiple developmental forms of each species and the creation of credible, species-specific reference sequence collection would be highly beneficial for further studies, as it would provide a useful tool for screening and identification of *Hepatozoon* species in a wide range of vertebrates and arthropods (Rigó et al., 2016).

6.2. Pathogens in the urban habitat

Urbanisation is a phenomenon, within the population shift from rural areas to the cities. In 2014 more than half of the population (54%) lived in urban areas, and it is expected that until 2050 this ratio will increase to 60% (United Nations, 2014*). Th*us, when big cities were designed, it was crucial to have relatively big green recreational areas in the concrete jungle. In smaller scale, our gardens also have a recreational function. Gardens are the places where people can use their imagination and creativity to make a nice and refreshing environment. On the other hand, this diverse patchy habitat provides a huge quantity of food for animals that can adapt to this urban environment. For example, planted ornamental shrubs and trees serve a good food source in city gardens. These decorative plants can also serve as shelter for several species. City people love small songbirds, therefore in wintertime provide extra food (sunflower seeds, nuts) to help the survival of these animals, which could be also beneficial for urbanised squirrels and other rodents. In addition, the leftover food from companion animals is a good opportunity, but the main food source insured by the city is the regenerative and inexhaustible food waste. In the European Union more than 88 million tonnes of food is wasted annually (around 173 kg/person/ year; estimated cost: 143 billion euros), which will likely rise (Stenmarck et al., 2016).

6.2.1. *Anaplasma phagocytophilum* and *Can*. N. mikurensis in urban hedgehogs

The low pathogen (*A. phagocytophilum* and *Can*. N. mikurensis) prevalence observed in the urban hedgehog population caught on the Margaret Island compared with that in rodents in other locations (Jahfari et al., 2012; Silaghi et al., 2012) might be caused by the usage of skin samples. Skin samples from rodents showed only 1.1% positivity in a study in Germany; however, average prevalence of *Candidatus* N. mikurensis in transudate, spleen, kidney, and liver samples from the same animals was 37.8%–51.1% (Silaghi et al., 2012). Although we did not test other organs, we hypothesize that prevalence of *Can.* N. mikurensis infection in urban hedgehogs is probably more than 2.3%.

We detected *A. phagocytophilum* in 67 (76.1%) of 88 urban hedgehogs. This prevalence was similarly high in European hedgehogs in Germany (Silaghi et al., 2011). *Ixodes ricinus* ticks

are more common than *I. hexagonus* ticks in this urban hedgehog population (Földvári et al., 2011). Thus, *I. ricinus* ticks can acquire these bacteria when feeding on hedgehogs and the risk for human infection with *A. phagocytophilum* in this park in Budapest is relatively high (Földvári et al., 2014).

6.2.2. Rickettsiae in field collected ticks

From the urban habitat 22 *R. monacensis* and 9 *R. helvetica* out of 534 questing *I. ricinus* were identified with the less sensitive conventional PCR and sequencing. Compared to the average ratio of these two rickettsiae in other European studies (Rizzoli et al., 2014; Spitalská et al., 2014) the relatively high prevalence of *R. monacensis* (originally described from a city park in Germany (Rizzoli et al., 2014)) appears unique probably as a consequence of the eco-epidemiology of the closed island park habitat (Földvári et al., 2014).

The qPCR specific for *Rickettsia* spp. was positive in 88 (16.5 %) out of 534 *I. ricinus* ticks from the urban and 41 (25.3 %) out of 162 ticks from the rural habitat. Prevalence of rickettsiae in *I. ricinus* did not differ significantly in the two study sites (Table 11).
Female I*. ricinus* ticks in the urban park were found to have a particularly high prevalence of *R. helvetica* (44.6 %) suggesting a higher infection risk when humans are bitten by this tick stage. The significantly higher prevalence of *R. helvetica* and *Rickettsia* spp. in the adult stages of *I. ricinus* compared to nymphs from the urban habitat (Fisher's exact test: $p < 0.05$) suggests the important role of transstadial infection in the eco-epidemiology of these pathogens (Table 11). From the urban habitat 22 *R. monacensis* and 9 *R. helvetica* out of 534 questing *I. ricinus* were identified with the less sensitive conventional PCR and sequencing (Szekeres et al., 2016a).

6.2.3. Pathogens detected in road-killed mammals and their ticks

The green areas of cities, like suburban forests, cemeteries and city parks are suitable habitats for several wildlife species. Urban mammals can serve as hosts for ticks and tick-borne pathogens with medical and veterinary importance (Rizzoli et al., 2014). Our study emphasizes the benefits of using roadkill to assess human risk of infection. We also shed light on the diversity and composition of tick-borne bacterial communities in road- and accidentally killed urban mammals.

We found six different pathogens in 90 collected tissue samples from eight accidentally died mammal species (Table 20.). Besides, several single infections (n=7), double (n=10), triple (n=5), fourfold (n=5) and one fivefold infections also occurred in the collected animals. Most of the co- and multiple infections (n=28) were in hedgehogs but double infection also occurred in the other collected mammal species (Lesser weasel, European red squirrel and house mouse) (Table 21.).

Among road-killed mammals, especially hedgehogs proved to be a good source of ixodid ticks: 124 ticks were removed from eight carcasses (111 *I. ricinus* and 13 *I. hexagonus*). Four different pathogens were identified with real-time PCR within these ectoparasites (Table 18.).

Borrelia afzelii was found in hedgehog skin and *I. ricinus* samples (female, male, nymph) and *B. spielmanii* in muscle samples from hedgehogs. Both *Borrelia* species cause erythema migrans in humans (Földvári et al., 2005). Similarly, to *E. europaeus, E. roumanicus* probably also has reservoir role for Lyme borreliosis spirochetes that has not yet been demonstrated with xenodiagnostic experiments. A recent study from the Czech Republic also identified *B. afzelii* as the most common genospecies in questing *I. ricinus* also within urban habitats (Kybicová et al., 2017).

We detected one *B. miyamotoi* positive spleen sample from a European red squirrel, a similar observation was recently reported from Belgium (Ruyts et al., 2017). The presence of *B. miyamotoi* and the high prevalence of *B. burgdorferi* s.l. (Pisanu et al., 2014) in red squirrels suggest this species might have role in the cycles of these *Borrelia* species. *Borrelia miyamotoi* is an emerging pathogen reported from many countries in ticks, hosts and also humans (Wagemakers et al., 2015). The number of patients reported is increasing in the Northern Hemisphere (USA, Russia, Japan, Germany, the Netherlands) (Boden et al., 2016; Chowdri et al., 2013; Gugliotta et al., 2013; Hovius et al., 2013; Krause et al., 2013; Platonov et al., 2011; Sato et al., 2014).

Anaplasma phagocytophilum ecotype I, which is the most prevalent human pathogenic ecotype, was found in Northern white-breasted hedgehog tissue samples and in *I. ricinus* and *I. hexagonus* ticks. Some of the removed ticks from the *A. phagocytophilum* positive hedgehogs were also infected with *A. phagocytophilum*. In addition, some hedgehogs were positive for *A. phagocytophilum* and the removed ticks from them were negative and there were some positive tick samples from negative hedgehogs as well. These findings are not surprising, since transmission efficacy during feeding might be less than 100% and *A. phagocytophilum* has transstadial transmission (Sonenshine and Roe, 2014). These two ectoparasite species are the most prevalent ticks feeding on hedgehogs and also have important role in the eco-epidemiology of Lyme-borreliosis (Rizzoli et al., 2014). In the urban hedgehogs, we found even higher (76%) *A. phagocytophilum* prevalence than in the present study (52%) (Földvári et al., 2014).The fact, that the human pathogenic *A. phagocytophilum* ecotype I was detected in hedgehogs, and with high prevalence in both tick species removed from them (mean minimum prevalence: 56%) emphasize the hedgehog's role in the *A. phagocytophilum* cycles (Jahfari et al., 2017). Our findings is, the first report of *A. phagocytophilum* in tissues from lesser shrew.

One roe deer fawn skin sample was positive with *A. phagocytophilum* specific real-time PCR. In roe deer *A. phagocytophilum* infection was reported in several countries from Europe (Jahfari et al., 2014). Our finding is interesting however, because the fawn was hit by car at an early age (approximately 2 weeks), thus it had short time-period to acquire infection via tick bite. During the dissection, no ectoparasite was found on this carcass. These circumstances suggest, that transplacental transmission is possible not just in sheep (Reppert et al., 2013) but also in wild ruminants like roe deer as well.

In *I. ricinus* nymphs and one larva pool *R. monacensis*, and in all stages of *I. ricinus* and *I. hexagonus* nymph samples *R. helvetica* was detected with relatively high prevalence. These two bacteria are human pathogens, belonging to the Mediterranean spotted fever group rickettsiae causing elevated fever, eschar and maculopapular rash (Bowmann and Nuttall, 2008). In hedgehogs, moles, a mouse, a lesser weasel and a stone marten rickettsiae were detected. This finding suggests these species might have a role in the urban cycle of these pathogens too. This is the first report about *Rickettsia* sp. infection in European moles and lesser weasel and about *R. helvetica* in stone marten.

Northern white-breasted hedgehogs, one house mouse, two European moles and a lesser weasel was *Bartonella* spp. specific PCR positive. The highest similarity with our *Bartonella* sp. sequences was found with *Bartonella taylorii* sequences from the same submitter and same host (Accession number: KT445922.1; KT445921.1; KT445919.1). After these first three, the other hits were “Uncultured” *Bartonella* sp. and *Bartonella* sp. sequences. Thus, we aligned with NCBI BLAS one of the three similar *B. taylorii* sequences and unfortunately it showed similarity only with themselves but none of the more than ten *B. taylorii* gltA sequences in the GenBank. For this reason, we considered our sequences as *Bartonella* sp.

In *I. hexagonus* nymphs we found for the first-time *R. helvetica* and *Rickettsia* sp. pathogens. There was a report about rickettsiae infection in *I. ricinus* and *I. hexagonus* but unfortunately it was not clear, whether *I. hexagonus* truly carried any *Rickettsia* pathogens (Giroud et al., 1965). The relatively high *A. phagocytophilum* (min. prev.:12/13=92%), *R. helvetica* (min. prev.: 3/13=23%) and *Rickettsia* sp. (min. prev.: 6/13=46%) positivity among *I. hexagonus* samples suggest that, this tick species can have an important role in the endophilic pathogen cycle of tick-borne pathogens as we have shown in the natural habitat section for *I. acuminatus*. All *I. hexagonus* were removed from two *A. phagocytophilum, R. helvetica* and *Rickettsia sp*. real-time PCR positive hedgehogs (code: H4 and H16). Xenodiagnostic experiments are needed to clarify the vector role of this tick species in the future.

7. Conclusions

The presence of the newly described human pathogen, *Borrelia miyamotoi* in a natural habitat with frequent human visitors has important public health implications. This study is the first report of this bacterium in wild *A. flavicollis* as well as in Hungary.

Apodemus flavicollis, A. agrarius and *My. glareolus* were found to be involved in the natural cycle of LB spirochetes. Our results suggest an important role of *I. acuminatus* ticks in the endophilic pathogen cycle of *B. afzelii*, similar to the role of *I. ricinus* in the exophilic pathogen cycle. Forestry workers, hunters, woodcutters, gamekeepersand hikers are especially exposed to ticks in areas of intense transmission of bacteria within enzootic cycles (i.e. forests). Consequently, they have to be considered as a high-risk population for tick-borne pathogens. In these high exposure groups, surveillance and prevention are the most crucial pillars of the protection against tick-borne pathogens like *B. burgdorferi* s.l., *B. miyamotoi* and *Rickettsia spp*. Neoehrlichiosis, granulocytic anaplasmosis and relapsing fever caused by *B. miyamotoi* have not been diagnosed in humans in Hungary. This finding is probably caused by diagnostic difficulties rather than absence of these pathogens in the environment.

We identified *B. miyamotoi* and *B. burgdorferi* s.l. spirochetes from samples (2011-2012) indicating that these pathogens seem to have a stable cycle in this area even surviving rodents that usually live for less than a year.

Infection with tick-borne pathogens cause predominantly non-characteristic symptoms. Laboratory cultivation and serologic detection of *Can.* N. mikurensis has not been successful, and this pathogen has not been identified in blood smears. Thus, accurate diagnosis of suspected cases requires suitable molecular methods. Parks can be considered points of contact for reservoir animals, pathogens, ticks, and humans. Our results indicate that *E. roumanicus* hedgehogs play a role in urban ecoepidemiology of at least two emerging human pathogens. To better understand the urban cycle of these pathogens, potential reservoir hosts, ticks collected from these hosts, and vegetation in parks should be investigated.

Our results showed considerable difference between the dominant rickettsial agents in the city park (*R. helvetica* and *R. monacensis*) and natural forest habitat (*R. raoultii*). This is due to the differences of these habitats in their vector and host diversity. In urban settings, usually *I. ricinus* dominates, whereas in natural habitats there is a more diverse tick community even visible in the small rural sample size of the present study. This more diverse tick community extends the range of possible human pathogenic rickettsiae, including newly emerging ones. Both our study sites have frequent human visitors: Margaret Island is a popular recreational and jogging park in the centre of Budapest and Gemenc is a popular hunting and hiking area with over 50,000 tourists per year. Since all rickettsiae (*R. helvetica, R. monacensis* and

R. raoultii) detected in this study are proven human pathogens (Fournier et al., 2000; Jado et al., 2007; Jia et al., 2014), we can conclude that despite the distinct eco-epidemiological traits, the risk (hazard and exposure) of acquiring rickettsial infections in both the urban and the rural study sites exists.

Our survey showed that accidentally killed animals are valuable resources in the investigation of the eco-epidemiology of tick-borne pathogens. Based on our molecular analyses hedgehogs, moles, shrews, squirrels, mice, martens and weasels are involved in the maintenance of one or several tick-borne pathogens in urban habitats. Due to the high motility of these urbanised mammals within and between human dwellings, we expect an enhanced spread of ticks and tick-borne pathogens wherever they are present. Further studies should investigate their relative contribution to the maintenance and spread of these pathogens and to specify their relative role in human tick bites and tick-borne diseases.

Using road-killed animals as source of tissue samples and ectoparasites provides both advantages and disadvantages. It is possible to collect samples not only from protected common species but also from inner organs of animals under protection and thus neglected from eco-epidemiological studies. In contrast, the main disadvantage is the varying condition of the carcasses. It is possible to collect dead animals in very good conditions but often there are dried samples without identifiable organs (except skin) and bodies fully flattened by cars. The PCR could be false-negative because of the high degree of degradation (inhibitors and DNA degradation) and the rise of decomposing bacteria. However, despite the highest degree of degradation it was possible to amplify DNA of *A. phagocytophilum*, *R. helvetica* and *Rickettsia* sp. in dried skin and muscle samples from road- and accidentally killed urban animals with real-time PCR. Therefore, even badly damaged carcasses might provide important eco-epidemiological information.

Some wild animal species (e.g. red foxes, martens) during the recent centuries have become not only urbanised but also became synanthropic species, and the number of these species might grow in the future. The cities take away bigger and bigger areas from the natural habitats while cities with growing food waste also serve as an inexhaustible and easily obtainable food source. Thus, more and more known and potential vertebrate reservoir species of *B. burgdorferi* s.l. might find suitable habitat in urban areas. *Ixodes ricinus* can also be found in parks and green areas in cities. With proper management of these areas, the suitable questing substrate and habitats for exophilic ticks can be minimised without harming other species or reducing minerals and organic material of the habitats. This management practices include reducing the undergrowth of shrubs and bushes, collecting the leaf litter in fall or after winter (composting and recycling), and cutting the loan short. To reduce the number of nidicolous (endophilic) ticks like *I. hexagonus*, the use of artificial nesting boxes for squirrels

and hedgehogs (especially in gardens), where the bedding of these nest can be sterilised and changed regularly, can be a possible way to reduce the number of ticks. These animals in urban and suburban areas are possible risk factors for humans to get infected from tick-borne pathogens. Nonetheless, with proper usage of repellents and a thorough self inspection after a walk in risky areas the hazard of infection could be minimised.

I hope, I could guide the curious reader out from the complex multi-level maze of the tick-borne pathogens and their relationship with hosts and vectors found in two different habitats. In addition, my dissertation may shed a little bit more light on this interesting topic and will hopefully also generate many good research questions for the future.

8. Overview of the new scientific results

The following scientific results of the presented dissertation are new to science

1. *Borrelia miyamotoi* can infect *Apodemus flavicollis*, the yellow necked field mouse, thus this species is a candidate reservoir.

2. *Ixodes acuminatus* ticks can establish a so called “endofilic pathogen cycle” without the contribution of *Ixodes ricinus* in host nest/borrows within the epidemiology of *Borrelia burgdorferi* s.l.

3. The relatively common but neglected protozoan haemoparasite *Hepatozoon erhardovae* was rediscovered in Gemenc and partial genetic data were obtained.

4. Northern white-breasted hedgehogs (*Erinaceus roumanicus*) are infected with *Anaplasma phagocytophilum* and *Can*. N. mikurensis, thus this insectivore species is a candidate reservoir for these pathogens, especially in urban habitats where they live in higher density.

5. In urban and natural habitats ticks harbour different *Rickettsia* species composition according to the collection site.

6. Road-killed carcasses are useful source to examine samples from protected species for the presence of tick-borne pathogens for further epidemiological studies.

7. Northern white-breasted hedgehogs (*Erinaceus roumanicus*) are infected with *Borrelia spielmanii* and *Bartonella* spp. which extends its known zoonotic importance.

8. Lesser weasel (*Mustela nivalis*) is infected with *Rickettsia* sp., and stone marten (*Martes foina*) is infected with *Rickettsia helvetica* pathogens. Both pathogens are potential human threat and these species can have role in the cycle of these causative agents.

9. References

Aguirrebengoa, K., Portillo, A., Santibáñez, S., Marín, J.J., Montejo, M., Oteo, J.A., 2008. Human Rickettsia sibirica mongolitimonae infection, Spain. Emerg. Infect. Dis. 14, 528–529.

Alberti, A., Zobba, R., Chessa, B., Addis, M.F., Sparagano, O., Parpaglia, M.L.P., Cubeddu, T., Pintori, G., Pittau, M., 2005. Equine and canine Anaplasma phagocytophilum strains isolated on the island of Sardinia (Italy) are phylogenetically related to pathogenic strains from the United States. Appl. Environ. Microbiol. 71, 6418–6422.

Angelakis, E., Billeter, S.A., Breitschwerdt, E.B., Chomel, B.B., Raoult, D., 2010. Potential for tick-borne bartonelloses. Emerg. Infect. Dis. 16, 385–391.

Arthur, D.R., 1968. British Ticks. Butterworths, London.

Arthur, D.R., 1953. The Host Relationships of *Ixodes hexagonus* Leach in Britain. Parasitology 43, 227-238 .

Aulagnier, S., Chevallier, J., Norwood, J., Varela, J.M., 2009. Mammals of Europe, North Africa and the Middle East. A&C Black London.

Aydin, M.F., Sevinc, F., Sevinc, M., 2015. Molecular detection and characterization of Hepatozoon spp. in dogs from the central part of Turkey. Ticks Tick. Borne. Dis. 6, 388–392.

Bajer, A., Welc-Falęciak, R., Bednarska, M., Alsarraf, M., Behnke-Borowczyk, J., Siński, E., Behnke, J.M., 2014. Long-Term Spatiotemporal Stability and Dynamic Changes in the Haemoparasite Community of Bank Voles (Myodes glareolus) in NE Poland. Microb. Ecol. 68, 196–211.

Barker, S.C., Murrel, A., 2004. Systematics and evolution of ticks with a list of valid genus and species names. Parasitology 129, S15–S36.

Bernasconi, M. V., Valsangiacomo, C., Balmelli, T., Péter, O., Piffaretti, J.C., 1997. Tick zoonoses in the southern part of Switzerland (Canton Ticino): Occurrence of Borrelia burgdorferi sensu late and Rickettsia sp. Eur. J. Epidemiol. 13, 209–215.

Boden, K., Lobenstein, S., Hermann, B., Margos, G., Fingerle, V., 2016. Borrelia miyamotoi – Associated Neuroborreliosis in Immunocompromised Person. Emerg. Infect. Dis. 22, 1617–1620.

Bolfíková, B., Hulva, P., 2012. Microevolution of sympatry: landscape genetics of hedgehogs Erinaceus europaeus and E. roumanicus in Central Europe. Heredity (Edinb). 108, 248–255.

Bowmann, A.S., Nuttall, P.A. (Eds.), 2008. Ticks: biology, disease and control. Cambridge University Press.

Bown, K.J., Begon, M., Bennett, M., Birtles, R.J., Burthe, S., Lambin, X., Telfer, S., Woldehiwet, Z., Ogden, N.H., 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, 404–410.

Bown, K.J., Lambin, X., Telford, G.R., Ogden, N.H., Telfer, S., Woldehiwet, Z., Birtles, R.J., 2008. Relative importance of Ixodes ricinus and Ixodes trianguliceps as vectors for Anaplasma phagocytophilum and Babesia microti in field vole (Microtus agrestis) populations. Appl. Environ. Microbiol. 74, 7118–25.

Bősze, S., 2007. Vörös mókus, in: Bihari, Z., Csorba, G., Heltai, M. (Eds.), Magyarország Emlőseinek Atlasza. Kossuth Kiadó, Budapest, pp. 136–137.

Briciu, V.T., Meyer, F., Sebah, D., Ţăţulescu, D.F., Coroiu, G., Lupşe, M., Carstina, D., Mihalca, A.D., Hizo-Teufel, C., Klier, C., Huber, I., Fingerle, V., 2014. Real-time PCR-based identification of Borrelia burgdorferi sensu lato species in ticks collected from humans in Romania. Ticks Tick. Borne. Dis. 5, 575–581.

Burri, C., Schumann, O., Schumann, C., Gern, L., 2014. Are Apodemus spp. mice and Myodes glareolus reservoirs for Borrelia miyamotoi, Candidatus Neoehrlichia mikurensis, Rickettsia helvetica, R. monacensis and Anaplasma phagocytophilum? Ticks Tick. Borne. Dis. 5, 245–251.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

Cetin, E., Sotoudeh, M., Auer, H., Stanek, G., 2006. Paradigm Burgenland: risk of Borrelia burgdorferi sensu lato infection indicated by variable seroprevalence rates in hunters. Wien. Klin. Wochenschr. 118, 677–681.

Choi, Y.J., Jang, W.J., Kim, J.H., Ryu, J.S., Lee, S.H., Park, K.H., Paik, H.S., Koh, Y.S., Choi, M.S., Kim, I.S., 2005. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg. Infect. Dis. 11, 237–244.

Chowdri, H.R., Gugliotta, J.L., Berardi, V.P., Goethert, H.K., Molloy, P.J., Sterling, S.L., Telford, S.R., 2013. Borrelia miyamotoi infection presenting as human granulocytic anaplasmosis: a case report. Ann. Intern. Med. 159, 21–27.

Chrudimská, T., Čeřovský, V., Slaninová, J., Rego, R.O.M., Grubhoffer, L., 2014. Defensin from the ornate sheep tick Dermacentor marginatus and its effect on Lyme borreliosis spirochetes. Dev. Comp. Immunol. 46, 165–170.

Cisarovsky, G., Schmid-Hempel, P., 2014. Few colonies of the host Bombus terrestris disproportionately affect the genetic diversity of its parasite, Crithidia bombi. Infect. Genet. Evol. 21, 192–197.

Cochez, C., Heyman, P., Heylen, D., Fonville, M., Hengeveld, P., Takken, W., Simons, L., Sprong, H., 2015. The Presence of Borrelia miyamotoi, A Relapsing Fever Spirochaete, in Questing Ixodes ricinus in Belgium and in The Netherlands. Zoonoses Public Health

62, 331–333.

Collares-Pereira, M., Couceiro, S., Franca, I., Kurtenbach, K., Scha, S.M., Baptista, S., Vieira, M.L., Cunha, C., 2004. First Isolation of Borrelia lusitaniae from a Human Patient. J. Clin. Microbiol. 42, 1316–1318.

Cosson, J.-F., Michelet, L., Chotte, J., Le Naour, E., Cote, M., Devillers, E., Poulle, M.-L., Huet, D., Galan, M., Geller, J., Moutailler, S., Vayssier-Taussat, M., 2014. Genetic characterization of the human relapsing fever spirochete Borrelia miyamotoi in vectors and animal reservoirs of Lyme disease spirochetes in France. Parasit. Vectors 7, 233.

Courtney, J.W., Kostelnik, L.M., Zeidner, N.S., Massung, R.F., 2004. Multiplex Real-Time PCR for Detection of Anaplasma phagocytophilum and Borrelia burgdorferi. J. Clin. Microbiol. Vol. 42, N, 3164–3168.

Craig, T.M., 2001a. Hepatozoon spp. and Hepatozoonosis, 2nd ed, Parasitic Diseases of Wild Mammals. Iowa State University Press, Ames.

Craig, T.M., 2001b. HEPATOZOON SPP. AND HEPATOZOONOSIS, 2nd ed, Parasitic Diseases of Wild Mammals. Iowa State University Press, Ames.

Craine, N.G., Nuttall, P.A., Marriott, A.C., Randolph, S.E., 1997. Role of grey squirrels and pheasants in the transmission of Borrelia burgdorferi sensu lato, the Lyme disease spirochaete, in the U.K. Folia Parasitol. (Praha). 44, 155–160.

Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A., Barba-Carretero, J.C., 2003. Molecular studies on Babesia, Theileria and Hepatozoon in southern Europe: Part II. Phylogenetic analysis and evolutionary history. Vet. Parasitol. 114, 173–194.

Criado-Fornelio, A., Ruas, J.L., Casado, N., Farias, N. a R., Soares, M.P., Müller, G., Brumt, J.G.W., Berne, M.E. a, Buling-Saraña, A., Barba-Carretero, J.C., 2006. New molecular data on mammalian Hepatozoon species (Apicomplexa: Adeleorina) from Brazil and Spain. J. Parasitol. 92, 93–99.

Crooks, K.R., 2002. Relative sensitivities of mammalian carnivores to habitat fragmentation. Conserv. Biol. 16, 488–502.

Crowder, C.D., Carolan, H.E., Rounds, M.A., Honig, V., Mothes, B., Haag, H., Nolte, O., Luft, B.J., Grubhoffer, L., Ecker, D.J., Schutzer, S.E., Eshoo, M.W., 2014. Prevalence of Borrelia miyamotoi in Ixodes Ticks in Europe and the United States. Emerg. Infect. Dis. 20, 1678–1682.

de Azevedo Gomes, L., Moraes, L.A., Figueira Aguiar, D.C., Tavares Dias, H.L., Sardinha Ribeiro, A.S., Piram do Couto Rocha, H., Teixeira Nunes, M.R., Costa Gonçalves, E., 2017. Genetic diversity of Hepatozoon spp. in Hydrochoerus hydrochaeris and Pecari tajacu from eastern Amazon. Ticks Tick. Borne. Dis.

de Bruin, A., van Leeuwen, A.D., Jahfari, S., Takken, W., Földvári, M., Dremmel, L., Sprong, H., Földvári, G., 2015. Vertical transmission of Bartonella schoenbuchensis in Lipoptena

cervi. Parasit. Vectors 8, 176.

De Sousa, R., Edouard-Fournier, P., Santos-Silva, M., Amaro, F., Bacellar, F., Raoult, D., 2006. Molecular detection of Rickettsia felis, Rickettsia typhi and two genotypes closely related to Bartonella elizabethae. Am. J. Trop. Med. Hyg. 75, 727–731.

Derdáková, M., Václav, R., Pangrácova-Blaňárová, L., Selyemová, D., Koči, J., Walder, G., Spitalská, E., 2014. Candidatus Neoehrlichia mikurensis and its co-circulation with Anaplasma phagocytophilum in Ixodes ricinus ticks across ecologically different habitats of Central Europe. Parasit. Vectors 7, 160.

Dickman, C.R., 1986. A method for censusing small mammals in urban habitats. J. Zool. 210, 631–636.

Doby, J.M., Betremieux, C., Lambert, M.C., Lorvelec, O., Rolland, C., Costil, C., 1991. Les micromammiferes forestiers reservoirs de germes pour Borrelia burgdorferi agent de la borreliose de Lyme? Etude serologique de 296 animaux dans l'ouest de la France. Rev Med Vet 142, 737–742.

Dubska, L., Literak, I., Kocianova, E., Taragelova, V., Sychra, O., 2009. Differential role of passerine birds in distribution of Borrelia spirochetes, based on data from ticks collected from birds during the postbreeding migration period in central Europe. Appl. Environ. Microbiol. 75, 596–602.

Dumitrache, M.O., Paştiu, A.I., Kalmár, Z., Mircean, V., Sándor, A.D., Gherman, C.M., Peştean, C., Mihalca, A.D., Cozma, V., 2013. Northern white-breasted hedgehogs Erinaceus roumanicus as hosts for ticks infected with Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in Romania. Ticks Tick. Borne. Dis. 4, 214–217.

Dumler, J.S., Choi, K.S., Garcia-Garcia, J.C., Barat, N.S., Scorpio, D.G., Garyu, J.W., Grab, D.J., Bakken, J.S., 2005. Human granulocytic anaplasmosis and Anaplasma phagocytophilum. Emerg. Infect. Dis. 11, 1828–1834.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acid Res. 32, 1792–1797.

Edouard, S., Parola, P., Socolovschi, C., Davoust, B., la Scola, B., Raoult, D., 2013. Clustered cases of rickettsia sibirica mongolitimonae infection, France. Emerg. Infect. Dis. 19, 337–338.

Egyed, L., Elő, P., Sréter-Lancz, Z., Széll, Z., Balogh, Z., Sréter, T., 2012. Seasonal activity and tick-borne pathogen infection rates of Ixodes ricinus ticks in Hungary. Ticks Tick. Borne. Dis. 3, 90–94.

Fontaine, J.J., Martin, T.E., 2006. Parent birds assess nest predation risk and adjust their reproductive strategies. Ecol. Lett. 9, 428–434.

Fournier, P.-E., Grunnenberger, F., Jaulhac, B., Gastinger, G., Raoult, D., 2000. Evidence of Rickettsia helvetica Infection in Humans, Eastern France. Emerg. Infect. Dis. 6, 389–

392.
Földvári, G., 2016. Life cycle and ecology of Ixodes ricinus: the roots of public health importance., in: Braks, M.A.H., van Wieren, S.E., Takken, W., Sprong, H. (Eds.), Ecology and Prevention of Lyme Borreliosis (Ecology and Control of Vector-Borne Diseases, Volume 4). Wageningen Academic Publishers, Wageningen, pp. 31–40.
Földvári, G., Farkas, R., 2005. [Review of literature relating to *Dermacentor reticulatus* (Acari: Ixodidae) and newer data on the occurrence in Hungary.] in Hungarian with English abstract. Magy. Állatorvosok Lapja 127, 289–298.
Földvári, G., Farkas, R., Lakos, A., 2005. Borrelia spielmanii Erythema Migrans, Hungary. Emerg. Infect. Dis. 11, 1794–1795.
Földvári, G., Jahfari, S., Rigó, K., Jablonszky, M., Szekeres, S., Majoros, G., Tóth, M., Molnár, V., Coipan, E.C., Sprong, H., 2014. Candidatus Neoehrlichia mikurensis and Anaplasma phagocytophilum in Urban Hedgehogs. Emerg. Infect. Dis. 20, 496–498.
Földvári, G., Rigó, K., Jablonszky, M., Biró, N., Majoros, G., Molnár, V., Tóth, M., 2011. Ticks and the city: ectoparasites of the Northern white-breasted hedgehog (Erinaceus roumanicus) in an urban park. Ticks Tick. Borne. Dis. 2, 231–234.
Földvári, G., Rigó, K., Lakos, A., 2013. Transmission of Rickettsia slovaca and Rickettsia raoultii by male Dermacentor marginatus and Dermacentor reticulatus ticks to humans. Diagn Microbiol Infect Dis 76, 378–389.
Földvári, G., Rigó, K., Majláthová, V., Majláth, I., Farkas, R., Pet'ko, B., 2009. Detection of Borrelia burgdorferi sensu lato in lizards and their ticks from Hungary. Vector-Borne Zoonotic Dis. 9, 331–336.
Földvári, G., Široký, P., Szekeres, S., Majoros, G., Sprong, H., 2016. Dermacentor reticulatus: a vector on the rise. Parasit. Vectors 9, 1–29.
Fukunaga, M., Koreki, Y., 1995. The flagellin gene of Borrelia miyamotoi sp. nov. and its phylogenetic relationship among Borrelia species. FEMS Microbiol. Lett. 134, 255–258.
Fukunaga, M., Takahashi, Y., Tsuruta, Y., Matsushita, O., Ralph, D., McClelland, M., Nakao, M., 1995. Genetic and phenotypic analysis of Borrelia miyamotoi sp. nov., isolated from the ixodid tick Ixodes persulcatus, the vector for Lyme disease in Japan. Int. J. Syst. Bacteriol. 45, 804–810.
Geller, J., Nazarova, L., Katargina, O., Järvekülg, L., Fomenko, N., Golovljova, I., 2012. Detection and genetic characterization of relapsing fever spirochete Borrelia miyamotoi in Estonian ticks. PLoS One 7, e51914.
Gern, L., Rouvinez, E., Toutoungi, L.N., Godroid, E., 1997. Transmission cycles of Borrelia burgdorferi sensu lato involving Ixodes ricinus and/or Ixodes hexagonus ticks and the European hedgehog, Erinaceus europeus, in suburban and urban areas in Switzerland,. Folia Parasitol. (Praha). 44, 309–314.

Gern, L., Sell, K., 2009. Isolation of Borrelia burgdorferi sensu lato from the skin of the European badger (Meles meles) in Switzerland. Vector-Borne Zoonotic Dis. 9, 207–208.

Gern, L., Toutoungi, L.N., Hu, C.M., Aeschlimann, A., 1991. Ixodes (Pholeoixodes) hexagonus, an efficient vector of Borrelia burgdorferi in the laboratory. Med. Vet. Entomol. 5, 431–435.

Gherman, C.M., Sándor, A.D., Kalmár, Z., Marinov, M., Mihalca, A.D., 2012. First report of Borrelia burgdorferi sensu lato in two threatened carnivores: the marbled polecat, Vormela peregusna and the European mink, Mustela lutreola (Mammalia: Mustelidae). BMC Vet. Res. 8, 137.

Gilles, J., Just, F.T., Silaghi, C., Pradel, I., Passos, L.M.F., Lengauer, H., Hellmann, K., Pfister, K., 2008. Rickettsia felis in fleas, Germany. Emerg. Infect. Dis. 14, 1294–1296.

Giroud, P., Capponi, M., Dumas, N., Rageau, J., 1965. Les Ixodes ricinus et hexagonus de France contiennent des agents rickettsiens ou proches. Comptes rendus Hebd. des seances l 'academie des Sci. 4874–4876.

Grankvist, A., Andersson, P.O., Mattsson, M., Sender, M., Vaht, K., Höper, L., Sakiniene, E., Trysberg, E., Stenson, M., Fehr, J., Pekova, S., Bogdan, C., Bloemberg, G., Wennerås, C., 2014. Infections with the tick-borne bacterium “candidatus neoehrlichia mikurensis” mimic noninfectious conditions in patients with B cell malignancies or autoimmune diseases. Clin. Infect. Dis. 58, 1716–1722.

Gray, J., Kahl, O., Lane, R.S., Stanek, G., 2002. Lyme Borreliosis Biology, Epidemiology and Control.

Grönwall, O., Pehrson, A., 1984. Nutrient content in fungi as a primary food of the red squirrel Sciurus vulgaris L. Oecologia 64, 230–231.

Gubbels, J.M., De Vos, A.P., Van Der Weide, M., Viseras, J., Schouls, L.M., De Vries, E., Jongejan, F., 1999. Simultaneous detection of bovine Theileria and Babesia species by reverse line blot hybridization. J. Clin. Microbiol. 37, 1782–1789.

Gugliotta, J.L., Goethert, H.K., Berardi, V.P., Telford, S.R. 3rd, 2013. Meningoencephalitis from Borrelia miyamotoi in an immunocompromised patient. N. Engl. J. Med. 368, 240–245.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst. Biol. 59, 307–321.

Gurnell, J., 1987. The natural history of squirrels. Christopher Helm London.

Guy, E.C., Stanek, G., 1991. Detection of Borrelia burgdorferi in patients with Lyme disease by the polymerase chain reaction. J. Clin. Pathol. 44, 610–611.

Hamšíková, Z., Silaghi, C., Rudolf, I., Venclíková, K., Mahríková, L., Slovák, M., Mendel, J., Blažejová, H., Berthová, L., Kocianová, E., Hubálek, Z., Schnittger, L., Kazimírová, M.,

2016. Molecular detection and phylogenetic analysis of Hepatozoon spp. in questing Ixodes ricinus ticks and rodents from Slovakia and Czech Republic. Parasitol. Res. 115, 3897–3904.

Hanincová, K., Schäfer, S.M., Etti, S., Sewell, H.S., Taragelová, V., Ziak, D., Labuda, M., Kurtenbach, K., 2003a. Association of Borrelia afzelii with rodents in Europe. Parasitology 126, S0031182002002548.

Hanincová, K., Taragelová, V., Koci, J., Schäfer, S.M., Hails, R., Ullmann, A.J., Piesman, J., Labuda, M., Kurtenbach, K., 2003b. Association of Borrelia garinii and B. valaisiana with songbirds in Slovakia. Appl. Environ. Microbiol. 69, 2825–2830.

Hansford, K.M., Fonville, M., Jahfari, S., Sprong, H., Medlock, J.M., 2015. Borrelia miyamotoi in host-seeking Ixodes ricinus ticks in England. Epidemiol. Infect. 143, 1079–1087.

Harkness, L.M., Drohan, A.E., Dickson, C.M., Smith, T.G., 2010. Experimental transmission of Hepatozoon clamatae (Apicomplexa: Adeleida) to the wood frog, Rana sylvatica, and to the mosquito Culex pipiens. J. Parasitol. 96, 434–436.

Harris, D.J., Maia, J.P.M.C., Perera, A., 2011. Molecular characterization of Hepatozoon species in reptiles from the Seychelles. J. Parasitol. 97, 106–110.

Herwaldt, B.L., Cacciò, S., Gherlinzoni, F., Aspöck, H., Slemenda, S.B., Piccaluga, P., Martinelli, G., Edelhofer, R., Hollenstein, U., Poletti, G., Pampiglione, S., Löschenberger, K., Tura, S., Pieniazek, N.J., 2003. Molecular Characterization of a Non – Babesia divergens Organism Causing Zoonotic Babesiosis in Europe. Emerg. Infect. Dis. 9, 94294–8.

Heylen, D., Tijsse, E., Fonville, M., Matthysen, E., Sprong, H., 2013. Transmission dynamics of Borrelia burgdorferi s.l. in a bird tick community. Environ. Microbiol. 15, 6636–73.

Hillyard, P., 1996. Ticks of North-West Europe. Field Studies Council, Shrewsbury.

Hornok, S., Földvári, G., Rigó, K., Meli, M.L., Tóth, M., Molnár, V., Gönczi, E., Farkas, R., Hofmann-Lehmann, R., 2014. Vector-borne agents detected in fleas of the northern white-breasted hedgehog. Vector Borne Zoonotic Dis. 14, 74–76.

Hornok, S., Meli, M.L., Gönczi, E., Hofmann-Lehmann, R., 2013. First evidence of Candidatus Neoehrlichia mikurensis in Hungary. Parasit. Vectors 6, 267.

Hornok, S., Sándor, A.D., Beck, R., Farkas, R., Beati, L., Kontschán, J., Takács, N., Földvári, G., Silaghi, C., Meyer-Kayser, E., Hodžić, A., Tomanović, S., Abdullah, S., Wall, R., Estrada-Peña, A., Duscher, G.G., Plantard, O., 2017. Contributions to the phylogeny of Ixodes (Pholeoixodes) canisuga, I. (Ph.) kaiseri, I. (Ph.) hexagonus and a simple pictorial key for the identification of their females. Parasit. Vectors 10, 1–12.

Hovius, J.W.R., de Wever, B., Sohne, M., Brouwer, M.C., Coumou, J., Wagemakers, A., Oei, A., Knol, H., Narasimhan, S., Hodiamont, C.J., Jahfari, S., Pals, S.T., Horlings, H.M., Fikrig, E., Sprong, H., van Oers, M.H.J., 2013. A case of meningoencephalitis by the

relapsing fever spirochaete Borrelia miyamotoi in Europe. Lancet 382, 658.

Hubbard, M.J., Baker, A.S., Cann, K.J., 1998. Distribution of Borrelia burgdorferi s.l. spirochaete DNA in British ticks (Argasidae and Ixodidae) since the 19th century, assessed by PCR. Med. Vet. Entomol. 12, 89–97.

Humair, P.F., Gern, L., 2000. The wild hidden face of Lyme borreliosis in Europe. Microbes Infect. 2, 915–922.

Humair, P.F., Gern, L., 1998. Relationship between Borrelia burgdorferi sensu lato species, red squirrels (Sciurus vulgaris) and Ixodes ricinus in enzootic areas in Switzerland. Acta Trop. 69, 213–227.

Humair, P.F., Peter, O., Wallich, R., Gern, L., 1995. Strain Variation of Lyme-Disease Spirochetes Isolated From Ixodes-Ricinus Ticks and Rodents Collected in 2 Endemic Areas in Switzerland. J. Med. Entomol. 32, 433–438.

Humair, P.F., Postic, D., Wallich, R., Gern, L., 1998. An avian reservoir (Turdus merula) of the Lyme borreliosis spirochetes. Zentralbl. Bakteriol. 287, 521–538.

Humair, P.F., Rais, O., Gern, L., 1999. Transmission of Borrelia afzelii from Apodemus mice and Clethrionomys voles to Ixodes ricinus ticks: differential transmission pattern and overwintering maintenance. Parasitology 118 (Pt 1, 33–42.

Humair, P.F., Turrian, N., Aeschlimann, A., Gern, L., 1993. Ixodes ricinus immatures on birds in focus of Lyme borreliosis. Folia Parasitol ogyca 40, 237–242.

Jackson, D.B., Green, R.E., 2000. The importance of the introduced hedgehog (Erinaceus europaeus) as a predator of the eggs of waders (Charadrii) on machair in South Uist, Scotland. Biol. Conserv. 93, 333–348.

Jado, I., Oteo, J.A., Aldámiz, M., Gil, H., Escudero, R., García-amil, C., Rodríguez-Moreno, I., Anda, P., 2007. Rickettsia monacensis and Human Disease, Spain. Emerg. Infect. Dis. 13, 1405–1407.

Jaenson, T.G.T., Jaenson, D.G.E., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden. Parasit. Vectors 5, 8.

Jahfari, S., Coipan, E.C., Fonville, M., Van Leeuwen, A.D., Hengeveld, P., Heylen, D., Heyman, P., Van Maanen, C., Butler, C.M., Földvári, G., Szekeres, S., Van Duijvendijk, G., Tack, W., Rijks, J.M., Van Der Giessen, J., Takken, W., Van Wieren, S.E., Takumi, K., Sprong, H., 2014. Circulation of four Anaplasma phagocytophilum ecotypes in Europe. Parasit. Vectors 7, 365.

Jahfari, S., Fonville, M., Hengeveld, P., Reusken, C., Scholte, E.-J., Takken, W., Heyman, P., Medlock, J., Heylen, D., Kleve, J., Sprong, H., 2012. Prevalence of Neoehrlichia mikurensis in ticks and rodents from North-west Europe. Parasit. Vectors 5, 74.

Jahfari, S., Ruyts, S.C., Frazer-Mendelewska, E., Jaarsma, R., Verheyen, K., Sprong, H.,

2017. Melting pot of tick-borne zoonoses: the European hedgehog contributes to the maintenance of various tick-borne diseases in natural cycles urban and suburban areas. Parasit. Vectors 10, 134.

Jansen, A., La Scola, B., Raoult, D., Lierz, M., Wichmann, O., Stark, K., Schneider, Thomas, 2008. Antibodies against Rickettsia spp. in Hunters, Germany. Emerg. Infect. Dis. 14, 1961–1963.

Jia, N., Zheng, Y., Huo, Q., Ni, X., Jiang, B., Chu, Y., Jiang, R., Jiang, J., Cao, W., 2014. Human Infections with Rickettsia rauoltii, China. Emerg. Infect. Dis. 20, 8–10.

Jones, C., Norbury, G., 2010. Feeding selectivity of introduced hedgehogs Erinaceus europaeus in a dryland habitat, South Island, New Zealand. Acta Theriol. (Warsz). 56, 45–51.

Kahl, O., Gern, L., Eisen, L., Lane, R.S., 2002. Ecological Research on Borrelia burgdorferi sensu lato: Terminology and Some Methodological Pitfalls, in: Gray, J., Kahl, O., Lane, R.S., Stanek, G. (Eds.), Lyme Borreliosis: Biology, Epidemiology and Control. CABI, Bristol, pp. 29–46.

Kahl, O., Janetzki, C., Gray, J.S., Stein, J., Bauch, R.J., 1992. Tick infection rates with Borrelia: Ixodes ricinus versus Haemaphysalis concinna and Dermacentor reticulatus in two locations in eastern Germany. Med Vet Entomol 6, 363–366.

Karbowiak, G., Rychlik, L., Nowakowski, W., Wita, I., 2005. Natural infections of small mammals with blood parasites on the borderland of boreal and temperate forest zones. Acta Theriol. (Warsz). 50, 31–42.

Kawahara, M., Rikihisa, Y., Isogai, E., Takahashi, M., Misumi, H., Suto, C., Shibata, S., Zhang, C., Tsuji, M., 2004. Ultrastructure and phylogenetic analysis of “Candidatus Neoehrlichia mikurensis” in the family Anaplasmataceae, isolated from wild rats and found in Ixodes ovatus ticks. Int. J. Syst. Evol. Microbiol. 54, 1837–43.

Khanakah, G., Kocianová, E., Vyrosteková, V., Rehácek, J., Kundi, M., Stanek, G., 2006. Seasonal variations in detecting Borrelia burgdorferi sensu lato in rodents from north eastern Austria. Wien. Klin. Wochenschr. 118, 754–758.

Kiewra, D., Stańczak, J., Richter, M., 2014. Ixodes ricinus ticks (Acari, Ixodidae) as a vector of Borrelia burgdorferi sensu lato and Borrelia miyamotoi in Lower Silesia, Poland--preliminary study. Ticks Tick. Borne. Dis. 5, 892–7.

Kiffner, C., Vor, T., Hagedorn, P., Niedrig, M., Rühe, F., 2011. Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. Parasitol. Res. 108, 323–335.

Krampitz, H.E., 1981. Development of Hepatozoon erhardovae Krampitz , 1964 (Protozoa : in experimental mammalian and arthropod II . Sexual development in fleas and sporozoite indices in xenodiagnosis *. Trans. R. Soc. Trop. Med. Hyg. 75, 155–157.

Krampitz, H.E., Wongchari, V., 1980. The development of Hepatozoon erhardovae in experimental mammalian and arthropod hosts. 1. Evaluation of suitable arthropod vectors., in: Fleas. Proceedings of the International Conference on Fleas, Ashton Wold, Peterborough, UK, 21-25 June 1977. AA Balkema., pp. 349–358.

Krause, P.J., Narasimhan, S., Wormser, G.P., Rollend, L., Fikrig, E., Lepore, T., Barbour, A., Fish, D., 2013. Human Borrelia miyamotoi Infection in the United States. N Engl J Med 368, 291–293.

Krawczyk, A.I., van Leeuwen, A.D., Jacobs-Reitsma, W., Wijnands, L.M., Bouw, E., Jahfari, S., van Hoek, A.H. a M., van der Giessen, J.W.B., Roelfsema, J.H., Kroes, M., Kleve, J., Dullemont, Y., Sprong, H., de Bruin, A., 2015. Presence of zoonotic agents in engorged ticks and hedgehog faeces from Erinaceus europaeus in (sub) urban areas. Parasit. Vectors 8.

Kubo, N., Arashima, Y., Yoshida, M., Kawabata, M., Nishinarita, S., Hayama, T., Sawada, S., Horie, T., Nakao, M., Miyamoto, K., Kawano, K., 1992. Qestionnaire Surveys of Cases of Tick Bite and Lyme Borreliosis in Hunters in Hokkaido with Reference to Detection of Anti-Borrelia burgdorferi Antibody. Intern. Med. 31, 1163–1168.

Kurtenbach, K., Carey, D., Hoodless, a N., Nuttall, P. a, Randolph, S.E., 1998a. Competence of pheasants as reservoirs for Lyme disease spirochetes. J. Med. Entomol. 35, 77–81.

Kurtenbach, K., Peacey, M., Rijpkema, S.G.T., Hoodless, N., Nuttall, P. a, Randolph, S.E., Hoodless, A.N., 1998b. Differential Transmission of the Genospecies of Borrelia burgdorferi Sensu Lato by Game Birds and Small Rodents in England Differential Transmission of the Genospecies of Borrelia burgdorferi Sensu Lato by Game Birds and Small Rodents in England. Appl. Environ. Microbiol. 64, 1169–1174.

Kurtenbach, K., Sewell, H.S., Ogden, N.H., Randolph, S.E., Nuttall, P.A., 1998c. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. 66, 1248–1251.

Kybicová, K., Baštová, K., Malý, M., 2017. Detection of Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in questing ticks Ixodes ricinus from the Czech Republic. Ticks Tick. Borne. Dis. 8, 483–487.

Laakkonen, J., Sukura, A., Oksanen, A., Henttonen, H., Soveri, T., 2001a. Haemogregarines of the genus Hepatozoon (Apicomplexa: Adeleina) in rodents from northern Europe. Folia Parasitol. (Praha). 48, 263–267.

Laakkonen, J., Sukura, a, Oksanen, a, Henttonen, H., Soveri, T., 2001b. Haemogregarines of the genus Hepatozoon (Apicomplexa: Adeleina) in rodents from northern Europe. Folia Parasitol. (Praha). 48, 263–7.

Lakos, A., 2009. Lyme borreliosis-lessons learnt from 25 years. Orv. Hetil. 150, 725–32.

Lakos, A., Igari, Z., Solymosi, N., 2012. Recent lesson from a clinical and seroepidemiological survey: low positive predictive value of Borrelia burgdorferi antibody testing in a high risk population. Adv. Med. Sci. 57, 356–363.

Lanszki, J., 2003. Feeding habits of stone martens in a Hungarian village and its surroundings. Folia Zool. 52, 367–377.

Lanszki, J., Körmendi, S., Hancz, C., Zalewski, A., 1999. Feeding habits and trophic niche overlap in a Carnivora community of Hungary. Acta Theriol. (Warsz). 4, 429–442.

Li, H., Jiang, J., Liu, W., Zheng, Y., Huo, Q., Tang, K., Zuo, S., Liu, K., Jiang, B., Yang, H., Cao, W., 2012. Human Infection with Candidatus Neoehrlichia mikurensis, China. Emerg. Infect. Dis. 18, 10–13.

Liebisch, G., Sohns, B., Bautsch, W., 1998. Detection and typing of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks attached to human skin by PCR. J. Clin. Microbiol. 36, 3355335–8.

Lorusso, V., Lia, R.P., Dantas-Torres, F., Mallia, E., Ravagnan, S., Capelli, G., Otranto, D., 2011. Ixodid ticks of road-killed wildlife species in southern Italy: new tick-host associations and locality records. Exp. Appl. Acarol. 55, 293–300.

Majláthová, V., Majláth, I., Derdáková, M., Víchová, B., Pet, B., 2006. Borrelia lusitaniae and Green Lizards (Lacerta viridis), Karst Region, Slovakia. Emerg. Infect. Dis. 12, 1895–1901.

Marié, J.-L., Davoust, B., Socolovschi, C., Raoult, D., Parola, P., 2011. Molecular detection of rickettsial agents in ticks and fleas collected from a European hedgehog (Erinaceus europaeus) in Marseilles, France. Comp. Immunol. Microbiol. Infect. Dis. 35, 77–79.

Matuschka, F.-R., Endepols, S., Richter, D., Spielman, A., 1997. Competence of Urban Rats as Reservoir Hosts for Lyme Disease Spirochetes. J. Med. Entomol. 34, 489–493.

Mayer, J., 1992. The vertebrates of Béda-Karapancsa landscape protection area, South Hungary. Dunántúli Dolg. Természetudományi Sor. 6, 257–272.

McDonald, R.A., Lariviere, S., 2001. Diseases and pathogens of mustela spp., with special reference to the biological control of introduced stoat Mustela erminea populations in New Zealand. J. R. Soc. New Zeal. 31, 721–744.

Medlock, J.M., Hansford, K.M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J.-C., Golovljova, I., Jaenson, T.G.T., Jensen, J.-K., Jensen, P.M., Kazimirova, M., Oteo, J. a, Papa, A., Pfister, K., Plantard, O., Randolph, S.E., Rizzoli, A., Santos-Silva, M.M., Sprong, H., Vial, L., Hendrickx, G., Zeller, H., Van Bortel, W., 2013. Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. Parasit. Vectors 6, 1.

Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., BÃ¸dker, R., Fach, P.,

Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. Front. Cell. Infect. Microbiol. 4, 103.

Moller, H., 1983. Foods and foraging behaviour of Red (Sciurus vulgaris) and Grey (Sciurus carolinensis) squirrels. Mamm. Rev. 13, 81–98.

Morán Cadenas, F., Rais, O., Humair, P.-F., Douet, V., Moret, J., Gern, L., 2007. Identification of host bloodmeal source and Borrelia burgdorferi sensu lato in field-collected Ixodes ricinus ticks in Chaumont (Switzerland). J. Med. Entomol. 44, 1109–1117.

Morris, P., 1973. Winter nests of the hedgehog (Erinaceus europaeus L.). Oecologia 11, 299–313.

Norman, A.F., Regnery, R., Jameson, P., Greene, C., Krause, D.C., 1995. Differentiation of Bartonella-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J. Clin. Microbiol. 33, 1797–1803.

Nosek, J., Sixl, W., 1972. Central-European Ticks (Ixodoidea) - Key for determination-. Mitt. Abt. Zool. Landesmus. Joanneum 1, 61–92.

Nováková, M., Víchová, B., Majláthová, V., Lesnáková, A., Pochybová, M., Pe'tko, B., 2010. First case of human granulocytic anaplasmosis from Slovakia. Ann Agric Env. Med 17, 173–175.

Nuttall, P. a, Paesen, G.C., Lawrie, C.H., Wang, H., 2000. Vector-host interactions in disease transmission. J. Mol. Microbiol. Biotechnol. 2, 381–386.

Oliver, J.H., 1989. Biology and systematics of ticks (Acari: Ixodida). Annu. Rev. Ecol. Syst. 20, 397–430.

Ostfeld, R.S., Levi, T., Jolles, A.E., Martin, L.B., Hosseini, P.R., Keesing, F., 2014. Life History and Demographic Drivers of Reservoir Competence for Three Tick-Borne Zoonotic Pathogens. PLoS One 9, e107387.

Oteo, J. a, Portillo, A., 2012. Tick-borne rickettsioses in Europe. Ticks Tick. Borne. Dis. 3, 271–278.

Overzier, E., Pfister, K., Thiel, C., Herb, I., Mahling, M., Silaghi, C., 2013. Diversity of Babesia and Rickettsia Species in Questing Ixodes ricinus : 13.

Pekova, S., Vydra, J., Kabickova, H., Frankova, S., Haugvicova, R., Mazal, O., Cmejla, R., Hardekopf, D.W., Jancuskova, T., Kozak, T., 2011. Candidatus Neoehrlichia mikurensis infection identified in 2 hematooncologic patients: Benefit of molecular techniques for rare pathogen detection. Diagn. Microbiol. Infect. Dis. 69, 266–270.

Pfäffle, M., Petney, T., Elgas, M., Skuballa, J., Taraschewski, H., 2009. Tick-induced blood loss leads to regenerative anaemia in the European hedgehog (Erinaceus europaeus). Parasitology 136, 443–452.

Pfäffle, M., Petney, T., Skuballa, J., Taraschewski, H., 2011. Comparative population

dynamics of a generalist (Ixodes ricinus) and specialist tick (I. hexagonus) species from European hedgehogs. Exp. Appl. Acarol. 54, 151–164.

Pinto, C.M., Helgen, K.M., Fleischer, R.C., Perkins, S.L., 2013. *Hepatozoon* Parasites (Apicomplexa: Adeleorina) in Bats. J. Parasitol. 99, 722–724.

Pisanu, B., Chapuis, J.-L., Dozières, A., Basset, F., Poux, V., Vourc'h, G., 2014. High prevalence of Borrelia burgdorferi s.l. in the European red squirrel Sciurus vulgaris in France. Ticks Tick. Borne. Dis. 5, 1–6.

Platonov, A.E., Karan, L.S., Kolyasnikova, N.M., Makhneva, N.A., Toporkova, M.G., Maleev, V. V, Fish, D., Krause, P.J., 2011. Humans Infected with Relapsing Fever Spirochete Borrelia miyamotoi,Russia. Emerg. Infect. Dis. 17, 1816–1823.

Platonov, A.E., Maleev, V. V, Karan', L.S., 2010. Relapsing borrelioses fevers: forgotten and new ones. Ter. Arkh. 82, 74–80.

Pugliese, A., Beltramo, T., Torre, D., 2007. Seroprevalence study of Tick-borne encephalitis , Borrelia burgdorferi , Dengue and Toscana virus in Turin Province. Cell Biochem. Funct. 185–188.

Ramos, J.M., Jado, I., Padilla, S., Masiá, M., Anda, P., Gutiérrez, F., 2013. Human infection with Rickettsia sibirica mongolitimonae, Spain, 2007-2011. Emerg. Infect. Dis. 19, 267–269.

Rao, H.X., Yu, J., Guo, P., Ma, Y.C., Liu, Q.Y., Jiao, M., Ma, Z.W., Ge, H., Wang, C.X., Song, X.P., Shi, Y., Li, D.M., 2015. Bartonella Species Detected in the Plateau Pikas (Ochotona curzoiae) from Qinghai Plateau in China. Biomed. Environ. Sci.

Raoult, D., Birtles, R.J., Montoya, M., Perez, E., Tissot-Dupont, H., Roux, V., Guerra, H., 1999. Survey of three bacterial louse-associated diseases among rural Andean communities in Peru: prevalence of epidemic typhus, trench fever, and relapsing fever. Clin. Infect. Dis. 29, 434–436.

Reeve, N., 1994. Hedgehogs. T & AD Poyser (Natural History), London.

Reppert, E., Galindo, R.C., Breshears, M.A., Kocan, K.M., Blouin, E.F., de la Fuente, J., 2013. Demonstration of Transplacental Transmission of a Human Isolate of Anaplasma phagocytophilum in an Experimentally Infected Sheep. Transbound. Emerg. Dis. 60, 93–96.

Richter, D., Schlee, D.B., Matuschka, F.-R., 2003. Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent. Emerg. Infect. Dis. 9, 697–701.

Richter, D., Schlee, D.B., Matuschka, F.R., 2011. Reservoir competence of various rodents for the Lyme disease spirochete Borrelia spielmanii. Appl. Environ. Microbiol. 77, 3565–3570.

Rigó, K., Gyuranecz, M., Tóth, A.G., Földvári, G., 2011. Detection of Borrelia burgdorferi Sensu Lato and Anaplasma phagocytophilum in small mammals and ectoparasites in

Hungary. Vector Borne Zoonotic Dis. 11, 1499–501.

Rigó, K., Majoros, G., Szekeres, S., Molnár, I., Jablonszky, M., Majláthová, V., Majláth, I., Földvári, G., 2016. Identification of Hepatozoon erhardovae Krampitz, 1964 from bank voles (Myodes glareolus) and fleas in Southern Hungary. Parasitol. Res. 115.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubalek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Spitalska, E., Kazimi-rová, M., 2014. Ixodes ricinus and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Front. Public Heal. 2, 251.

Rosický, B., 1957. Fauna of CSR. Volume 10, Fleas - Aphaniptera. 10, 439.

Rózsa, L., Reiczigel, J., Majoros, G., 2000. Quantifying parasites in samples of hosts. J. Parasitol. 86, 228–232.

Ruyts, S.C., Frazer-Mendelewska, E., Van Den Berge, K., Verheyen, K., Sprong, H., 2017. Molecular detection of tick-borne pathogens Borrelia afzelii, Borrelia miyamotoi and Anaplasma phagocytophilum in Eurasian red squirrels (Sciurus vulgaris). Eur. J. Wildl. Res. 63, 9–12.

Sato, K., Takano, A., Konnai, S., Nakao, M., Ito, T., Koyama, K., Kaneko, M., Ohnishi, M., Kawabata, H., 2014. Human infections with Borrelia miyamotoi, Japan. Emerg. Infect. Dis. 20, 1391–1393.

Schouls, L.M., Pol, I. Van De, Rijpkema, S.G.T., Schot, C.S., 1999. Detection and Identification of Ehrlichia , Borrelia burgdorferi Sensu Lato , and Bartonella Species in Dutch Ixodes ricinus Ticks Detection and Identification of Ehrlichia , Borrelia burgdorferi Sensu Lato , and Bartonella Species in Dutch Ixodes ricinus. J. Clin. Microbiol. 37, 2215–2222.

Scoles, G.A., Papero, M., Beati, L., Fish, D., 2001. A Relapsing Fever Group Spirochete Transmitted by Ixodes scapularis Ticks. Vector Borne Zoonotic Dis. 1, 21–34.

Sebek, Z., 1978. Blood Parasites of Small Mammals in Western Hungary. Parasitol. Hung.

Silaghi, C., Skuballa, J., Thiel, C., Pfister, K., Petney, T., Pfäffle, M., Taraschewski, H., Passos, L.M.F., 2011. The European hedgehog (Erinaceus europaeus) – A suitable reservoir for variants of Anaplasma phagocytophilum? Ticks Tick. Borne. Dis. 3, 49–54.

Silaghi, C., Woll, D., Mahling, M., Pfister, K., Pfeffer, M., 2012. Candidatus Neoehrlichia mikurensis in rodents in an area with sympatric existence of the hard ticks Ixodes ricinus and Dermacentor reticulatus, Germany. Parasit. Vectors 5, 285.

Skuballa, J., Oehme, R., Hartelt, K., Petney, T., Bücher, T., Kimmig, P., Taraschewski, H., 2007. European Hedgehogs as Hosts for Borrelia spp., Germany. Emerg. Infect. Dis. 13, 952–953.

Skuballa, J., Petney, T., Pfäffle, M., Oehme, R., Hartelt, K., Fingerle, V., Kimmig, P., Taraschewski, H., 2012. Occurrence of different Borrelia burgdorferi sensu lato

genospecies including B. afzelii, B. bavariensis, and B. spielmanii in hedgehogs (Erinaceus spp.) in Europe. Ticks Tick. Borne. Dis. 3, 8–13.

Sloboda, M., Kamler, M., Bulantová, J., Votýpka, J., Modrý, D., 2007. A new species of Hepatozoon (Apicomplexa: Adeleorina) from Python regius (Serpentes: Pythonidae) and its experimental transmission by a mosquito vector. J. Parasitol. 93, 1189–1198.

Smith, T.G., 1996. The Genus Hepatozoon (Apicomplexa : Adeleina). J. Parasitol. 82, 565–585.

Socolovschi, C., Mediannikov, O., Raoult, D., Parola, P., 2009. The relationship between spotted fever group Rickettsiae and Ixodid ticks. Vet. Res. 40, 1–20.

Sonenshine, D., Roe, R. (Eds.), 2014. Biology of Ticks. Oxford University Press, Oxford, UK.

Spitalská, E., Boldis, V., Derdáková, M., Selyemová, D., Rusnáková Tarageľová, V., 2014. Rickettsial infection in Ixodes ricinus ticks in urban and natural habitats of Slovakia. Ticks Tick. Borne. Dis. 5, 161–165.

Stanek, G., Fingerle, V., Hunfeld, K.-P., Jaulhac, B., Kaiser, R., Krause, A., Kristoferitsch, W., O'Connell, S., Ornstein, K., Strle, F., Gray, J., 2011. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin. Microbiol. Infect. 17, 69–79.

Stanek, G., Wormser, G.P., Gray, J., Strle, F., 2012. Lyme borreliosis. Lancet 379, 461–473.

Stenmarck, Å., Jensen, C., Quested, T., Moates, G., 2016. Estimates of European food waste levels.

Stenos, J., Graves, S.R., Unsworth, N.B., 2005. A highly sensitive and specific real-time PCR assay for the detection of spotted fever and typhus group Rickettsiae. Am. J. Trop. Med. Hyg. 73, 1083–1085.

Stuen, S., Granquist, E.G., Silaghi, C., 2013. Anaplasma phagocytophilum--a widespread multi-host pathogen with highly adaptive strategies. Front. Cell. Infect. Microbiol. 3, 31.

Szabó, I., 1975. Fleas – Siphonaptera. Fauna Hungariae 15, 1–97.

Szekeres, S., Coipan, E.C., Rigó, K., Majoros, G., Jahfari, S., Sprong, H., 2015a. Candidatus Neoehrlichia mikurensis and Anaplasma phagocytophilum in natural rodent and tick communities in Southern Hungary. Ticks Tick Borne Dis 6.

Szekeres, S., Coipan, E.C., Rigó, K., Majoros, G., Jahfari, S., Sprong, H., Földvári, G., 2015b. Eco-epidemiology of Borrelia miyamotoi and Lyme borreliosis spirochetes in a popular hunting and recreational forest area in Hungary. Parasit. Vectors 8, 309.

Szekeres, S., Docters van Leeuwen, A., Rigó, K., Jablonszky, M., Majoros, G., Sprong, H., Földvári, G., 2016a. Prevalence and diversity of human pathogenic rickettsiae in urban versus rural habitats, Hungary. Exp. Appl. Acarol. 68, 223–226.

Szekeres, S., Majláthová, V., Majláth, I., Földvári, G., 2016b. Neglected hosts : the role of lacertid lizards and medium-sized mammals in the eco-epidemiology of Lyme borreliosis, in: Braks, M.A.H., van Wieren, S.E., Takken, W., Sprong, H. (Eds.), Ecology

and Prevention of Lyme Borreliosis (Ecology and Control of Vector-Borne Diseases, Volume 4). Wageningen Academic Publishers, Wageningen, pp. 103–126.

Taragelová, V., Koči, J., Hanincová, K., Kurtenbach, K., Derdáková, M., Ogden, N.H., Literák, I., Kocianová, E., Labuda, M., 2008. Blackbirds and song thrushes constitute a key reservoir of Borrelia garinii, the causative agent of borreliosis in Central Europe. Appl. Environ. Microbiol. 74, 1289–1293.

The R Development Core Team, 2010. R : A Language and Environment for Statistical Computing.

Tijsse-Klasen, E., Koopmans, M.P.G., Sprong, H., 2014. Tick-borne pathogen - reversed and conventional discovery of disease. Front. public Heal. 2, 73.

Tomanović, S., Chochlakis, D., Radulović, Z., Milutinović, M., Cakić, S., Mihaljica, D., Tselentis, Y., Psaroulaki, A., 2013. Analysis of pathogen co-occurrence in host-seeking adult hard ticks from Serbia. Exp. Appl. Acarol. 59, 367–376.

Tóth-Ronkay, M., Bajor, Z., Bárány, A., Földvári, G., Görföl, T., Halpern, B., Leél-Őssy, S., Mészáros, R., Péntek, A.L., Tóth, B., Tóth, Z., Vörös, J., 2015. Budapest, in: Kelcey, J.G. (Ed.), Vertebrates and Invertebrates of European Cities: Selected Non-Avian Fauna. Springer Verlag, Berlin, pp. 26–73.

Tóth, M., Bárány, A., Szenci, P., 2011. A nyest Budapesten *. ÁLLATTANI KÖZLEMÉNYEK 96, 39–59.

Tóth Apáthy, M., 1998. Data to the diet of the urban stone marten (Martes foina Erxleben) in Budapest. Opusc. Zool. 113–118.

Toutoungi, L.N., Gern, L., Aeschlimann, A., Debrot, S., 1991. A propos du genre Pholeoixodes, parasite des carnivores. Acarologia 32, 311–328.

Vichová, B., Majláthová, V., Nováková, M., Stanko, M., Hviščová, I., Pangrácová, L., Chrudimský, T., Čurlík, J., Petko, B., 2014. Anaplasma infections in ticks and reservoir host from Slovakia. Infect. Genet. Evol. 22, 265–272.

Vichová, B., Majláthová, V., Nováková, M., Straka, M., Petko, B., 2010. First Molecular Detection of Anaplasma phagocytophilum in European Brown Bear (Ursus arctos). VECTOR-BORNE ZOONOTIC Dis. 10, 543–545.

Visser, M., Rehbein, S., Wiedemann, C., 2001. Species of flea (Siphonaptera) infesting pets and hedgehogs in Germany. J. Vet. Med. Ser. B 48, 197–202.

Wagemakers, A., Staarink, P.J., Sprong, H., Hovius, J.W.R., 2015. Borrelia miyamotoi: a widespread tick-borne relapsing fever spirochete. Trends Parasitol. 31, 260–269.

Wauters, L.A., Dhondt, A.A., 1987. Activity budget and foraging behaviour of the red squirrel (Sciurus vulgaris Linnaeus, 1758) in a coniferous habitat. Zeitschrift für Säugetierkd. 52, 341–353.

Wauters, L., Swinnen, C., Dhondt, A.A., 1992. Activity budget and foraging behaviour of red

squirrels (Sciurus vulgaris) in coniferous and deciduous habitats. J. Zool. 227, 71–86.

Wirtgen, M., Nahayo, a, Linden, a, Losson, B., Garigliany, M., Desmecht, D., 2011. Detection of Anaplasma phagocytophilum in Dermacentor reticulatus ticks. Vet. Rec. 168, 195.

World's population increasingly urban with more than half living in urban areas [WWW Document], 2014. . United Nations.

Yabsley, M.J., Murphy, S.M., Luttrell, M.P., Wilcox, B.R., Howerth, E.W., Munderloh, U.G., 2008. Characterization of "Candidatus Neoehrlichia lotoris" (family Anaplasmataceae) from raccoons (Procyon lotor). Int. J. Syst. Evol. Microbiol. 58, 2794–2798.

Yalden, D.W., 1976. The food of the Hedgehog in England. Acta Theriol. (Warsz). 21, 401–424.

Ying, B., Kosoy, M.Y., Maupin, G.O., Tsuchiya, K.R., Gage, K.L., 2002. Genetic and ecologic characteristics of Bartonella communities in rodents in Southern China. Am. J. Trop. Med. Hyg. 66, 622–627.

Zöldi, V., Juhász, A., Nagy, C., Papp, Z., Egyed, L., 2013. Tick-borne encephalitis and Lyme disease in Hungary: the epidemiological situation between 1998 and 2008. Vector Borne Zoonotic Dis. 13, 256–265.

10. Scientific publications

Own full text publications in peer-reviewed with impact factor assigned

Papers in the topic of the dissertation:

Szekeres S., Lakos A., Földvári G.: *Borrelia miyamotoi*: egy újabb, humán patogén, kullancs által terjesztett, visszatérő lázat okozó baktérium, (*Borrelia miyamotoi*: a recently identified human pathogenic tick-borne relapsing fever spirochete) ORVOSI HETILAP 158: pp. 1124-1130. (2017) IF (2016): 0,349

Rigó K., Majoros G., **Szekeres S**., Molnár I., Jablonszky M., Majláthová V., Majláth I., Földvári G.: Identification of *Hepatozoon erhardovae* Krampitz, 1964 from bank voles (*Myodes glareolus*) and fleas in Southern Hungary, PARASITOLOGY RESEARCH 115:(6) pp. 2409-2413. (2016) IF: 2,329

Szekeres S., Docters van Leeuwen A., Rigó K., Jablonszky M., Majoros G., Sprong H., Földvári G.: Prevalence and diversity of human pathogenic rickettsiae in urban versus rural habitats, Hungary, EXPERIMENTAL AND APPLIED ACAROLOGY 68:(2) pp. 223-226. (2016) IF: 1,760

Szekeres S., Coipan E.C., Rigó K., Majoros G., Jahfari S., Sprong H., Földvári G.: *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* in natural rodent and tick communities in Southern Hungary, TICKS AND TICK-BORNE DISEASES 6: pp. 111-116. (2015) IF: 2,690

Szekeres S., Coipan E.C., Rigó K., Majoros G., Jahfari S., Sprong H., Földvári G.: Eco-epidemiology of *Borrelia miyamotoi* and Lyme borreliosis spirochetes in a popular hunting and recreational forest area in Hungary, PARASITES AND VECTORS 8: Paper 309. 8 p. (2015) IF:3,234

Földvári G., Jahfari S., Rigó K., **Szekeres S**., Jablonszky M., Majoros G., Tóth M., Molnár V., Coipan E.C., Sprong H.: *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* in urban hedgehogs, EMERGING INFECTIOUS DISEASES 20: pp. 496-498. (2014) IF: 6,751

Papers in other topics:

Reuter G., Boros Á., Földvári G., **Szekeres S**., Mátics R., Kapusinszky B., Delwart E., Pankovics P.: Dicipivirus (family Picornaviridae) in wild Northern white-breasted hedgehog (*Erinaceus roumanicus*), ARCHIVES OF VIROLOGY pp. 1-7. (2017) IF (2016): 1,813

Szekeres S., Lügner J., Fingerle V., Margos G., Földvári G.: Prevalence of *Borrelia miyamotoi* and *Borrelia burgdorferi* sensu lato in questing ticks from a recreational coniferous forest of East Saxony, Germany, TICKS AND TICK-BORNE DISEASES 8: pp. 920-927. (2017) IF(2016): 3,230

Földvári G., Siroky P., Majoros G., **Szekeres S**., Sprong H.: *Dermacentor reticulatus*: a vector on the rise, PARASITES AND VECTORS 9: Paper 314. 29 p. (2016) IF: 3,035

Jahfari S., Coipan E.C., Fonville M., Docters van Leeuwen A., Hengeveld P.D., Heylen D., Heyman P., van Maanen C., Butler C.M., Földvári G., **Szekeres S**., van Duijvendijk G., Tack W., Rijks J.M., van der Giessen J., Takken W., van Wieren S.E., Takumi K., Sprong H.: Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe, PARASITES AND VECTORS 7:(1) Paper 365. 11 p. (2014) IF: 3,430

Vilisics F., **Szekeres S**., Hornung E.: Size dependent differences in litter consumption of Isopods: preliminary results, ZOOKEYS 176: pp. 247-259. (2012) IF: 0,864

International scientific book chapter:

Szekeres S., Majláthová V., Majláth I., Földvári G.: Neglected hosts: The role of lacertid lizards and medium-sized mammals in the eco-epidemiology of Lyme borreliosis, In: M A H Braks, S E van Wieren, W Takken, H Sprong (ed.), Ecology and prevention of Lyme borreliosis. 300 p., Wageningen: Wageningen Academic Publishers, 2016. (Ecology and Control of Vector-borne diseases; 4.) (ISBN:978-90-8686-293-1), 2015

Oral presentation at international conferences:

Földvári G., **Szekeres S.**, Majoros G., Juhász A., Mándoki M., Hornok S., Fok É., Sprong H.: Tick global, act local. Emergence and elimination of a *Rickettsia massiliae*-infected *Rhipicephalus sanguineus* population in Central-Europe: lecture at the One Health 9th Tick and Tick-borne Pathogen Conference & 1st Asia Pacific Rickettsia Conference, Cairns, Australia, 2017.

Szekeres S., Docters van Leeuven A., Majoros G., Sprong H., Földvári G.: Vector-borne pathogens in accidentally died and road-killed mammals in urban habitats, Hungary: lecture at the One Health 9th Tick and Tick-borne Pathogen Conference & 1st Asia Pacific Rickettsia Conference, Cairns, Australia, 2017.

Földvári G., Jahfari S., Rigó K., Jablonszky M., **Szekeres S**., Majoros G., Tóth M., Molnár V., Coipan E.C., Sprong H.: Urban hedgehogs as potential risk factors for tick-borne zoonotic bacteria in a city park, Budapest, In: V4 Parasitological Meeting – Parasites in the Heart of Europe. Stará Lesna, Slovakia,

Földvári G., Jahfari S., Rigó K., Jablonszky M., **Szekeres S**., Majoros G., Tóth M., Molnár V., Coipan E.C., Sprong H.: Tick-borne zoonotic bacteria associated with urban hedgehogs in a city park, Budapest, In: 8th Ticks and Tick-borne Pathogens Conference. Cape Town, Republic of South Africa,

Szekeres S., Rigó K., Majoros G., Coipan E.C., Jahfari S., Sprong H., Földvári G.: Ticks and rodents with *Anaplasma phagocytophilum* and Candidatus Neoehrlichia mikurensis infection in Southern Hungary, V4 Parasitological Meeting – Parasites in the Heart of Europe, Stará Lesna, Slovakia,

Poster presentations at international conferences:

Szekeres S., Docters van Leeuwen A., Rigó K., Jablonszky M., Majoros G., Sprong H., Földvári G.: Differences in risk of rickettsial infection between rural and urban field-collected ticks in Hungary: poster at the One Health 9th Tick and Tick-borne Pathogen Conference & 1st Asia Pacific Rickettsia Conference, Cairns, Australia, 2017.

Szekeres S., Rigó K., Majoros G., Coipan E.C., Jahfari S., Sprong H., Földvári G.: Ticks and rodents with *Anaplasma phagocytophilum* and *Candidatus* Neoehrlichia mikurensis infection in Southern Hungary, In: 8th Ticks and Tick-borne Pathogens Conference. Cape Town, Republic of South Africa, 2017.

Földvári G., **Szekeres S**., Coipan E.C., Rigó K., Majoros G., Jahfari S., Sprong H.: Eco-epidemiology of *Borrelia miyamotoi* and Lyme borreliosis spirochetes in a popular hunting and recreational forest area in Hungary, 14th International Conference on Lyme Borreliosis and Other Tick-Borne Diseases, Vienna, Austria 2015

11. Acknowledgements

Firstly, I would like thank Gábor Földvári, who is my mentor from the beginning of my MSc thesis work. He gave me many opportunities to practice the substantial aspect of the researcher's life. It was my pleasure to go every time to field trips or conferences with him. He made useful suggestions for the preparation of my publications and my dissertation as well. He also supported me in my research trips, scholarships and conferences to foreign countries.

I would also like to thank Krisztina Rigó, who was the former PhD student of Gábor, also my supervisor during my MSc work. She taught me how to do dissection, PCR and other molecular practices and kindly helped me by the sample collection in different parts of my dissertation.Mónika Jablonszky who showed me some of the DNA extraction methods and also helped us in the sample collection.

My former parasitology teacher Gábor Majoros was always there when I had some questions about some interesting facts in the life of parasites, animals, geology etc. He and Alexandra Juhász helped us in sample collection from the natural and urban habitats. Gábor Majoros performed the morphological analysis of the rodent spleen smear samples.

To my Dutch friends, Hein Sprong and his team, Elena Claudia Coipan, Manoj Fonville, Arieke Docters van Leuween and Setareh Jahfari in the Netherlands was crucial pillar of my work. There I learned how to perform real-time PCR and sequence analysis on my samples. Hein Sprong helped me in my foreign research trips as supervisor, providing laboratory and laboratory equipment in the Netherlands. Dank je well!

I would like to thank also for the Hungarian-Slovakian (HuSk) conference team in Cape Town, Republic of South Africa and in Cairns, Australia for the best conferences of my life. Hi Bruce! (Shermanka, Irwinka, Doris, RalPhD and Bruce this greeting is just for You. 😊)

Zsolt Lang, my biomathematics and biostatistics teacher who was always helpful and supportive with the statistical analysis of the data.

Mária Ronkayné-Tóth and her many students and volunteers who helped us capturing hedgehogs on Margaret Island. Her help was essential in organising the sample collections with many participants.

The many volunteers who helped the road-killed sample collections all around Hungary from urban habitats.

Boglárka Krizsán, Mónika Gajdos, Evelin Tóth, Jen Lügner and Tamás Gabelics veterinary- and biology MSc students helped me in the procession of the samples (DNA extraction, PCR). Thanks a lot for the nice atmosphere during the laboratory works.

Professor Róbert Farkas the former head, nowadays the curator of the Department of Parasitology and Zoology, UVM who provided laboratory to perform the present research and a desk to work in the Department.

Sample collection was approved from both habitats by the Middle-Danube-Valley Inspectorate for Environmental Protection, Nature Conservation and Water Management, Hungary.

I thank Nóra Takács, Barbara Flaisz, Krisztina Szőke, Anikó Czene, Zsusza Bartos, Veronika Tóth and Balázs Tánczos for the nice working environment in the past and nowadays.

All my BSc, MSc classmates and many friends who were there when we struggled together and we needed each other the most.

I would like to thank for the financial support of the city council of Hajdúböszörmény, the NKB and Kutatókar grant of the UVM (former SZIU), the EurNegVec Cost Action TD1303, the Campus Hungary, Campus Mundi and Eötvös Scholarships.

Last but not least, I am grateful to my family who always supported and encouraged me to achieve my goals.