




# Ticks and their epidemiological role in Slovakia: from the past till present

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Received: 26 April 2021 / Accepted: 6 July 2021 / Published online: 17 September 2021  
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## Abstract

In Slovakia, 22 tick species have been found to occur to date. Among them, *Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus* and marginally *Haemaphysalis concinna*, *H. inermis* and *H. punctata* have been identified as the species of public health relevance. Ticks in Slovakia were found to harbour and transmit zoonotic and/or potentially zoonotic agents such as tick-borne encephalitis virus (TBEV), spirochaetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex, the relapsing fever spirochaete *Borrelia miyamotoi*, bacteria belonging to the orders Rickettsiales (*Rickettsia* spp., *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*), Legionellales (*Coxiella burnetii*), and Thiotrichales (*Francisella tularensis*), and *Babesia* spp. parasites (order Piroplasmida). *Ixodes ricinus* is the principal vector of the largest variety of microorganisms including viruses, bacteria and piroplasms. TBEV, *B. burgdorferi* s.l., rickettsiae of the spotted fever group, *C. burnetii* and *F. tularensis* have been found to cause serious diseases in humans, whereas *B. miyamotoi*, *A. phagocytophilum*, *N. mikurensis*, *Babesia microti*, and *B. venatorum* pose lower or potential risk to humans. Distribution of TBEV has a focal character. During the last few decades, new tick-borne encephalitis (TBE) foci and their spread to new areas have been registered and TBE incidence rates have increased. Moreover, Slovakia reports the highest rates of alimentary TBE infections among the European countries. Lyme borreliosis (LB) spirochaetes are spread throughout the distribution range of *I. ricinus*. Incidence rates of LB have shown a slightly increasing trend since 2010. Only a few sporadic cases of human rickettsiosis, anaplasmosis and babesiosis have been confirmed thus far in Slovakia. The latest large outbreaks of Q fever and tularaemia were recorded in 1993 and 1967, respectively. Since then, a few human cases of Q fever have been reported almost each year. Changes in the epidemiological characteristics and clinical forms of tularaemia have been observed during the last few decades. Global changes and development of modern molecular tools led to the discovery and identification of emerging or new tick-borne microorganisms and symbionts with unknown zoonotic potential. In this review, we provide a historical overview of research on ticks and tick-borne pathogens in Slovakia with the most important milestones and recent findings, and outline future directions in the investigation of ticks as ectoparasites and vectors of zoonotic agents and in the study of tick-borne diseases.

**Keywords** Tick-borne pathogens · Tick-borne encephalitis virus · *Borrelia* · *Anaplasma* · *Rickettsia* · *Coxiella burnetii* · *Francisella tularensis* · *Babesia*

## Introduction

Slovakia is located in the heart of Europe and has a long tradition in the research of the ecology of ticks and epidemiology of tick-borne diseases (TBD). The presence of 22 tick species has been confirmed in the country to date (Rosický 1953; Černý 1972; Nosek et al. 1982b; Bona and Stanko 2013; Capek et al. 2014; Karbowski et al. 2020; Didyk et al. 2021). Some of the recorded tick species, i.e., *Hyalomma marginatum* sensu lato, *Rhipicephalus sanguineus* sensu lato, and *Ixodes frontalis* (Panzer, 1798), have been transported by migratory birds to Central Europe and Slovakia or introduced

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by dogs while visiting the Mediterranean countries with their owners. However, the likelihood of establishing viable populations of the introduced species in Slovakia is rather low. A significant proportion of the Slovak tick fauna is represented by species with host specificity to selected groups of birds and mammals. Only randomly and rarely do they come into contact with humans, e.g. *Argas vespertilionis* Latreille, 1796, *Ixodes vespertilionis* Koch, 1844 and *Ixodes simplex* Neumann, 1906, parasitizing cave bats (Dusbábek 1972). All stages of *Ixodes trianguliceps* Birula, 1895 infest small mammals, *Ixodes laguri* Olenov, 1929 feeds on ground squirrels (Černý 1990), and *Ixodes apronophorus* Schulze, 1924 feeds mainly on the water vole *Arvicola amphibius* (Linnaeus, 1758) (Černý 1957; Černý 1972). Other examples of a narrow host specificity are e.g. the ornithophilic species *Ixodes arboricola* Schulze and Schlottke, 1929, parasitizing birds nesting in tree cavities or *Ixodes lividus* Koch, 1844, a specific parasite of the sand martin *Riparia riparia* (Linnaeus, 1758) nesting in river bank hollows and cavities (Filippova 1977; Krumpál et al. 1995; Estrada-Peña et al. 2017).

In epidemiological terms, considering their role as ectoparasites and vectors of pathogens for humans and domestic animals, in Slovakia there are six important tick species, namely *Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus*, *Haemaphysalis inermis*, *H. concinna* and *H. punctata*. All of them are external three-host ticks which are seeking for hosts on the vegetation. During their life cycle (larva - nymph - adult) they change their host groups (mainly different bird and mammalian species). This way, they can transmit different agents of viral, bacterial or protozoan infections by vertical and transstadial passage as well as horizontally.

## Brief history of tick research

In Slovakia, targeted and systematic research of ticks started after World War II. A significant number of comprehensive parasitological studies in the 1950s and early 1960s resulted from the research on the outbreak of tick-borne encephalitis (TBE) caused by tick-borne encephalitis virus (TBEV) in the natural focus in Rožňava in eastern (E) Slovakia. The TBE outbreak, reported in April 1951, was related to the consumption of raw milk. Research results were summarized in a monograph by Blaškovič (1954) and in other publications (e.g. Rosický et al. 1954; Bárdoš et al. 1954; Libíková et al. 1954). Since then, multidisciplinary teams involving virologists, zoologists, parasitologists, ecologists, epidemiologists and clinicians have explored natural foci of TBE and of other zoonoses caused by tick-borne bacteria and protozoan parasites. Simultaneously, an intensive research of bionomics, distribution and seasonal dynamics of *I. ricinus* (Mačička 1955; Korbel 1956; Nosek 1958) was initiated along with the research of, at that time less-known, species of the

*Dermacentor* and *Haemaphysalis* genera (Rosický 1952; Mačička et al. 1955; Mačička et al. 1956; Mačička 1958; Nosek 1972; Nosek 1973). The published studies offered also practical information on the seasonal patterns of tick infestation on domestic livestock and the predilection sites on their bodies (Mačička and Rosický 1956; Černý 1960). Another important reason for starting intensive research on the ecology and bionomics of ticks in that period was the effort of applied research with outputs in agricultural practice, especially in the breeding of domestic animals on pastures with reduced tick populations (Mačička and Rosický 1954; Rosický and Mačička 1955; Rosický and Mačička 1956; Mačička 1956). A specific series of studies dealt with the negative role of shrubs in pastures as they, in contrast to meadows, allow ticks to survive and thrive on (Rosický and Ryšavý 1955; Černý 1959).

Since the 1950s, dozens of papers have been published on the role of reptiles, birds and mammals as tick hosts and reservoirs of viral and bacterial pathogens in natural foci of diseases. The studies were carried out by researchers in various Slovak institutions, mainly institutes of the Slovak Academy of Sciences (Institute of Virology, Institute of Parasitology and Institute of Zoology), Comenius University (Medical Faculty, Faculty of Natural Sciences), University of Veterinary Medicine and Pharmacy, and others.

A number of contributions dealing with the role of ticks as vectors of TBEV and Tribeč virus were published (Libíková et al. 1964; Libíková et al. 1965; Grešíková and Nosek 1966; Grešíková and Nosek 1967; Grešíková et al. 1968; Kožuch et al. 1969a; Kožuch et al. 1973; Grešíková and Nosek 1981; Grešíková et al. 1983; Grešíková et al. 1986; Hubálek et al. 1986; Grešíková et al. 1987; Hubálek et al. 1987; Kožuch et al. 1987). Simultaneously, the roles of ticks as vectors and reservoirs of bacterial (*Francisella tularensis*, *Coxiella burnetii*, *Rickettsia* spp., *Anaplasma phagocytophilum* and *Borrelia* spp.) as well as protozoan (*Babesia* spp.) pathogens were studied and confirmed (e.g. Řeháček et al. 1970; Guryčová and Letkovský 1973; Řeháček et al. 1976; Guryčová et al. 1982; Kožuch et al. 1987; Kmety et al. 1987; Guryčová and Výrosteková 1989; Kmety et al. 1990; Výrosteková et al. 1991; Prokopčáková et al. 1992; Kocianová et al. 1993; Drgoňová and Řeháček 1995; Guryčová et al. 1995; Gern et al. 1999; Špitalská and Kocianová 2002; Špitalska et al. 2002; Smetanová et al. 2006; Blaňarová et al. 2016). Results of the most important tick-related research in former Czechoslovakia (i.e. Czech Republic and Slovakia) in the period after World War II, particularly focused on methods of tick sampling, bionomics of ticks and their survival and distribution in nature, occurrence of ticks in towns, predators and symbionts of ticks, usage of acaricides and repellents in tick control, were summarized in a monograph by Stanko and Slovák (2019).

In addition to investigations of natural foci of TBD, important research has been carried out in Slovakia on tick-host-pathogen interactions and mechanisms of pathogen, particularly TBEV transmission (Labuda et al. 1993c; Randolph et al. 1999). Since the 1990s, special attention has been paid to the exploration of the biological and pharmacological properties of tick saliva, which represents a rich source of diverse molecules that play roles in feeding and survival strategies of ticks and in transmission of pathogens, but can also be explored as potential sources to design pharmaceuticals for treatment of human diseases (Hajnická et al. 2001; Hajnicka et al. 2005; Koh et al. 2007; Hajnicka et al. 2011; Štibrániová et al. 2019). Slovak scientists have also been involved in the research of anti-tick and transmission blocking vaccines to control tick infestation and prevent transmission of TBD (Labuda et al. 2006; Rego et al. 2019). Studies of microbiomes of epidemiologically important tick species (*I. ricinus*, *D. reticulatus*, *D. marginatus*) (Kmet' and Čaplová 2019; Zhang et al. 2019a) and of the mitochondrial genome of *D. marginatus* by using next generation sequencing techniques have been initiated recently (Zhang et al. 2019b). And last but not least, Slovak scientists promoted the research of tick neuroendocrine networks, a field that was neglected in the past (Šimo et al. 2012; Šimo et al. 2014).

## Tick species of public health relevance

### *Ixodes ricinus* (Linnaeus, 1758)

*Ixodes ricinus*, the castor bean tick, is a generalist ectoparasite infesting a large number of mammalian, avian and reptilian species. It is the principal vector of a wide variety of pathogenic microorganism (viruses, bacteria, protozoans) (Černý 1972; Rizzoli et al. 2014) and is distributed throughout Europe, including Slovakia. Recently, the distribution area of *I. ricinus* in Europe has spread to higher altitudes and northern latitudes (Medlock et al. 2013). Bionomics, seasonal occurrence, vertical distribution, range of hosts for the developmental stages and of *I. ricinus* in Slovakia have been continually studied since the 1950s. It has been shown that individual developmental stages of *I. ricinus* infest a wide range of vertebrate species (Fig. 1). Larvae, nymphs and very rarely adult ticks were reported from 31 species of small mammals - rodents (Rodentia) and insectivores (Insectivora) (Rosický 1953; Turček 1954; Mačička 1955; Grulich 1960; Černý 1961; Kožuch et al. 1967a; Nosek et al. 1967b; Kiefer et al. 1981; Stanko and Ambros 1985; Kožuch et al. 1987; Labuda et al. 1989; Stanko and Ambros 1989; Kováčik and Dudich 1990; Stanko 1995; Stanko and Miklisová 1995; Hanincová et al. 2003a; Minichová et al. 2017). In addition, larvae and nymphs were found to infest four species of lizards (Grulich et al. 1957; Černý 1961; Řeháček et al. 1961; Lác et al. 1971; Majláth et al. 1998; Majláthová et al. 2006; Václav et al. 2011;

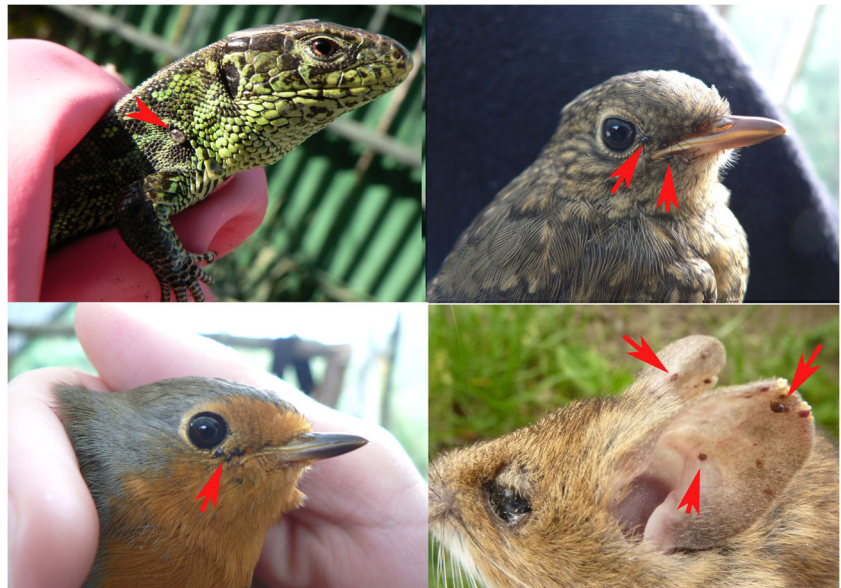
Kočiková et al. 2018) and more than 50 bird species (Rosický 1953; Rosický and Balát 1954; Turček 1954; Černý 1961; Nosek et al. 1967b; Ernek et al. 1968; Černý 1972; Nosek et al. 1972b; Hanincová et al. 2003b; Taragel'ová et al. 2005; Špitalská et al. 2006; Berthová et al. 2016). Carnivores (mustelids, foxes, badgers), wild ungulates (Artiodactyla) such as roe deer *Capreolus capreolus* (Linnaeus, 1758), fallow deer *Dama dama* (Linnaeus, 1758), red deer *Cervus elaphus* Linnaeus, 1758, mouflon *Ovis gmelini musimon* Pallas, 1762, wild boar *Sus scrofa* Linnaeus, 1758, as well as domestic animals (especially cattle, horses, sheep, goats, dogs) play an important role as hosts of all developmental stages of *I. ricinus*, but especially adults (Rosický et al. 1954; Turček 1953; Turček 1954; Mačička and Nosek 1958; Černý 1960; Černý 1961; Rosický et al. 1961; Nosek et al. 1967b; Nosek et al. 1972b; Černý 1975; Peťko and Stanko 1991; Smetanová et al. 2006; Stefanidesova et al. 2008; Kazimírová et al. 2018).

*Ixodes ricinus* may show a unimodal or bimodal pattern of seasonality. The character of seasonal activity is significantly influenced by microclimatic factors, especially by temperature, air humidity and soil moisture, mainly during summer. In most areas it presents a bimodal pattern with adults and nymphs peaking in spring (April, May) and in autumn (September, October). Higher peaks usually prevail during spring months (Černý et al. 1965; Řeháček 1981; Koči et al. 2009; Pangráčová et al. 2013; Kazimírová et al. 2016).

*Ixodes ricinus* was found to occur in all suitable types of habitats with sufficient humidity, i.e. in deciduous and mixed forests and shrubland. Its expansion to urban parks and gardens and increasing population densities in urban and suburban habitats have also been observed during the last decade (Pangráčová et al. 2013; Kazimírová et al. 2016; Rosà et al. 2018). Up to altitudes of 600–800 m asl., the occurrence of *I. ricinus* is abundant, but above 1000 m a.s.l. it is rare, although the upper limit of its distribution in Slovakia has moved up to 1250 m since the 1980s (Lukan et al. 2010; Peťko et al. 2011). However, ticks feeding on hosts, particularly small mammals and hunted animals, were recorded up to the elevation of 1600 m asl. (Rosický 1953; Mačička 1955; Dyk 1957; Bárdoš et al. 1959; Černý 1972; Nosek and Krippel 1974).

In Slovakia, *I. ricinus* was confirmed as vector of a variety zoonotic disease agents, i.e. TBEV, spirochaetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex, rickettsiae of the spotted fever group (SFG) (*Rickettsia helvetica*, *R. monacensis*), *C. burnetii* and *F. tularensis*, and of emerging and neglected pathogens that pose potential risk to humans, i.e. *Borrelia miyamotoi*, *A. phagocytophilum*, *Neoehrlichia mikurensis*, *Babesia microti* and *B. venatorum*.

**Fig. 1** Infestation of vertebrate hosts with larvae and nymphs of *Ixodes ricinus* (red arrows indicate the position of the ticks). *Lacerta agilis* (top left), *Turdus* sp. (top right), *Erithacus rubecula* (bottom left), *Apodemus flavicollis* (bottom right). Photos: Veronika Rusňáková Taragel'ová, Michal Stanko



#### *Dermacentor* spp.

In parallel with the research on *I. ricinus*, investigations on the bionomics and ecology of species of the genera *Dermacentor* and *Haemaphysalis* were also carried out in Slovakia. This research was especially interesting, because these species did not occur in Bohemia, or occurred locally in southern Moravia (*D. reticulatus* and *H. concinna*). Attention was paid to the geographic distribution of *Dermacentor* spp., their seasonal dynamics and host spectra of individual developmental stages. Both, the ornate dog tick, *D. reticulatus*, and the ornate sheep tick, *D. marginatus*, have ecologically limited and mosaic-like patterns of distribution, but the ecological requirements of the two species differ. While *D. reticulatus* prefers a moderately moist climate at more northern latitudes, *D. marginatus* is adapted to a warmer and drier climate at more southern latitudes in Europe; the distribution of the two species was found to overlap between 41–51° N (Rubel et al. 2016).

*Dermacentor reticulatus* (Fabricius, 1794) prefers inundated forests and usually occurs in synusia with *H. concinna* and *I. ricinus*. Its adults can tolerate extreme conditions of the environment and survive even under water during floods for several weeks (Földvári et al. 2016). Between the 1950s and 1970s, the distribution range of *D. reticulatus* in Slovakia was found to be limited to river basins in the southeast (SE) (the Latorica River) and the southwest (SW) (the Morava and Danube rivers) (Mačička et al. 1955; Mačička et al. 1956; Nosek 1972; Řeháček 1981). However, during the last few decades, changes in the distribution of *D. reticulatus* and its expansion to new areas have been noticed in Europe (Földvári et al. 2016), including Slovakia, where the species extended its range about 200 km further northward and by 300 m into higher altitudes (Bullová et al. 2009).

*Dermacentor marginatus* (Sulzer, 1776) occurs in two basic habitat types, in the forest steppe region in synusia with *H. inermis*, *H. punctata* and *I. ricinus*, and in the Slovak karst region in the same synusia or without *H. inermis* (Nosek 1972; Nosek and Krippel 1974).

Adults of *D. marginatus* and *D. reticulatus* are active in spring, usually from middle March. Peaks of activity can be observed in spring, from the beginning of April to the first decade of May, and in autumn (September and October). The activity of larvae and nymphs of both species was registered in summer months, from June to August (Mačička et al. 1955; Mačička et al. 1956; Nosek 1972; Nosek 1979; Daniel et al. 1980; Řeháček 1981). Hosts of *D. reticulatus* adults include cattle, horses, sheep, goats, pigs, wild boar, red deer, roe deer, fallow deer, dogs, foxes, hedgehogs, hares and rabbits. Five shrew species (Insectivora), seven rodent species (Rodentia), hares and rabbits have been confirmed as hosts of larvae and nymphs. Nymphs were also found to infest red deer, roe deer, goats, weasels, polecats and one bird species, the Eurasian jay *Garrulus glandarius* (Linnaeus, 1758) (Rosický 1952; Mačička et al. 1956; Nosek 1958; Černý 1972; Nosek 1972]. Adults of *D. marginatus* were found infesting cattle, goats, sheep, pig, wild boar, red deer, roe deer, fallow deer, horse, dogs, foxes and hedgehogs. Larvae and nymphs were recorded on goats, red deer, roe deer, dogs, weasels, polecats, weasels, polecats, wildcats, six shrew species, twelve rodent species, hares and rabbits (Mačička et al. 1955; Černý 1972; Nosek 1972; Nosek et al. 1972b; Heglasová et al. 2020).

*Dermacentor* spp. are known as vectors of the SFG rickettsiae (*Rickettsia raoultii* and *R. slovaca*), piroplasms of veterinary importance (*Babesia canis*) and contribute to the

circulation of *F. tularensis* and *C. burnetii*, whereas their role in transmission of TBEV is suggested.

#### *Haemaphysalis* spp.

Three species of the genus *Haemaphysalis* occur in the territory of Slovakia, mostly in the southern and central part of the country. Host-seeking ticks rarely occur in the same sites and at the same time, because individual species, particularly adults, have different habitat requirements, host preferences as well as seasonal dynamics.

The winter tick, *Haemaphysalis inermis* Birula, 1895, represents a very primitive species in the genus, which manifests in morphological features (e.g. according to structure of basis capituli, by elongate palpi without palpal spur in all stages), short feeding times in larvae and nymphs (only a few hours); larvae prefer lizards as hosts (Nosek 1973; Nosek 1981). *Haemaphysalis inermis* occurs in the southern regions of Slovakia, delimited approximately by the 8 °C annual isotherm and 600–700 mm isohyets. The maximum activity of *H. inermis* adults was registered in cold months, from October to May, while juvenile stages are seeking for hosts on the vegetation during the summer months (from April to August) (Mačička 1958; Nosek 1972; Řeháček et al. 1976). *Haemaphysalis inermis* larvae were found on the sand lizard *Lacerta agilis* Linnaeus, 1758 and the green lizard *Lacerta viridis* (Laurenti, 1768), on birds, e.g. the grey partridge *Perdix perdix* (Linnaeus, 1758), the spotted flycatcher *Muscicapa striata* (Pallas, 1764), the willow warbler *Phyloscopus trochilus* (Linnaeus, 1758), on two insectivore species, i.e. the European mole *Talpa europaea* Linnaeus, 1758, and the common shrew *Sorex araneus* Linnaeus, 1758, on six rodent species, i.e. the yellow-necked mouse *Apodemus flavicollis* (Melchior, 1834), the wood mouse *Apodemus sylvaticus* (Linnaeus, 1758), the bank vole *Myodes glareolus* (Schreber, 1780), the common vole *Microtus arvalis* Pallas, 1778, the European pine vole *Microtus subterraneus* (Selys-Longchamps, 1836), the hazel dormouse *Muscardinus avellanarius* (Linnaeus, 1758), on hares (*Lepus europaeus* Pallas, 1778) and wild boar. Nymphs feed on the same reptilian and mammalian species as larvae, and in addition on dogs, foxes, hedgehogs, red deer, roe deer, sheep, cattle and goats. Known hosts of adult ticks are wild boar, cattle, sheep, goat, red deer, roe deer, fallow deer, horses, hedgehogs, brown hares, rabbits and man (Rosický 1953; Mačička and Rosický 1954; Turček 1954; Mačička 1958; Černý 1972; Nosek et al. 1972b; Heglasová et al. 2020).

*Haemaphysalis concinna* Koch, 1844, the relict tick, is a widely distributed species in forests of temperate Eurasia

(Nosek 1971a; Filippova 1977; Estrada-Peña et al. 2017; Rubel et al. 2018). The distribution of *H. concinna* in Slovakia is limited by the same annual isotherm and isohyets as that of *H. inermis*, but the two species prefer different habitats. *Haemaphysalis concinna* inhabits light and humid deciduous forests and mixed hornbeam-oak forests with shrubby undergrowth, forest clearings and margins of oak forests, it frequently occurs on lake coast and in river basins (Nosek 1971a; Nosek 1972). Adult ticks are active from middle April to August. Larvae are active from the end of May to October, nymphs occur on vegetation from middle April until October (Rosický 1953; Nosek 1958; Nosek 1971a; Nosek 1972). According to Nosek (1971a), birds and small mammals, such as the European mole, shrews and voles, yellow-necked mouse, rabbits and brown hare are very frequent hosts of immature stages. Larvae and nymphs were recorded on two species of lizards, 23 species of birds, four species of insectivores and nine species of rodents. Nymphs were also found on stoats, dogs, hedgehogs, red deer and roe deer. Ungulates are very important hosts for adult *H. concinna*; infestations were confirmed for red deer, roe deer, fallow deer, mouflon, sheep, goat, cattle, and in addition also for badgers, foxes, dogs and hedgehogs (Rosický 1953; Turček 1953; Rosický and Balát 1954; Grulich et al. 1957; Kožuch et al. 1967a; Nosek et al. 1967b; Nosek 1971a; Nosek 1972; Kazimírová et al. 2018; Heglasová et al. 2020).

*Haemaphysalis punctata* Canestrini et Fanzago, 1878, the red sheep tick, is an ecologically very adaptable species and tolerates different climatic conditions. It can be found from cold to mild and humid climates and in dry habitats. In Slovakia, it is restricted to areas with annual isotherms of 7–9 °C and isohyets of 650–1000 mm (Nosek 1972; Nosek 1971b; Nosek and Krippel 1974). Two lizard species, 19 bird species, two shrew species, nine rodent species, as well as rabbits and hares have been confirmed as hosts of immature stages of this species. Nymphs were collected from dogs, foxes, mustelids, badgers, horses, roe deer, cattle, sheep and goats. Adult ticks mainly feed on wild ungulates (wild boar, red deer, roe deer, fallow deer), on domestic animals (horses, cattle, sheep, goats, dogs), on lagomorphs, hedgehogs and foxes (Rosický 1953; Nosek et al. 1967b; Nosek 1971b; Černý 1972; Nosek 1972; Nosek et al. 1972b). Depending on the geographic region, the seasonal activity of *H. punctata* is variable (Estrada-Peña et al. 2017). Larvae are active in the summer months from June to September. Nymphs show a bimodal activity pattern with the first peak from the beginning or middle of April to the end of June and again from the beginning of August to the middle October. In southern Slovakia, adults of *H. punctata*

quest on the vegetation from the end of March to June and again in October (Nosek 1971b; Nosek 1972).

In Slovakia *Haemaphysalis* spp. are involved in maintenance of TBEV and *F. tularensis* in natural foci and possibly in transmission of *C. burnetii*.

## Tick-borne pathogens and diseases

### Tick-borne encephalitis virus

TBE is a viral disease of the central nervous system and can result in long-term neurological symptoms with a potentially fatal outcome. TBE is endemic over a wide area comprising temperate regions of Europe and northeastern Asia up to Japan (Nuttall and Labuda 1994; Ruzek et al. 2019; Riccardi et al. 2019). TBE has been a notifiable disease in the European Union since 2012 and 28 countries, including Slovakia, are under the surveillance of the European Centre for Disease Prevention and Control (ECDC) (Beaute et al. 2018). Over the past four decades, the number of human TBE cases in endemic regions of Europe has increased, the risk areas have spread north- and westward (Beaute et al. 2018; Ruzek et al. 2019; Riccardi et al. 2019), and new foci have been discovered in higher altitudes (Danielová et al. 2009; Holzmann et al. 2009). Global climatic changes, changes in agricultural practices and urbanisation that affect vector and vertebrate host populations, and changes in human behaviour involving increased outdoor activities and travelling to endemic areas are considered to be the main factors affecting shifts in distribution of infected ticks and increased TBE incidence (Beaute et al. 2018; Kunze et al. 2018).

TBEV, the etiological agent of the disease, is a member of the genus *Flavivirus* (family Flaviviridae). The TBEV species includes three subtypes: Far Eastern (FE), Siberian (Sib) and European (Eu), among which TBEV-Eu is the least virulent (Gresikova and Calisher 1988; Ruzek et al. 2019). Majority of human TBEV infections are acquired through bites of infected ticks, but local outbreaks of alimentary infections due to consumption of raw goat, sheep or cow milk or unpasteurized dairy products also occur (Bojanić Rašović 2018). The first isolation of TBEV (Eu) in Central Europe was reported from former Czechoslovakia in 1949 (Rampas and Gallia 1949). This finding, together with the outbreak of alimentary TBEV infections in Rožňava (E Slovakia) in 1951 initiated a series of comprehensive multidisciplinary follow up studies on the mechanisms of circulation and persistence of TBEV in natural foci, on the ecology of vectors and reservoir hosts, on characterization of virus strains, and on the epidemiology of the disease (Blaškovič 1954; Rosický et al. 1954; Bárdoš et al. 1954; Libíková et al. 1954; Blaškovič 1961; Blaškovič 1967; Bárdoš 1965; Grešíková 1972; Grešíková 1999/1999). These findings were of high priority, exceeded the boundaries of

former Czechoslovakia, and were the starting point of an intensive and long-lasting multilateral and international collaboration of Slovak scientists.

In natural foci, TBEV is maintained in a cycle involving ticks and vertebrate reservoir hosts that amplify the virus (Michelitsch et al. 2019). Humans are not part of this cycle and represent dead-end hosts. Virus circulation and prevalence in the tick population is determined by the duration of viremia in the vertebrate hosts as well as by the presence and abundance of virus-immune hosts in particular regions (Blaškovič 1961; Blaškovič 1967). However, ticks can also be considered as reservoirs of the virus (Řeháček 1965) as laboratory studies demonstrated that experimentally infected *I. ricinus* larvae can maintain the virus during winter (Řeháček 1960), the virus titers are higher in ticks kept under field conditions (Kožuch and Nosek 1985), and the virus can be detected in salivary glands of females at least 120 days post-infection (Slovák et al. 2014).

Climatic conditions that favour co-incident transmission between infected *I. ricinus* nymphs and non-infected larvae attached to the same host (co-feeding transmission) also play a crucial role in TBEV persistence in natural foci (Labuda and Randolph 1999; Randolph et al. 1999). Moreover, increasing daytime and weekly average temperatures were shown to extend tick feeding and increase the chance of TBEV transmission (Daniel et al. 2018). Transstadial transmission in ticks plays an important role in virus maintenance, whereas transovarial transmission is low and thus not efficient (Řeháček 1962; Riccardi et al. 2019). Transmission of the virus from infected to non-infected ticks that co-feed on the same non-viraemic host (non-viraemic transmission = NVT) is another important mechanism for supporting virus circulation (Randolph 2009) and was extensively studied for TBEV-Eu strains under laboratory conditions by Labuda et al. (1993a, 1993c). Transmission of the virus was found to be enhanced by factors in tick salivary glands (saliva-assisted transmission = SAT) and was experimentally proved not only for *I. ricinus*, but also for *D. reticulatus* and *Rhipicephalus appendiculatus* Neumann, 1901 (Labuda et al. 1993b). In addition, vertical transmission of TBEV between generations of infected reservoir hosts (Bakhvalova et al. 2009) and prolonged latent infections in rodents (Tonteri et al. 2011) can provide long-term persistence of the virus in the mammalian hosts without involvement of the vector ticks.

In Slovakia, *I. ricinus* is the principal vector of TBEV and small mammals (e.g. rodents, insectivores) are its reservoirs and amplifying hosts (Blaškovič and Nosek 1972; Černý 1975; Kožuch et al. 1990) (Table 1). The prevalence of infection in ticks in natural foci is generally low (up to 5%), depending on the region and character of the focus, but can be higher in microfoci (Grešíková 1972; Labuda et al. 2002; Cagnacci et al. 2012). Since the 1950s, virus isolates have been obtained from questing *I. ricinus* nymphs and adults

**Table 1** Viruses and spirochaetes detected in field-collected ticks in Slovakia, their reservoir hosts and relevance for public health

Microorganism	Tick species	Reservoir hosts	Public health relevance	References
TBEV	<i>Ixodes ricinus</i> *	Small mammals: mainly rodents - <i>Apodemus</i> spp., <i>Myodes glareolus</i> Insectivores	Yes+	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
	<i>Haemaphysalis inermis</i>			15
	<i>Haemaphysalis concinna</i>			16
Tribeč virus	<i>I. ricinus</i>	Small rodents	Yes	2
Uukuniemi virus	<i>I. ricinus</i>	Small rodents	Yes	2
Murine	<i>I. ricinus</i>	Small rodents	No	17, 18
gammaherpesvirus 68	<i>H. concinna</i>			19
	<i>Dermacentor reticulatus</i>			20
<i>Borrelia burgdorferi</i> s.l.	<i>I. ricinus</i> *		Yes	
<i>B. afzelii</i>		Small rodents	Yes+	21, 22, 23, 24, 25, 26, 27, 28, 29, 30
<i>B. garinii</i>		Birds	Yes+	23, 24, 25, 26, 29, 31, 32, 33
<i>B. valaisiana</i>		Birds	No	23, 24, 26, 27, 29, 32
<i>B. burgdorferi</i> s.s.		Squirrels	Yes+	22, 24, 25, 28, 29, 30, 33
<i>B. lusitanae</i>		Reptiles (lizards)	No	23, 26, 28, 29, 34, 35
<i>B. spielmanii</i>		Small rodents, dormice	Yes	23, 29
<i>B. bavariensis</i>		Small rodents	Yes	22, 24
<i>B. bissettii</i>		Rodents	No	32
<i>Borrelia myiamotoi</i>	<i>I. ricinus</i> *	Small rodents	Yes	24, 29, 36, 37

\*Competent vector for human-pathogenic strains; + Confirmed human cases in Slovakia

References: 1 – Labuda et al. (2002), 2 – Grešíková (1972), 3 – Bárdoš (1965), 4 – Kožuch et al. (1990), 5 – Cagnacci et al. (2012), 6 – Grešíková (1975), 7 – Grešíková and Nosek (1967), 8 – Nosek et al. (1970), 9 – Nosek et al. (1982a, 1982b), 10 – Kožuch et al. (1967a, 1967b), 11 – Kožuch et al. (1995), 12 – Ernek et al. (1963), 13 – Grulich (1960), 14 – Nosek and Grulich (1967), 15 – Grešíková and Nosek (1966), 16 – Riedl et al. (1971), 17 – Ficová et al. (2011), 18 – Kúdelová et al. (2018), 19 – Vrbová et al. (2016), 20 – Kúdelová et al. (2015), 21 – Hanincová et al. (2003a), 22 – Lenčáková et al. (2006), 23 – Chvostáč et al. (2018), 24 – Hamšíková et al. (2017), 25 – Štěpánová-Tresová et al. (2000), 26 – Gem et al. (1999), 27 – Pangráčová et al. (2013), 28 – Rusňáková Taragel'ová et al. (2016), 29 – Vaculová et al. (2019), 30 – Smetanová et al. (2007), 31 – Drgoňová and Řeháček (1995), 32 – Hanincová et al. (2003b), 33 – Taragel'ová et al. (2008), 34 – Majláthová et al. (2006), 35 – Václav et al. (2011), 36 – Subramanian et al. (2012), 37 – Venczel et al. (2016)

(Grešíková and Nosek 1967; Grešíková 1972; Grešíková 1975; Labuda et al. 2002). The virus has been isolated also from brains and other organs of different rodent and insectivore species captured during monitoring of TBE foci in the country, and sera of these animals contained antibodies against TBEV (Bárdoš 1965; Kožuch et al. 1967a; Nosek et al. 1970; Nosek et al. 1982a; Kožuch et al. 1990; Kožuch et al. 1995). Mice (*Apodemus* spp.) are among the most important hosts. Mice populations are generally abundant, have a high reproduction rate and short life-span, are infested with subadult stages of *I. ricinus* (Černý 1975), and are chronically infected with the virus which was found to persist in mice and voles even during winter (Kožuch et al. 1990). Bank voles that are also frequently infested with *I. ricinus*, show lower viremia (Ernek et al. 1963; Malkova et al. 1965) and seroprevalence

(Kožuch et al. 1990) than mice, but they probably can contribute to the maintenance of TBEV foci (Ernek et al. 1963). The difference in susceptibility to TBEV infection and NVT efficiency between mice and voles was proved also experimentally in studies involving naïve wild rodents and TBEV-infected *I. ricinus* ticks (Labuda et al. 1993c]. These studies showed higher acquisition of the virus by uninfected ticks co-feeding with infected ones on susceptible *Apodemus* spp. in spite of very low or undetectable levels of viraemia. In contrast, co-feeding transmission was lower in viraemic bank voles (Labuda et al. 1993c). The differences in the capacity of voles and mice to support NVT have been explained by delayed dissemination of TBEV in the skin of bank voles from the attachment sites of infected *I. ricinus* ticks in comparison with more rapid virus dissemination in the skin of mice

(Malkova et al. 1965; Labuda et al. 1996). Moreover, not only naïve, but also virus-immune wild rodents were found to support NVT and it has been suggested that they can participate in the transmission of TBEV in natural foci (Labuda et al. 1997). Insectivores (shrews, moles, hedgehogs) that maintain more stable populations than rodents have also been suggested as reservoir hosts of TBEV in Slovakia (Grulich 1960; Kožuch et al. 1967a; Nosek and Grulich 1967). TBEV was found to persist during hibernation and subsequently replicate in hedgehogs and dormice (Kožuch et al. 1963) and in bats (Nosek et al. 1961), but hedgehogs do not support NVT in the laboratory (Labuda et al. 1993c).

Large vertebrates, especially free-living ruminants, are sources of blood and can support abundant local tick populations (Kiffner et al. 2010; Rizzoli et al. 2014). Thus, large vertebrates, mainly deer, play an important role in maintenance of the *I. ricinus* life cycle, but do not serve as amplifying hosts for TBEV (Keesing et al. 2006). Findings of a recent study highlighted the complexity of interactions between deer, rodents, *I. ricinus* and TBEV on a local level in Italy and Slovakia, and supported the possibility of a dilution effect for TBE by roe deer (Cagnacci et al. 2012). Free-living and also domestic animals have been found to contain antibodies to TBEV in their sera and serve as sentinels for the presence of the virus in natural foci (Ernek et al. 1967; Trávníček et al. 1999; Labuda et al. 2002; Sláviková et al. 2019).

Lizards and birds are among natural hosts of *I. ricinus*, but probably do not participate in the natural circulation of TBEV, although lizards can develop clinical signs of infection and produce antibodies against the virus in the laboratory (Grešíková-Kohútová and Albrecht 1959; Sekeyová and Grešíková 1979). Birds are important in geographic spreading of the virus, mainly through attached infected ticks (Blaškovič 1961; Ernek et al. 1968). This assumption has been demonstrated experimentally for a few bird species which either did not develop, or developed moderate viremia (Grešíková 1972). Pheasants (*Phasianus colchicus* Linnaeus, 1758) do not support NVT of TBEV through infected *I. ricinus* ticks (Labuda et al. 1993c). In contrast, TBEV could be recovered from experimentally infected mallards (*Anas platyrhynchos* Linnaeus, 1758) (Ernek et al. 1969), although these findings have limited ecological significance. Recently, TBEV RNA was detected in the brain of a common buzzard *Buteo buteo* (Linnaeus, 1758) (Csank et al. 2016), which may suggest spill-over of the virus to predatory birds. However, the importance of birds as sentinels for TBEV has been proved based on serological studies performed on, e.g., passerine birds (Ernek et al. 1968; Csank et al. 2018; Csank et al. 2019) or species of the Anseriformes order (Ernek et al. 1975).

Presence of TBEV has been repeatedly confirmed in *D. reticulatus* collected in different parts of Europe (Földvári et al. 2016; Chitimia-Dobler et al. 2019). In Slovakia, TBEV

has been detected in and isolated from *H. inermis* (Grešíková and Nosek 1966) and *H. concinna* (Riedl et al. 1971) (Table 1). The competence to harbour and transmit the virus in natural foci has been suggested for *Ixodes hexagonus* Leach, 1915 and *I. trianguliceps* (Nosek and Grulich 1967; Grešíková 1972) and has been demonstrated under laboratory conditions for, e.g., *H. concinna* (Kožuch and Nosek 1980), *H. inermis* (Kožuch et al. 1967b; Nosek et al. 1972a; Nosek et al. 1986), *D. reticulatus* (Nosek et al. 1984; Ličková et al. 2020), *D. marginatus* (Kožuch and Nosek 1971; Nosek et al. 1972a), the bird tick *I. arboricola* (Lichard and Kožuch 1967), and even for non-indigenous tick species such as *Haemaphysalis spinigera* Neumann, 1897, *Haemaphysalis turturis* Nuttall and Warburton, 1915 (Nosek et al. 1967a) and *R. appendiculatus* (Labuda et al. 1993b). These findings suggest that majority of ixodid species can potentially transmit TBEV, but the real range of natural vectors is limited by climatic factors, the efficiency of the vectors and their preferences for particular vertebrate hosts (Gresikova and Calisher 1988; Nuttall and Labuda 1994, 1994).

Modern molecular techniques enabled further characterisation of virus strains formerly isolated from ticks and rodents in Slovakia, comparison of nucleotide sequences of strains isolated from vectors and hosts, and contributed to the understanding of local genetic diversity and history of evolution and spread of TBEV strains in Central Europe (Weidmann et al. 2013; Frey et al. 2014). Similarity of the isolates from *I. ricinus* and bank vole with the Neudoerfl strain and high degree of identity of the stains from the vector and bank vole were revealed, suggesting no specific association of TBEV strains to hosts (Frey et al. 2014). Moreover, phylogenetic analyses indicated a recent spread of virus strains in Central Europe from east to the west, particularly from the Czech Republic and Slovakia to Germany via Danube River system (Weidmann et al. 2013).

TBE foci in Slovakia are scattered throughout the country and were identified based on an extensive longitudinal monitoring of TBEV in ticks, vertebrate hosts and humans. This monitoring was carried out over a period of about 40 years, beginning with the end of the 1950s (Blaškovič 1967; Nosek et al. 1970; Grešíková 1972; Blaškovič and Nosek 1972; Nosek et al. 1978; Labuda et al. 2002), and resulted in the identification of endemic areas for TBE that were concentrated in the western, southern, and eastern parts of the country (Bárdoš et al. 1959; Blaškovič and Nosek 1965; Kožuch et al. 1967a; Kožuch et al. 1969a, 1969b; Kožuch et al. 1982; Grešíková et al. 1986; Kožuch et al. 1987; Kohl et al. 1989; Kožuch et al. 1990; Labuda et al. 2002). TBEV foci were divided into the Carpathian and Pannonian type. The former type was located in the southern slopes of the western Carpathians, characterized by deciduous forests, rich herbaceous undergrowth, well-developed layer of litter and high population density of ticks and small mammals. The latter



type was located in lowlands along the Danube River, in patches of forests within agricultural landscape. There is evidence that during the last few decades, new TBE foci have been established, e.g. in E Slovakia (Labuda et al. 2002), foci have spread from lowlands to sub-mountainous areas (Lukan et al. 2010), and incidence rates have increased (Svihrova et al. 2011; Beaute et al. 2018) (Fig. 2).

TBE has become a reportable disease in former Czechoslovakia in 1952. Current information on the disease incidence on monthly and yearly basis, geographical distribution, age and gender distribution, seasonality, and trends over the last 10 years are available on the website of the Regional Authority of Public Health of the Slovak Republic providing surveillance of the communicable diseases in Slovakia and in the Epidemiological Information System (EPIS) (<https://www.epis.sk>). Recently, TBE cases have been reported from each region, with high incidence rates in the Banskobystrický, Žilinský and Trenčiansky regions. The number of reported TBE human cases has had an increasing trend since 2000, with the highest morbidity (3.4 / 100,000 inhabitants) in 2020 (Fig. 2; <http://www.uvzsr.sk>). Among European countries, Slovakia reports the highest rate of alimentary infections due to the presence of the virus in the milk of infected goats, sheep or cows, with an increasing trend since 2007 (Kerlik et al. 2018; <http://www.uvzsr.sk>). Memorable is the already mentioned outbreak of alimentary infection in Rožňava in 1951, during which over 600 people acquired TBEV through consumption of infected goat milk (Blaškovič 1954). Since then, the mechanisms of alimentary infections and their epidemiology have been extensively studied (reviewed in Grešíková 1972; Grešíková 1999), and sporadic cases or smaller local outbreaks have been reported almost every year (Labuda et al. 2002; Kerlik et al. 2018; Dorko et al. 2018; <http://www.uvzsr.sk>). Recently, the largest outbreak occurred in Košice/Nižný Klatov (E Slovakia) in May 2016 during which 500 persons were exposed to contaminated sheep milk or cheese and 44 contracted the disease (Dorko et al. 2018). This event

has called for further attention that should be paid to TBE in Slovakia.

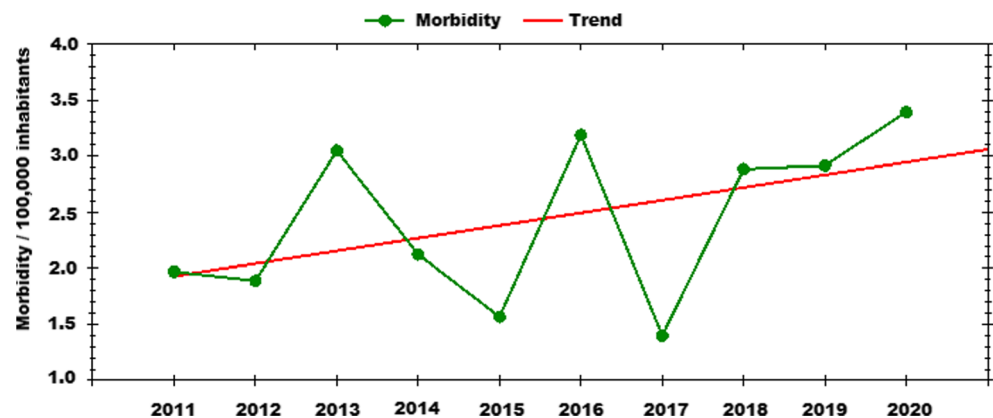
### Other viruses detected in ticks

In addition to TBEV, the arboviruses Tribeč and Uukuniemi have been sporadically detected and isolated from ticks and vertebrates in Slovakia (Table 1) and antibodies have been detected in several free-living vertebrates. However, the public health relevance of these viruses has not been fully explained and is probably low (Grešíková 1972).

Tribeč virus and the closely related Železná studnička and Lipovník viruses that belong to the Kemerovo group were isolated from *I. ricinus*, small mammals and blood of sentinel goats during the 1960s and were further characterized (for review see Grešíková 1972). *Ixodes ricinus* was identified as the vector of these viruses and small mammals are probably their reservoirs. Uukuniemi virus is an orbivirus and was isolated from *I. ricinus* and *A. flavicollis*. Antibodies against Tribeč virus have recently been detected in migrating adult and juvenile passerine birds in E Slovakia, possibly indicating local infection (Csank et al. 2019).

Murine Gammaherpesvirus 68 (MHV-68), a DNA virus (genus *Rhadinovirus*, subfamily *Gammaherpesvirinae*, family *Herpesviridae*, order *Herpesvirales*) is a natural pathogen of rodents (Mistříková et al. 2000). MHV-68 is genetically similar to human pathogens such as Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus and can be used in animal models of pathogenesis (Rajčáni and Kúdelová 2007). In rodent populations, the virus is transmitted directly, mainly through the intranasal route. However, during the last decade, the presence of MHV-68 has repeatedly been detected by molecular methods in ticks of different species and developmental stages in Slovakia (Table 1) and the question has been raised if ticks can serve as vectors of the virus (Kúdelová and Štibrániová 2019). The first report dates back to 2011, when the presence of viral DNA was detected for the first time in *I. ricinus* subadults collected from green lizards (Ficová et al. 2011) and subsequently in questing *D. reticulatus* (Kúdelová

**Fig. 2** Incidence of human tick-borne encephalitis cases in Slovakia. Trend over 2011–2020. Modified from <https://www.epis.sk>



et al. 2015), *H. concinna* (Vrbová et al. 2016), and *I. ricinus* adults (Kúdelová et al. 2018). The presence of life virus in different organs of *D. reticulatus* adults was also reported (Kudelova et al. 2017) and its transmission by *I. ricinus* to laboratory mice and survival in ticks was demonstrated (Hajnická et al. 2017).

### ***Borrelia* spirochaetes**

*Borrelia* spirochetes belong to the most common and epidemiologically the most important bacteria that are transmitted by ticks. Genus *Borrelia* is formed by two groups which are morphologically indistinguishable but differ in the vectors, clinical symptomatic in patients and ecology. Members of the *B. burgdorferi* s.l. group are the causative agents of Lyme borreliosis (LB), which is the most common tick-borne disease in Europe (Hubálek 2009; Stanek and Reiter 2011). The only recognized vectors of this group are hard ticks from the *I. ricinus* complex (Barbour and Fish 1993). The second group is represented by more than 20 spirochetes that cause relapsing fever and are transmitted mostly by soft ticks (Barbour and Hayes 1986).

**Lyme borreliosis group** At the time of its discovery about 40 years ago, *B. burgdorferi* was thought to be a single species (Burgdorfer et al. 1982). Currently, *B. burgdorferi* s.l. forms a complex of 22 genospecies (Margos et al. 2015; Pritt et al. 2016; Margos et al. 2017), out of which 10 are present in Europe: *B. burgdorferi* sensu stricto (s.s.), *B. garinii* (Baranton et al. 1992), *B. afzelii* (Canica et al. 1993), *B. lusitaniae* (Lefleche et al. 1997), *B. valaisiana* (Wang et al. 1997), *B. bissettii* (Postic et al. 1998), *B. spielmanii* (Richter et al. 2006), *B. bavariensis* (Margos et al. 2009), *B. finlandensis* (Casjens et al. 2011), and *B. turdi* (Norte et al. 2012). Human disease in Europe is caused by *B. burgdorferi* s.s., *B. spielmanii*, *B. afzelii*, *B. garinii*, *B. bavariensis* and *B. bissettii*. Lyme neuroborreliosis caused mostly by *B. garinii* has been monitored by the epidemiological surveillance of ECDC in the European Surveillance System (TESSy) since 2019 (<https://www.ecdc.europa.eu/en/news-events/ecdc-comment-european-commission-updates-communicable-disease-surveillance-list-lyme>). All genospecies of *B. burgdorferi* s.l. are maintained in natural foci via zoonotic transmission cycles involving vertebrate reservoir hosts and ixodid ticks. *Ixodes ricinus* is the principal vector of *B. burgdorferi* s.l. in Europe (Humair and Gern 2000; Hanincová et al. 2003a; Hanincová et al. 2003b; Majláthová et al. 2006; Taragel'ová et al. 2008). It is well accepted that genetic variability within the *B. burgdorferi* s.l. complex is associated with different clinical outcome in patients (Van Dam et al. 1993) as well as with different reservoir hosts (Humair and Gern 2000; Kurtenbach et al. 2002). The association to the reservoir hosts is given by the response

of the specific host complement system to different *Borrelia* genospecies (Kurtenbach et al. 2002). The reported mean prevalence of *B. burgdorferi* s.l. in questing ticks in Europe is around 15% (Strnad et al. 2017) with the highest rates in central Europe and southern part of Scandinavia (Estrada-Peña et al. 2018).

After the discovery of *B. burgdorferi* in the 1980s, research on these spirochaetes in Slovakia has been initiated in the early 1990s and was primarily focused on detections of borreliae in field-collected ticks by dark-field microscopy and on seroscreening in patients (Kmety et al. 1990; Kocianová et al. 1993; Drgoňová and Řeháček 1995). Following the pioneer study by Kmety et al. (1990), 2,857 questing ticks collected in Bratislava in 1991 were analysed by dark-field microscopy with the total prevalence of 17.8% and the first isolated strains that belonged to *B. garinii* (Drgoňová and Řeháček 1995). The seroprevalence in suspected patients with LB was 21.8% in western (W) Slovakia (Drgoňová and Řeháček 1995) and 14.6% in E Slovakia (Štefančíková et al. 2001). It soon became clear that a large portion of questing *I. ricinus* harboured borreliae and infected ticks were found in all explored areas of Slovakia. Immunofluorescence detection followed by isolation of *Borrelia* from ticks in W Slovakia revealed presence of *B. burgdorferi* s.l. in 49% of the analysed ticks. Isolated strains were represented by *B. afzelii* (68%), *B. garinii* (14%), *B. valaisiana* (14%) and *B. lusitaniae* (Gern et al. 1999). Average prevalence rates of *B. burgdorferi* s.l. in *I. ricinus* studied in two distinct geographical regions of E Slovakia during four years were 4.8%, 17.2%, 15.5%, 14.2%, respectively (Štepanová-Tresová et al. 2000). *Borrelia* isolates obtained from E Slovakia were identified using immunoblotting with monoclonal antibodies as *B. burgdorferi* s.s., *B. garinii*, mixture of *B. garinii* with *B. burgdorferi* s.s. and *B. burgdorferi* s.s. with *B. afzelii*. Presence of *B. burgdorferi* s.s. is not very common in Slovakia, however, its high prevalence (31.3%) in E Slovakia was confirmed by restriction fragment length polymorphism analysis (RFLP) of the *ospA* gene by Lenčáková et al. (2006).

With the broad development of PCR based techniques in the 1990s and early 2000s, more and more methods were implemented in the direct borrelial detection from tick and host DNA. Ecological studies to assess the importance of various wild-living vertebrates as natural reservoirs in the circulation of specific borrelial genospecies have been conducted in Slovakia since the early 2000s (Hanincová et al. 2003a; Hanincová et al. 2003b). Hanincová et al. (2003a), based on field studies, confirmed the association of *B. afzelii* with rodents, particularly yellow-necked mouse (*A. flavicollis*) and

bank vole (*M. glareolus*) as reservoir hosts. *Apodemus flavicollis* had higher infestation rates with ticks than *M. glareolus*, however, the infectivity for feeding ticks was higher in voles than in mice (Hanincová et al. 2003a). In SW Slovakia, Hamšíková et al. (2017) detected borrelia also in other rodent species such as *M. arvalis*, *M. subterraneus* and *A. sylvaticus*. *Apodemus flavicollis* had a lower prevalence of borrelia than *M. glareolus*. The dominant genospecies was again *B. afzelii*, but *B. bavariensis* and *B. garinii* were also detected. The importance of rodents as reservoir hosts of other pathogenic borrelial genospecies such as *B. burgdorferi* s.s., *B. bavariensis* and *B. spielmanii* was confirmed in various studies around Europe (Humair et al. 1998; Huegli et al. 2002; Richter et al. 2004). The role of avian hosts, especially of ground-foraging passerines, in the maintenance of borrelial infection is now indisputable. It has been established throughout Europe, including Slovakia, that birds constitute an important component of the reservoir host community of *B. burgdorferi* s.l. (Olsén et al. 1995; Humair et al. 1998; Hanincová et al. 2003b; Taragel'ová et al. 2008; Mtierová et al. 2020). In Slovakia, passerines especially black-birds (*Turdus merula* Linnaeus, 1758) and song-trushes (*Turdus philomelos* Brehm, 1831) have been shown to be important reservoir hosts for *B. garinii* and *B. valaisiana* in natural as well as urban habitats (Hanincová et al. 2003b; Taragel'ová et al. 2008; Chvostáč et al. 2018; Mtierová et al. 2020). Chvostáč et al. (2018) confirmed the importance of birds in the predominant circulation of *B. garinii* and *B. valaisiana* in questing ticks in an urban forest with a low abundance of rodents. Birds may act also as minor reservoir hosts for *B. lusitaniae* (Poupon et al. 2006), however, lizards are the main reservoirs for this genospecies. This was also confirmed based on analyses of feeding ticks and skin biopsies from green lizards in Slovakia by Majláthová et al. (2006) and Václav et al. (2011) (Table 1).

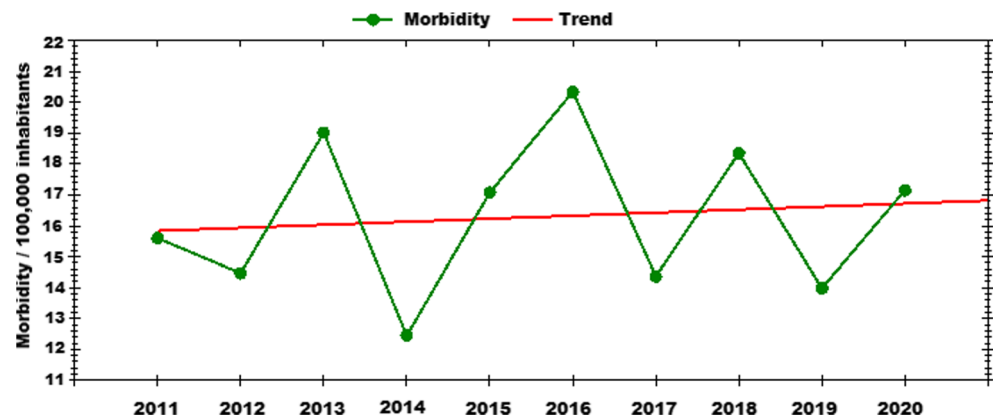
*Borrelia* prevalence in questing ticks was found to vary from 4.4% in a suburban forest in northern (N) Slovakia (Pangráčová et al. 2013) up to 53.2% in a suburban forest in E Slovakia (Venczel et al. 2016). The prevalence in urban parks was found to be generally lower than in natural forests or other sylvatic areas (Pangráčová et al. 2013; Venczel et al. 2016; Rusňáková Taragel'ová et al. 2016; Chvostáč et al. 2018; Rosà et al. 2018; Vaculová et al. 2019]. However, in an urban park in Malacky, a small town in W Slovakia, the overall prevalence of around 20% resembled a natural type of habitat (Hanincová et al. 2003a; Taragel'ová et al. 2008).

The genetic variability within *B. burgdorferi* s.l. complex has been studied mostly by PCR-RFLP or PCR-RLB (reverse line blot) of 5S-23 rRNA intergenic spacer followed by sequencing, whereby eight genospecies were detected with *B. afzelii* or *B. garinii* as the most prevalent, depending on the habitat composition, followed by *B. valaisiana* (Hanincová et al. 2003a; Hanincová et al. 2003b;

Majláthová et al. 2006; Smetanová et al. 2007; Taragel'ová et al. 2008; Bazovská et al. 2011; Pangráčová et al. 2013; Rusňáková Taragel'ová et al. 2016; Hamšíková et al. 2017; Chvostáč et al. 2018; Vaculová et al. 2019; Mtierová et al. 2020). The intraspecific variability and local population structure of *B. garinii* using MLST in questing as well as bird-feeding *I. ricinus* ticks in natural wetland in Slovakia was recently studied by Mtierová et al. (2020). The authors revealed presence of ten sequence types (STs) in bird feeding ticks and three in questing ticks. Only one ST was present in both questing and feeding *I. ricinus*. Four STs were detected for the first time. A distinct borrelial genospecies structure was observed in a mountain region in central Slovakia where *B. lusitaniae*, a common Mediterranean genospecies, prevailed in questing ticks (Rusňáková Taragel'ová et al. 2016), whereas lower prevalence of this genospecies was detected in questing ticks from urban and rural habitats of W Slovakia (Gern et al. 1999; Chvostáč et al. 2018; Vaculová et al. 2019] and in a xerothermic karst area in SE Slovakia (Majláthová et al. 2006). *Borrelia burgdorferi* s.s. which is not common in central Europe was detected in questing ticks in various habitats, but with lower prevalence than the three dominant genospecies (Smetanová et al. 2007; Taragel'ová et al. 2008; Rusňáková Taragel'ová et al. 2016; Chvostáč et al. 2018; Vaculová et al. 2019). In contrast, more than 60% of positive questing nymphs from suburban and natural forests of E Slovakia carried *B. burgdorferi* s.s. and also a high prevalence of *B. bavariensis* (ospA 4 type of *B. garinii*) was detected using PCR-RFLP of *ospA* gene (Lenčáková et al. 2006). *Borrelia bavariensis* has been rarely found in ticks in Slovakia. This might be due to the typing method, since in most cases PCR-RFLP or RLB of 5S-23S rRNA gene was used and by these techniques without further sequencing it is not possible to distinguish *B. garinii* from *B. bavariensis*. Later, when sequencing of *B. garinii* in *Borrelia* positive ticks was applied, *B. bavariensis* was detected in some of the samples from SW Slovakia (Hamšíková et al. 2017), however, with much lower prevalence than from E Slovakia (Lenčáková et al. 2006). *Borrelia spielmanii* was found only in a few, mostly urban areas of Slovakia, accounting for up to 4% of *Borrelia* positive questing ticks (Chvostáč et al. 2018; Vaculová et al. 2019). Presence of *B. bissetii*, a rare genospecies for Europe, was confirmed in Slovakia only once, in a questing tick from urban park Malacky in W Slovakia (Hanincová et al. 2003b).

In Slovakia, LB is a reportable disease since 1987 (<http://www.vzbb.sk/index.php>) with the overall morbidity of up to 20 cases per 100,000 inhabitants, with a slightly increasing trend during the last ten years (Fig. 3). More than 80% of the reported cases represent acute forms of LB with erythema migrans as the most common clinical symptom ([www.epis.sk](http://www.epis.sk)). Districts with higher urbanization in the southern part of

**Fig. 3** Incidence of human Lyme borreliosis cases in Slovakia. Trend over 2011–2020. Modified from <https://www.epis.sk>



the country and in areas with high mountains in the northeast have lower number of cases as opposed to areas with high broadleaf-vegetation cover in central and NW Slovakia, where over 50 cases per 100,000 inhabitants per year were reported ([www.epis.sk](http://www.epis.sk)). However, the numbers of real cases are probably higher.

Seroprevalence against *Borrelia* in healthy population as well as in patients with clinical borreliosis was found to differ according to the used method. Lenčáková et al. (2008) compared three serological tests in 74 patients with clinically confirmed LB. The recombinant line immunoblot had the highest sensitivity (93.2%) over ELISA (90.5%) and IFA (64.9%). VlsE proteins in immunoblots proved to have the best diagnostic value in testing IgG antibodies from patients with Lyme arthritis and IgM antibodies from patients with erythema migrans (EM). Schwarzová et al. (2009) isolated *Borrelia* from patients with clinically confirmed disseminated neuroborreliosis or EM with neurological symptoms. They obtained isolates from nine blood samples and one cerebrospinal fluid (CSF) sample: eight cultures were represented by *B. garinii*, one belonged to *B. afzelii* and one to *B. burgdorferi* s.s., seven patients did not develop antibodies against *Borrelia*. Similarly, Bazovská et al. (2011) detected *Borrelia* by PCR in CSF in 17 out of 32 patients with neuroborreliosis. The dominant genospecies was *B. garinii* (8), followed by *B. burgdorferi* s.s. (4) and *B. afzelii* (3). Bazovská et al. (2011) also detected borrelia in patients in whom neuroborreliosis was dubious and did not meet fully the criteria for neuroborreliosis.

In veterinary medicine, borrelial infection can cause symptoms mostly in dogs presenting with fever, arthritis and other musculoskeletal problems (Krupka and Straubinger 2010). In Slovakia, mainly serological studies have been conducted, confirming that dogs are exposed to infected ticks (Štefančíková et al. 1998; Čabanová et al. 2015). Obviously, borrelia infected ticks can be collected from dogs (Selyemová et al. 2013), however, profound clinical data are lacking.

To summarise, LB in Slovakia is a common multifactorial disease presenting mostly in early manifestation with EM, or

if diagnosed later, mostly as neuroborreliosis (Schwarzová et al. 2009; Bazovská et al. 2011). Borrelial infection prevalence in the vector *I. ricinus* tick is among the highest in Europe (Van Dam et al. 1993; Lenčáková et al. 2006), however, it varies between habitats and years, and depends on the presence of reservoir hosts. Even though urban habitats have lower prevalence of borrelia in ticks they still pose a significant risk for acquiring infection in humans.

***Borrelia miyamotoi*** *Borrelia miyamotoi* belongs to the relapsing fever spirochetes. It is the only known species from this group that is transmitted by hard ticks of the genus *Ixodes*. Since its discovery in 1995 in *I. persulcatus* from Japan it was detected in *Ixodes* spp. in the USA, Asia and Europe. Thus, it shares the same vectors with the LB spirochetes (Fukunaga and Koreki 1995; Scoles et al. 2001; Richter et al. 2003). Its pathogenicity was confirmed in Russia by Platonov et al. (2011). Possible co-infection of *B. burgdorferi* s.l. and *B. miyamotoi* was described in patients from Japan (Sato et al. 2014). The prevalence in ticks across Europe is between 0–3% (Crowder et al. 2014; Kjelland et al. 2015). The most important reservoir hosts are probably rodents (Burri et al. 2014). Presence of this pathogen in Slovakia was confirmed for the first time by Subramanian et al. (2012), however, its distribution, genetic variability and ecology are still not clear. The prevalence in questing ticks was studied in E, SE, SW Slovakia and in mountains in northcentral Slovakia. It was not detected in every studied site and the prevalence ranged from 0 to 3% (Venczel et al. 2016; Hamšíková et al. 2017; Vaculová et al. 2019). Hamšíková et al. (2017) detected *B. miyamotoi* in 1.7% of questing *I. ricinus* ticks from both urban and sylvatic habitats of SW Slovakia without significant difference in the studied sites. Moreover, *B. miyamotoi* was detected in skin biopsies of 9.3% of *A. flavicollis* and 4.4% of *M. glareolus* (Hamšíková et al. 2017), which is in agreement with Burri et al. (2014), suggesting the reservoir role of rodents in the circulation of this bacterium in natural foci. However, the public health relevance of this pathogen in Slovakia is unknown. In SE Slovakia, *B. miyamotoi* was

found in skin biopsy of *M. arvalis*, in 1.9 % of questing *I. ricinus*, and in 3.4 % of rodent-attached ticks – *I. ricinus* larvae and nymphs and for the first time in a *H. inermis* larva. Fleas collected from rodents were *B. miyamotoi* negative (Heglasová et al. 2020).

### ***Anaplasma phagocytophilum***

*Anaplasma phagocytophilum* is a gram negative intracellular alpha-proteobacterium that belongs to the genus *Anaplasma* and family Anaplasmataceae (Dumler et al. 2001). It infects neutrophils and is mostly associated with diseases of ruminants, horses, dogs and also humans, causing fever, flu-like symptoms, abortions and lower production in farm animals. Vectors are ticks of the *I. ricinus* complex (Nováková et al. 2010). It is interesting that human granulocytic anaplasmosis (HGA) cases are more common in North America (Adams et al. 2014), while in Europe their incidence is much lower (Lotric-Furlan et al. 1998; Oteo et al. 2000; Kristensen et al. 2001; Lotric-Furlan et al. 2006). On the other hand, in Europe anaplasmosis is common in domesticated ruminants such as sheep, goats, cattle as well as in horses and dogs and in wild living ungulates (Stuen et al. 2013). The high degree of clinical diversity is attributed to circulation of heterogeneous genetic variants that are adapted to specific reservoir hosts and specific vector ticks. This was also confirmed for Slovakia (Derdáková et al. 2011; Víchová et al. 2014; Blaňarová et al. 2014). While some strains are involved in human and animal diseases, other strains are involved only in diseases affecting animals or are not pathogenic (Massung et al. 2002; Massung et al. 2005). In Europe, *A. phagocytophilum* is transmitted mostly by *I. ricinus* (Strle 2004). Occasional vector of some genotypes associated with rodents is *I. trianguliceps* (Blaňarová et al. 2014; Bown et al. 2009; Baráková et al. 2014). Špitalská and Kocianová (2002) recorded the agents of *A. phagocytophila* group in ticks in Slovakia for the first time. In order to understand the ecology of *A. phagocytophilum*, Blaňarová et al. (2014) analysed the genetic variability of different *A. phagocytophilum* strains in questing and feeding *I. ricinus* and *I. trianguliceps* collected from vegetation and rodents as well as in the blood and biological samples from rodents. The bacterial DNA was detected in questing and host feeding *I. ricinus* from all studied sites, as well as in *I. trianguliceps* feeding on rodents and in rodents' ear and spleen biopsies. Prevalence of *A. phagocytophilum* in areas with rodents was much lower than in areas without rodents (Blaňarová et al. 2014; Chvostáč et al. 2018; Svitáková et al. 2015). In areas where *I. trianguliceps* ticks were absent,

*A. phagocytophilum* was not present in rodents (Blaňarová et al. 2014). Phylogenetic analysis based on four genetic loci in positive samples have shown that *A. phagocytophilum* genotypes in questing *I. ricinus* and feeding *I. ricinus* from ungulates, birds and dogs were distinct from genotypes found in rodents and feeding *I. trianguliceps* (Baráková et al. 2014). This confirmed previous findings from the UK (Bown et al. 2009) that *A. phagocytophilum* strains have specific associations with two vectors and different reservoir hosts. Unlike in the USA, *A. phagocytophilum* genotypes that are associated with European rodents are probably transmitted solely by *I. trianguliceps*, therefore these strains may be not of risk for humans, considering the narrow host selection behaviour and feeding preference of this tick species (Blaňarová et al. 2014). The main reservoir hosts for *A. phagocytophilum* in Slovakia are wild living ungulates (Smetanová et al. 2006; Stefanidesova et al. 2008; Štefanidesová et al. 2011; Víchová et al. 2014; Kazimírová et al. 2018) (Table 2). Kazimírová et al. (2018) reported very high prevalence of *A. phagocytophilum* in cervids from SW Slovakia where locally 100% of red deer, 95.4% of fallow deer and 92.9% of roe deer were infected. Moreover, they detected 88.9% prevalence in mouflon and 28.2% in wild boar. Strains from red deer, fallow deer, and mouflon clustered together with strains from wild ruminants, cattle, horses, hedgehogs, dogs, or foxes, representing the variants that can cause disease in domestic animals. Strains from wild boar clustered with strains from human patients suggesting the role of wild boar as a reservoir hosts of pathogenic strains for humans (Kazimírová et al. 2018). These findings are in accordance with previous findings in wild and domestic ruminants including sheep and goats from Slovakia, where the strains from small domestic ruminants belonged to the clade with strains from wild ungulates (Derdáková et al. 2011; Víchová et al. 2014). Presence of *A. phagocytophilum* in Slovakia was also reported in other vertebrate species, i.e. the European brown bear (*Ursus arctos* Linnaeus, 1758), chamois (*Rupicapra rupicapra* Linnaeus, 1758) and dogs (Víchová et al. 2010; Majláthová et al. 2011; Víchová et al. 2014). The importance of birds in the circulation of *A. phagocytophilum* is still disputable as genetically different strains from those in mammals were detected in ticks from birds in Italy (Baráková et al. 2014). In Slovakia, *A. phagocytophilum* was detected in great cormorants (*Phalacrocorax carbo sinensis* Staunton, 1796) (Víchová et al. 2016) pointing to the possible role of migratory birds in dispersal of the bacterium.

The prevalence of *A. phagocytophilum* in questing ticks differs geographically and depends on the presence of

**Table 2** Bacteria, piroplasms and other parasites detected in field-collected ticks in Slovakia, their reservoir hosts and relevance for public health

Microorganism	Tick species	Reservoir hosts	Public health relevance	References
<i>Anaplasma phagocytophilum</i>	<i>Ixodes ricinus</i> *	Free living ungulates, mainly cervids, mouflon, chamois, small domestic ruminants, cattle, dog, horse	Yes+	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16
	<i>Ixodes trianguliceps</i>	Small rodents	No	11
<i>Anaplasma ovis</i>	-	Small ruminants: sheep, goats	No	8
<i>Neoehrlichia mikurensis</i>	<i>I. ricinus</i> *	Small rodents	Yes	2, 17, 18, 19
<i>Rickettsia slovacica</i>	<i>Dermacentor marginatus</i> *	?	Yes+	20, 21, 22, 23, 24, 25, 26
	<i>D. reticulatus</i> *	?	Yes+	21, 27, 24, 26, 28, 29, 73
<i>Rickettsia raoultii</i>	<i>D. marginatus</i> *, <i>D. reticulatus</i> *	?	Yes+	21, 27, 24, 26, 28, 29, 73
<i>Rickettsia helvetica</i>	<i>Haemaphysalis inermis</i>	?	Yes+	10, 12, 25, 26, 30, 31, 32, 33, 34, 35
	<i>I. ricinus</i> *	?	Yes	10, 12, 25, 26, 28, 32, 33, 36, 37, 38
<i>Rickettsia monacensis</i>	<i>I. ricinus</i> *	?	No	39
<i>Rickettsia felis</i>	<i>I. ricinus</i>	?	?	10, 25, 26, 37, 40, 41
<i>Rickettsia</i> spp.	<i>I. ricinus</i> , <i>I. arboricola</i> , <i>I. hexagonus</i> , <i>H. inermis</i> , <i>H. concinna</i>	?	Yes+	10, 26, 37, 42, 43, 44
<i>Coxiella burnetii</i>	<i>I. ricinus</i> , <i>D. reticulatus</i> , <i>D. marginatus</i> , <i>H. concinna</i> , <i>H. punctata</i> , <i>H. inermis</i>	Domestic ruminants, pets, free-living animals, birds, fish, reptiles	No	26
<i>Coxiella</i> -like endosymbionts	<i>D. reticulatus</i> , <i>I. ricinus</i>		Yes+	45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55
<i>Francisella tularensis</i>	<i>I. ricinus</i> *	Small rodents, <i>Rattus norvegicus</i> , <i>Cricetus cricetus</i> , shrews, <i>Mustela nivalis</i> , hares		45, 56
	<i>I. trianguliceps</i>			46, 47, 48, 53, 57
	<i>D. reticulatus</i> *			46, 47, 48
	<i>H. concinna</i>		No	26
<i>Francisella</i> -like endosymbionts	<i>D. reticulatus</i> , <i>I. ricinus</i>		Yes	18, 28, 58, 59
<i>Babesia microti</i>	<i>I. ricinus</i> *	Small rodents	Yes	28, 58, 59
<i>Babesia venatorum</i>	<i>I. ricinus</i> *	Cervids	No	6, 28, 60, 61
<i>Babesia canis</i>	<i>D. reticulatus</i>	Dogs, wild canines	No	58
<i>Babesia capreoli</i>	<i>I. ricinus</i>	Cervids	No	58
<i>B. odocoilei</i>	<i>I. ricinus</i>	Cervids	No	10
<i>Babesia motasi</i> -like	<i>H. concinna</i>	?	No	58
<i>Babesia</i> sp. 1 and 2 (Eurasia)	<i>H. concinna</i>	?	No	62, 63
<i>Arsenophonus nasoniae</i>	<i>I. ricinus</i>	<i>Ixodiphagus hookeri</i> ?	?	31, 64
<i>Spiroplasma</i> sp.	<i>I. ricinus</i>	?	No	65
<i>Candidatus Cryptoplasma</i> sp. REP	<i>I. ricinus</i>	Lizards	No	10, 58, 66
<i>Theileria</i> spp.	<i>I. ricinus</i> , <i>H. concinna</i>	Cervids	No	67
<i>Hepatozoon canis</i>	<i>I. ricinus</i>	Canidae	No	68
<i>Nosema slovacica</i>	<i>I. ricinus</i> , <i>D. reticulatus</i>	Tick pathogen	No	68
<i>Unikaryon ixodis</i>	<i>I. ricinus</i>	Tick pathogen	No	68

**Table 2** (continued)

Microorganism	Tick species	Reservoir hosts	Public health relevance	References
<i>Trypanosoma</i> sp.	<i>I. ricinus</i>	?	?	69, 70
<i>Ixodiphagus hookeri</i>	<i>I. ricinus</i> , <i>H. concinna</i>	Tick parasitoid	No	62, 71, 72

\*Competent vector for human-pathogenic strains; + Confirmed human cases in Slovakia; ? unknown or not confirmed

References: 1 – Špitalská and Kocianová (2002), 2 – Derdákova et al. (2014), 3 – Smetanová et al. (2006), 4 – Stefanidesova et al. (2008), 5 – Vichová et al. (2010), 6 – Majláthová et al. (2011), 7 – Štefanidesová et al. (2011), 8 – Derdákova et al. (2011), 9 – Vichová et al. (2014), 10 – Kazimirová et al. (2018), 11 – Blaňarová et al. (2014), 12 – Chvostáč et al. (2018), 13 – Svitáľková et al. (2015), 14 – Pangráčová et al. (2013), 15 – Vaculová et al. (2019), 16 – Hamšíková et al. (2019), 17 – Špitalská et al. (2008a), 18 – Blaňarová et al. (2016), 19 – Svitáľková Hamšíková et al. (2016), 20 – Řeháček (1984), 21 – Špitalská et al. (2012), 22 – Brezina et al. (1969), 23 – Sekeyová et al. (1998), 24 – Radzijejskaja et al. (2015), 25 – Minichová et al. (2017), 26 – Špitalská et al. (2018), 27 – Boldiš et al. (2008), 28 – Švehlová et al. (2014), 29 – Špitalská et al. (2017), 30 – Sekeyová et al. (2012b), 31 – Subramanian et al. (2012), 32 – Špitalská et al. (2014), 33 – Špitalská et al. (2016), 34 – Heglasová et al. (2018), 35 – Václav et al. (2011), 36 – Sekeyová et al. (2000), 37 – Berthová et al. (2016), 38 – Sekeyová et al. (2003), 39 – Danchenko et al. (2018), 40 – Nováková et al. (2015), 41 – Vichová et al. (2018), 42 – Řeháček et al. (1991), 43 – Řeháček and Tarasevich (1988), 44 – Špitalská and Kocianová (2002), 45 – Guryčová and Letkovský (1973), 46 – Kmety et al. (1990), 47 – Výrosteková et al. (1991), 48 – Guryčová et al. (1995), 49 – Guryčová et al. (2003), 50 – Guryčová et al. (1982), 51 – Kožuch et al. (1987), 52 – Kožuch et al. (1995), 53 – Výrosteková et al. (2001), 54 – Výrosteková (1993), 55 – Výrosteková (1994), 56 – Guryčová and Výrosteková (1989), 57 – Guryčová (1998), 58 – Hamšíková et al. (2016a), 59 – Vichová et al. (2013), 60 – Chandoga et al. (2002), 61 – Duh et al. (2006), 62 – Boháčsová et al. (2016), 63 – Mediannikov et al. (2012), 64 – Bell-Sakyi et al. (2015), 65 – Kočíková et al. (2018), 66 – Černý (1958), 67 – Hamšíková et al. (2016b), 68 – Weiser et al. (1999), 69 – Rehacek et al. (1974), 70 Luu et al. (2020), 71 – Řeháček and Kocianová (1992), 72 – Slovák (20032003), 73 – Quarti et al. (2021)

competent reservoir hosts. In Europe, the prevalence in *I. ricinus* ticks ranges from 0.4–66.7% (Blanco and Oteo 2002). In Slovakia it varied from 0.6% up to 30%, with higher prevalence in urban areas than in natural habitats (Pangráčová et al. 2013; Derdákova et al. 2014; Blaňarová et al. 2014; Svitáľková et al. 2015; Chvostáč et al. 2018; Vaculová et al. 2019]. It has also been confirmed that the proportion of *I. ricinus* infected with variants of *A. phagocytophilum* that are not pathogenic to humans was higher in sites where roe deer were present than in sites with absence of these animals (Hamšíková et al. 2019).

HGA is not among the communicable diseases in Slovakia. Human cases are rare (Avdičová et al. 2019), but their part may be misdiagnosed or remain undiagnosed. Up to date only two patients with clinical symptomatic of arthralgia, fever and myalgia have been laboratory confirmed by positive findings of morulae in the granulocytes and positive PCR of the blood (Nováková et al. 2010; Špitalská et al. 2021a). Antibodies against *A. phagocytophilum* were found in sera of 25% patients with a history of tick bite in central Slovakia. The most frequent clinical symptoms in these patients were cephalalgia, arthralgia, myalgia, fever, exanthema, neurological symptoms and lymphadenopathy (Kocianová et al. 2008).

Studies from Slovakia support the theory that the genetic variability of *A. phagocytophilum* contributes to the zoonotic potential of the pathogen and the ecology of *A. phagocytophilum* seems to be more complex than it was thought before.

### *Neoehrlichia mikurensis*

*Neoehrlichia mikurensis* is an emerging tick-borne pathogenic bacterium from the family Anaplasmataceae, which was only recently cultured (Wass et al. 2019). In 1999, it was detected for the first time in *I. ricinus* from the Netherlands and described as *Ehrlichia*-like microorganism (Schouls et al. 1999). Later, it was detected in rodents and *Ixodes ovatus* Neumann, 1899 in Japan and described as a new species within Anaplasmataceae family (Kawahara et al. 2004). Based on phylogenetic analyses of various genetic markers it was related to *Ehrlichia*-like microorganisms detected in questing ticks, ticks feeding on birds and in rodents from various regions of Europe and Asia (Kawahara et al. 2004; Brouqui et al. 2003; Špitalská et al. 2008a). *Neoehrlichia mikurensis* was detected mainly in immunocompromised patients with septicaemia and fever (Fehr et al. 2010; Welinder-Olsson et al. 2010; Peková et al. 2011). Moreover, it was detected in a chronically neutropenic dog from Germany (Diniz et al. 2011). Rodents have been proposed as reservoir hosts since they develop systemic infection (Kawahara et al. 2004) and are able to transmit it to xenodiagnostic ticks (Burri et al. 2014). In Europe, the main vector is *I. ricinus* with an infection prevalence ranging between 0.1–24.3% (reviewed in Portillo et al. 2018). In Slovakia, *N. mikurensis* was detected in ticks from various habitats including urban parks, urban, suburban forests and natural forests with the prevalence between 0.5% in a suburban forest in E Slovakia (Blaňarová et al. 2016) to 11.6% in a farmland lowland pine forest in SW Slovakia (Derdákova et al. 2014). In another study from

SW Slovakia, Svitáľková Hamšíková et al. (2016) found a significantly higher prevalence of *N. mikurensis* in *I. ricinus* and rodents in a natural site compared to an urban/suburban site. Blaňarová et al. (2016) detected *N. mikurensis* not only in questing *I. ricinus* but also in 1.6% of tested spleens from rodents and in 0.3% of rodent feeding *I. ricinus* larvae. In addition, 3.3% of rodent feeding *I. trianguliceps* carried *N. mikurensis* as well, pointing to a possible role of this nidicolous tick in the ecology of *N. mikurensis*. Moreover, the authors detected the bacterium in a foetus of a *N. mikurensis* negative *A. flavicollis* female. Phylogenetic analysis of two genetic markers showed a high degree of genetic identity of this bacterium in Slovakia (Blaňarová et al. 2016) as well as its identity with the human pathogenic strain from Europe (Svitáľková Hamšíková et al. 2016). The data from Slovakia confirm the wide distribution of *N. mikurensis* in various habitats with rodents as important reservoir hosts of this emerging pathogen.

### *Rickettsia* species

Rickettsioses are diseases caused by Gram-negative obligate intracellular bacteria belonging to genus *Rickettsia*. They are among the oldest known vector-borne zoonoses. The genus *Rickettsia* belongs to the order Rickettsiales and contains species classified into the ancestral group (*R. belii* and *R. canadensis*), the typhus group (TGR - *R. prowazekii* and *R. typhi*), the spotted fever group (SFGR - *R. rickettsii*, *R. conorii*, *R. africae*, *R. sibirica*, *R. slovaca*, *R. honei*, *R. japonica*, *R. australis*, *R. akari*, *R. felis*, *R. aeschlimannii*, *R. helvetica*, *R. monacensis*, *R. massiliae*, *R. rhipicephali*, *R. montanensis*, *R. parkeri*, *R. peacockii*, *R. asiatica*, *R. heilongjiangensis*, *R. hoogstraalii*, *R. raoultii*, and *R. tamurae*). Rickettsial genomes are small, ranging from 1.11 Mb for *R. typhi* to 2.1 Mb for the “*Rickettsia* endosymbiont of *Ixodes scapularis*.” Their G+C content is low, ranging from 29% (*R. typhi*) to 33% (*Rickettsia* endosymbiont of *I. scapularis*) (Fournier et al. 2003; Parola et al. 2013). The pathogenicity of rickettsiae is different. Fournier et al. (2009) hypothesized that genome degradation in rickettsiae was associated with increasing virulence by comparing the genome of *R. africae*, a mildly pathogenic bacterium in humans, to those of the more pathogenic species *R. conorii*, *R. rickettsii*, and *R. prowazekii*. Rickettsiae are associated with arthropods, ticks, lice, fleas and mites, SFGR are mainly transmitted by ticks and TGR by lice and/or fleas.

The study of rickettsiae in Slovakia dates back to the 1960s when mainly serological methods were applied. In addition, for detection of rickettsial agents the microscopic method, the haemocyte test, was employed (Řeháček et al. 1971), which was routinely used also in the 2000s (Řeháček et al. 1976; Řeháček et al. 1990; Řeháček et al. 1991; Špitalska et al. 2002;

Řeháček et al. 1997; Boldiš et al. 2008). Disadvantage of the haemocyte test is that it can indicate only the presence of microorganisms with rickettsial morphology, but cannot differentiate species of *Rickettsia*, *Coxiella*, *Rickettsia*-like and *Coxiella*-like microorganisms. Similarly, due to the antigenic cross reactions among rickettsial species within the same group, serological techniques cannot distinguish rickettsial species from each another (Raoult 2004). At the end of the 1990s, molecular biology methods (PCR, PCR-RFLP, real time PCR, sequencing, methods based on amplification of fragments of *16S* rRNA, *23S* RNA, *gltA*, *ompA*, *ompB*, *sca4*, *17-kDa* genes) were introduced into the Slovak laboratory at the Institute of Virology.

Rickettsioses caused by SFG rickettsiae are reportable to the Regional Authority of Public Health of the Slovak Republic, but only sporadic human cases have been confirmed thus far (<https://www.uvzsr.sk>; Avdičová et al. 2019; Špitalská et al. 2021a).

***Rickettsia slovaca*** *Rickettsia slovaca* was the first rickettsial species identified in Slovakia. It was isolated from *D. marginatus* collected in central Slovakia (Brezina et al. 1969). Firstly, it was classified as a species closely related to *R. sibirica* (indicated as strain “B” and strain “D”), but later it was identified as a new species (Úrvolgyi and Brezina 1978). *Rickettsia slovaca* was shown to be transovarially transmitted and commonly found in ticks (Řeháček 1984). In 1998, *R. slovaca* was finally accepted as a separate taxonomic species (Sekeyová et al. 1998) on the basis of the distinctive clinical, epidemiological, phenotypic and genotypic features. For easy, quick and cheap differentiation of *R. slovaca* from other rickettsiae a method based on PCR-RFLP of the *sca4* gene using *HaeIII* restriction enzyme was designed (Špitalská et al. 2008b). Boldiš et al. (2009a, 2009b) showed ultrastructural changes within host cells, caused by infection with two isolates of *R. slovaca*, a strain isolated from questing *D. marginatus* collected in central Slovakia (wild type) and a high passage of strain “B”, and contributed to the understanding of the life cycle of the bacterium. The authors identified the highest point of multiplication of these isolates in different cell lines, which was achieved two days earlier by the wild type. The generation times of *R. slovaca* *in vivo* in *D. marginatus* and *I. ricinus* ticks were prolonged compared to the generation time in mammalian cell lines. Ticks were considered a more appropriate environment and the number of rickettsial DNA copies was much higher in *D. marginatus*, which confirmed its importance in maintaining of *R. slovaca* (Boldiš and Špitalská 2010). The presence of *R. slovaca* was found in 20.6–24.3% *D. marginatus* ticks and only in 0.7–8.1% of *D. reticulatus* ticks collected from vegetation and vertebrate hosts in W, central and E Slovakia (Špitalská et al. 2012; Radzijeuskaja et al. 2015; Minichová et al. 2017; Špitalská et al. 2018) (Table 2). In a



habitat with sympatric occurrence of *D. reticulatus* and *D. marginatus*, *R. slovacica* was confirmed in skin biopsy of *A. flavicollis* (Heglasová et al. 2018).

The pathogenicity of *R. slovacica* for humans was confirmed by Raoult et al. (1997). The incubation period is 5–10 days with clinical symptoms like scalp eschar, neck lymphadenopathy, asthenia, headache, painful adenopathies, and sometimes low fever, rash and face edema. Antibiotic treatment is successful, however alopecia lasts for several months (Parola et al. 2013). For the disease the terms TIBOLA (Tick-borne lymphadenopathy), DEBONEL (Dermacentor-borne-necrosis-erythemalymphadenopathy) and SENLAT (scalp eschar and neck lymphadenopathy after a tick bite) are used. Infections were recorded in different countries of Europe and in former Czechoslovakia the first clinically and serologically confirmed case was described by Mittermayer et al. (1980). Sekeyova et al. (2012) showed infection with *R. slovacica* in sera obtained from hospitalized patients with suspected TBD by immunofluorescence assay and PCR based on *16S* rRNA and *gltA* genes.

***Rickettsia raoultii*** *Rickettsia raoultii* is another rickettsial species present in *Dermacentor* spp. in Slovakia (Table 2). The first molecular evidence of *R. raoultii* was provided by Boldiš et al. (2008), with prevalence 8.1–8.6% and 22.3–50% in *D. marginatus* and *D. reticulatus*, respectively. *Dermacentor reticulatus* were found to be more infected with *R. raoultii* (86.7–98% and 94.2% infection in ticks from vegetation and hosts, respectively) in W and central Slovakia (Špitalská et al. 2012; Špitalská et al. 2018) than in E Slovakia (14.2%) (Radzijejskaja et al. 2015). In a site of SW Slovakia, with sympatric occurrence of three tick species (*D. reticulatus*, *I. ricinus* and *H. concinna*), 96.9% of *D. reticulatus* were infected with *R. raoultii* (Švehlová et al. 2014). Recently, *R. raoultii* was detected in questing *H. inermis* in the Slovak Karst National Park (SE Slovakia) in an area with sympatric occurrence with *D. reticulatus* and *D. marginatus*, suggesting a potential reservoir role of this tick species for *R. raoultii* (Quarti et al. 2021). Isolation of *R. raoultii* was attempted from haemolymph-positive *D. marginatus* ticks. Curves of its intracellular growth were modelled with lag, exponential, stationary and death phases with the highest point of multiplication on the 7th and 8th day post infection in mammalian cell lines. Experimental infection of guinea pigs through *R. raoultii*-positive ticks showed that infection was present in all organs and increased in blood samples during four weeks (Špitalská et al. 2012). *Rickettsia raoultii* is less pathogenic to humans than *R. slovacica* (Parola et al. 2009). Human infection with *R. raoultii* was indicated in sera by immunofluorescence assay and PCR (Sekeyova et al. 2012). The most complex case of human *R. raoultii* infection was described by

Špitalská et al. (2017, 2021a) in a seven-year-old girl bitten by a *D. reticulatus* female on her head. Rash, laryngitis, lymphadenopathy and high fever (38.9 °C) developed ca seven after tick attachment. Seroprevalence evaluated by ELISA showed the presence of IgG antibodies against SFGR and by *R. raoultii*-specific TaqMan real time PCR the presence of bacterial DNA was confirmed in both, the serum and the tick.

***Rickettsia helvetica*** *Rickettsia helvetica* is a species commonly found in *I. ricinus*. It was isolated for the first time in Switzerland (Burgdorfer et al. 1979). In Slovakia, the first isolation was carried out from questing *I. ricinus* collected in the district Podunajske Biskupice (SW Slovakia) (Sekeyová et al. 2012b). Subsequently, DNA of *R. helvetica* was detected repeatedly in questing *I. ricinus* from urban and suburban habitats of W and N Slovakia, natural sites in mountain forests in N and W Slovakia, in an agricultural site in E Slovakia, and in natural and rural areas in central Slovakia (Subramanian et al. 2012; Špitalská et al. 2014; Špitalská et al. 2016; Minichová et al. 2017; Chvostáč et al. 2018; Špitalská et al. 2018) (Table 2). The bacterium was present in ticks during the whole season and in all developmental stages and the prevalence of infection varied between tick developmental stages, sites and seasons. *Rickettsia helvetica* was the dominant rickettsial species in *I. ricinus* nymphs, females and males in an agricultural site of E Slovakia, a rural area in central Slovakia, and in suburban habitats of W Slovakia (Subramanian et al. 2012; Špitalská et al. 2016; Minichová et al. 2017; Chvostáč et al. 2018; Špitalská et al. 2018). Moreover, *R. helvetica* dominated also in *I. ricinus* larvae and nymphs collected from passerine birds such as the great tit *Parus major* Linnaeus, 1758, the European robin *Erithacus rubecula* (Linnaeus, 1758), the common chaffinch *Fringilla coelebs* Linnaeus, 1758, the dunnock *Prunella europaea* Linnaeus, 1758 and the common blackbird *T. merula* (Berthová et al. 2016; Chvostáč et al. 2018). The presence of DNA of this pathogen was recorded also in blood samples of birds, i.e. the great tit, the Eurasian blackcap *Sylvia atricapilla* (Linnaeus, 1758), the Eurasian blue tit *Cyanistes caeruleus* (Linnaeus, 1758), the common chaffinch, the Eurasian tree sparrow *Passer montanus* (Linnaeus, 1758), the dunnock, the common blackbird, the common reed bunting *Emberiza schoeniculus* (Linnaeus, 1758) and the European robin. The great tit was found as the most infested species with *I. ricinus*, carried *R. helvetica* positive larvae and nymphs and was found to be rickettsiemic in its blood. We hypothesized that synanthropic birds could play a role as carriers of infected ticks and thus could ensure the geographical distribution and maintenance of *R. helvetica* in nature (Berthová et al. 2016).

*Rickettsia helvetica* was also present in rodent-attached *I. ricinus* larvae and nymphs removed from *A. flavicollis*, *M. glareolus* and *M. arvalis*, and in ear biopsies and spleen of rodents that were not infested with ticks, i.e. *A. flavicollis*

females which were trapped in suburban and natural sites of SW Slovakia (Minichová et al. 2017). Rickettsial DNA was also detected in blood samples of *M. glareolus* and *A. flavicollis* in SW Slovakia (Mit'ková et al. 2015), and in ear biopsies from striped field mouse *Apodemus agrarius* (Pallas, 1771), *A. flavicollis*, *M. glareolus* and *M. arvalis* captured in E Slovakia (Heglasová et al. 2018; Špitalská et al. 2020). In addition, the bacterium was recorded in a *Ctenophthalmus agyrtes* (Heller, 1896) flea male collected from *A. agrarius*, in *C. solutus* Jordan et Rothschild, 1920 and *Megabothris turbidus* (Rothschild, 1909) collected from *A. flavicollis* and *M. glareolus* from E Slovakia (Špitalská et al. 2015; Špitalská et al. 2020), and in *Laelaps agilis* C. L. Koch, 1836, *L. jettmari* Vitzthum, 1930, *Haemogamasus nidi* Michael, 1892, *Hirsutiella zachvatkini* (Schluger, 1948), and *Kepkatrombicula storkani* (Daniel, 1956) mites parasitizing small mammals in SW and E Slovakia (Mit'ková et al. 2015; Špitalská et al. 2020).

*Rickettsia helvetica* was identified also in *I. ricinus* (all developmental stages) that were removed from hunted free-living ungulates (*C. capreolus*, *C. elaphus*, *D. dama*, *O. musimon*) in SW Slovakia (Kazimírová et al. 2018). Infection in a spleen sample of *C. capreolus* from central Slovakia was confirmed by Stefanidesova et al. (2008). The bacterium was also identified in *I. ricinus* removed from dogs (Selyemová et al. 2013; Lieskovská et al. 2018) and from free-living green lizards (Václav et al. 2011).

Results from the mentioned studies suggest that birds, lizards, small mammals, ungulates and dogs are good sentinels for the presence of *R. helvetica*, they provide a food source for ticks and ensure the circulation and geographical dispersion of rickettsiae.

This bacterium was recorded in ticks of all developmental stages that fed on humans (Špitalská et al. 2021b). Human infections with *R. helvetica* were documented by serology-based diagnoses and molecular tools in some European countries including Slovakia (Sekeyová et al. 2012). Patients had mild, self-limited illness associated with headache and myalgia and less frequently rash or eschar (Parola et al. 2013).

***Rickettsia monacensis*** *Rickettsia monacensis* is another species identified in *I. ricinus* in Slovakia. In 2000, two rickettsial strains: *Rickettsia* sp. “IRS3” and “IRS4” were detected in *I. ricinus* collected in NE and SW Slovakia (Sekeyová et al. 2000; Sekeyová et al. 2003). Sequences of the 16S rRNA, *gltA* and *ompA* encoding genes of both strains were shown to be nearly identical, closely related to *R. helvetica*, but distinct from those of all other known rickettsiae. In Germany, the species was isolated, characterized and named as *R. monacensis* (Simser et al. 2002). DNA of *R. monacensis* was identified in questing *I. ricinus* collected in urban and suburban sites of W and N Slovakia, natural sites of W

Slovakia, in a mountain forest in N Slovakia, in an agricultural site and rural site in E and central Slovakia (Švehlová et al. 2014; Špitalská et al. 2014; Špitalská et al. 2016; Minichová et al. 2017; Chvostáč et al. 2018; Špitalská et al. 2018) (Table 2). The bacterium was detected in all developmental stages of *I. ricinus* (Švehlová et al. 2014; Minichová et al. 2017; Chvostáč et al. 2018; Špitalská et al. 2018). It was also recorded in *I. ricinus* nymphs collected from birds (*E. rubecula*, *T. merula*) trapped near the Drienovec village (an ornithological station, part of the Slovak Karst National Park in E Slovakia) (Berthová et al. 2016), in *I. ricinus* larvae feeding on *A. flavicollis* and *M. glareolus* from SW and central Slovakia (Minichová et al. 2017), in *I. ricinus* larvae collected from *D. dama* in SW Slovakia (Kazimírová et al. 2018) and in *I. ricinus* feeding on dogs in W Slovakia (Lieskovská et al. 2018). Furthermore, DNA of *R. monacensis* was identified in a pool of *H. zachvatkini* mites infesting rodents in SW Slovakia (Mit'ková et al. 2015).

Additionally, DNA of *R. helvetica* and *R. monacensis* was amplified in adult *Ixodiphagus hookeri* (Howard, 1908) wasps parasitizing *I. ricinus* ticks. The presence of rickettsiae in wasps may be related either with the bacterial DNA taken up from the ticks or with the role of wasps in circulation of rickettsiae among tick vectors (Boháčsová et al. 2016).

Human cases have not been confirmed in Slovakia, and infections were identified only in few patients by using molecular tools and by isolation of the rickettsial strain by shell vial culture. Fever, flu-like symptoms and eschar were present in the patients (Parola et al. 2013).

***Rickettsia felis*** *Rickettsia felis* is primarily associated with its reservoir and vector, the cat flea *Ctenocephalides felis* (Bouché, 1835). It causes infection in humans, known as flea-borne spotted fever or cat-flea typhus (Abdad et al. 2011). It has not been reported from Slovakia, but a *Rickettsia* sp. closely related to *R. felis* was detected for the first time in a *Ctenophthalmus solutus* Jordan and Rothschild, 1920 flea removed from *A. agrarius* (Špitalská et al. 2015) and in an ear biopsy of *A. flavicollis* (Heglasová et al. 2018), both trapped in E Slovakia. Recently, a *R. felis* strain has been successfully isolated from *I. ricinus* (Danchenko et al. 2018).

***Rickettsia* spp.** DNA of *Rickettsia* genetically very close, although not identical to *R. africae* was identified in *Ceratophyllus garei* Rothschild, 1902 flea collected from reed warbler *Acrocephalus scirpaceus* (Hermann, 1804), a passerine migratory bird returning from Africa (Sekeyová et al. 2012a). *Rickettsia africae* is the etiological agent of African tick bite fever, which is in Europe only sporadically described, mostly detected in travellers returning from Africa and no human case has been recorded in Slovakia to this time.

Analyses of partial sequences of *gltA*, *ompA*, *ompB* and *htrA* genes showed the presence of *Candidatus R. vini* in *I. arboricola* nymphs collected from *P. major* nesting in central Slovakia (Nováková et al. 2015). An unidentified *Rickettsia* sp. was found in an *I. ricinus* female collected from *S. europaea* mist-netted in a suburban habitat in W Slovakia. Sequencing of the *gltA* gene fragment showed a 100% identity with *Candidatus R. vini* (Berthová et al. 2016).

Other unidentified *Rickettsia* species were found in questing ticks. Partial sequencing of the *gltA* gene showed 100% identity with *Candidatus R. mendelii* in questing *I. ricinus* females and nymphs collected in an urban habitat in W Slovakia (Minichová et al. 2017), and 99% identity with *Candidatus R. hungarica* in *H. inermis* from E Slovakia (Špitalská et al. 2018).

Unidentified *Rickettsia* species were also found in a questing *I. ricinus* male from suburban and natural habitats of W Slovakia, in an *I. ricinus* larva removed from *A. flavicollis* in a rural habitat of central Slovakia (Minichová et al. 2017), in *Eulaelaps stabularis* (Koch, 1839), *Laelaps hilaris* C. L. Koch, 1836, *L. agilis*, *H. nidi*, *L. jettmari* mites fed on small mammals in E Slovakia (Špitalská et al. 2020), in *I. ricinus* larvae, nymphs and males fed on ungulates in SW Slovakia (Kazimírová et al. 2018), and in *Archaeopsylla erinacei* (Bouché, 1835) flea, *I. ricinus*, *I. hexagonus*, and *H. concinna* ticks parasitizing red fox *Vulpes vulpes* (Linnaeus, 1758) populations (Vichová et al. 2018).

*Rickettsia* endosymbionts were identified by sequencing of 16S rRNA in a female *Ctenophthalmus uncinatus* (Wagner, 1898) flea collected from *M. glareolus* in E Slovakia (Špitalská et al. 2015), and by sequencing of *gltA* gene in *Ctenophthalmus assimilis* (Taschenberg, 1880) from *M. arvalis*, and in *M. turbidus* from *A. agrarius*, *A. flavicollis* and *M. glareolus* in E Slovakia (Špitalská et al. 2020).

### ***Coxiella burnetii***

Q fever, a zoonosis in humans, coxiellosis in animals, is caused by the Gram-negative coccobacillus *C. burnetii*. It is an intracellular pathogen replicating in eukaryotic cells, its target cells are macrophages located in body tissues and monocytes circulating in the blood stream (Porter et al. 2011). The genus *Coxiella* was moved from Rickettsiales to the order Legionellales and belongs now to the family Coxiellaceae. The chromosome varies in size from 1.5 to 2.4 Mbp, and is highly variable among different *C. burnetii* strains (Porter et al. 2011). The circular genome of *C. burnetii*, Nine Mile phase I RSA493 has a length of 1.99 Mbp (Seshadri et al. 2003). The bacterium undergoes antigenic variations. Bacteria isolated from natural sources are defined as virulent forms, are characterized by full-length lipopolysaccharide (LPS) molecules with an O-specific side chain, survive inside monocytes

and macrophages and represent the phase I variant (Abnave et al. 2017). After serial *in vitro* passages of the virulent *C. burnetii* in embryonated hen eggs or tissue culture in the laboratory, phase shift may occur and results in a truncated LPS molecules. This avirulent form is referred to as phase II, it does not exist in the natural environment. Phase II LPS has the same Lipid A as phase I, but lacks the *C. burnetii*-specific carbohydrates virenose and dihydrohydroxystreptose (Toman et al. 2009). This phase shift has an enormous impact on the serological host response and is important for serological diagnosis and vaccine development. The phase variation phenomenon has been studied at the Institute of Virology for decades (Kazár et al. 1975; Schramek et al. 1985; Skultety et al. 1996; Toman et al. 1998; Ftacek et al. 2000; Toman et al. 2003a; Toman et al. 2003b; Vadovic et al. 2005; Lukacova et al. 2008; Toman and Vadovič 2011; Flores-Ramirez et al. 2017).

*Coxiella burnetii* primarily infects domestic ruminants (cattle, sheep, goats), pets and also wild mammals, birds, fish and reptiles. In animals, the infection is asymptomatic but induces abortions in livestock. The most important route of infection in animals and humans is inhalation of bacteria contaminated dust, and the oral route is considered of secondary importance. Infected animals spread *C. burnetii* into the environment by milk, faeces, urine, saliva, vaginal secretion, placenta, or amniotic fluids (Porter et al. 2011). Arthropods have also been found infected. Ticks are considered to be natural primary reservoirs of *C. burnetii* and over 40 species have been found naturally infected worldwide (Reusse 1960; Řeháček and Tarasevich 1988; Parola and Raoult 2001). Ticks transmit the bacteria not only horizontally in saliva and faeces, but also vertically – transovarially and transstadially. In the laboratory at the Institute of Virology in Bratislava, the ability of the tortoise tick *Hyalomma aegyptium* (Linnaeus, 1758) to maintain natural foci of Q fever was experimentally investigated and the results showed that this tick species has indisputable potential in the epidemiology of Q fever (Široký et al. 2010). Human infections through tick bites have rarely been reported. The presence of *C. burnetii* in ticks from Slovakia has also been investigated (Table 2). By inoculation to yolk sacks of embryonated hen eggs, six strains of *C. burnetii* were recovered from *I. ricinus* and other strains from *D. reticulatus*, *D. marginatus*, *H. concinna*, *H. punctata* and *H. inermis* ticks collected in different habitats in lowlands and submontane regions of Slovakia (Řeháček et al. 1991). Comparison of virulence of *C. burnetii* isolates from bovine milk and from ticks showed that mice infected with the milk isolate demonstrated a lower number of *C. burnetii* particles in the spleen than those originating from ticks, which supports the hypothesis that strains circulating in cattle herds without a prolonged or extensive tick intervention gradually decrease their virulence (Kocianová et al. 2001). The tick species probably does not play a significant role. The presence of *C. burnetii* was

also demonstrated in questing *I. ricinus* and *H. concinna* ticks from SW Slovakia by PCR-RFLP based on *com1* gene (Špitalská and Kocianová 2002). *Coxiella burnetii* positive birds (*E. rubecula* and *S. atricapilla*) were captured during spring migration in Drienovec in 2012 and *C. burnetii* positive *I. ricinus* larvae and nymphs were collected from *E. rubecula*, *P. major*, *S. europaea* and *P. montanus* birds. The great tit was recorded as the most infested bird species with *I. ricinus* and carried *C. burnetii* positive larvae and nymphs. Similar to *R. helvetica*, it has been hypothesized that synanthropic birds could also carry infected ticks and ensure the geographical distribution and maintenance of *C. burnetii* in nature (Berthová et al. 2016). In contrast to birds, presence of *C. burnetii* was not confirmed in rodent-attached ticks and rodents (Minichová et al. 2017). However, *C. burnetii*-infected *I. ricinus* (all developmental stages) were found on cervids and mouflon hunted in forests of SW Slovakia (Kazimírová et al. 2018).

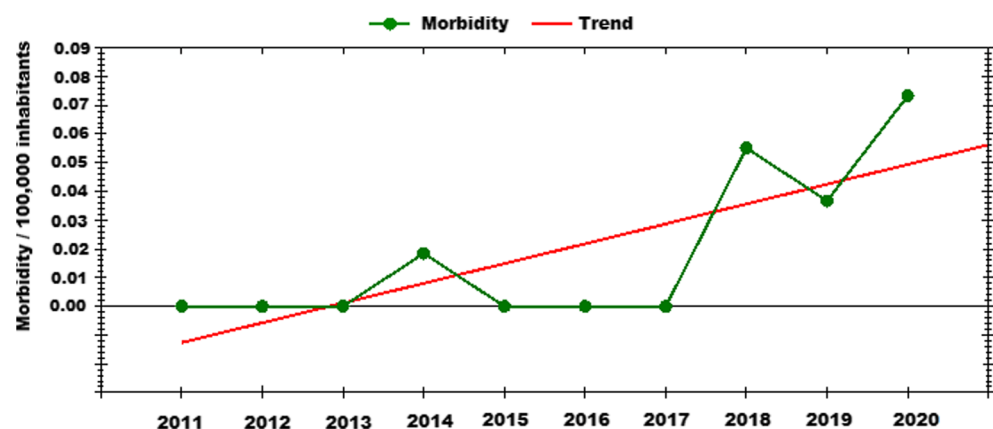
The presence of pathogenic *C. burnetii*, but also of *Coxiella*-like endosymbionts (CLEs) was monitored in questing ticks and ticks fed on humans originating from sites in W, E and N Slovakia (Špitalská et al. 2018; Špitalská et al. 2021b). The presence of *C. burnetii* DNA was detected in *D. reticulatus* and *I. ricinus*. *Haemaphysalis inermis* were *C. burnetii*-negative, but showed the highest prevalence of CLEs. Sequencing of the *groEL* gene fragments and phylogenetic analysis confirmed that the CLEs sequences did not group with the pathogenic *C. burnetii* and were identical to a *Coxiella* endosymbiont previously identified in different tick species as *Rhipicephalus geigy* (Aeschlimann and Morel, 1965) (isolate Rgei1), *Coxiella* endosymbiont of *Ornithodoros sonrai* Sautet and Witkowski, 1943 (isolate Oson1), *Rickettsiella* endosymbiont of *Ixodes ventralloii* Gil Collado, 1936 (isolate ixoventa6), and *Rickettsiella* endosymbiont of *I. arboricola* (isolate ixoarbo827).

The incubation period of Q fever is one to three weeks, depending on size of the inoculum, geographical area, route and the time of infection (Porter et al. 2011). Approximately 60% of humans remain

asymptomatic or with self-limiting illness associated with fever, fatigue, headache and myalgia, while 40% manifest clinical signs such as hepatitis and/or pneumonia (Maurin and Raoult 1999). Mortality rate of acute Q fever is estimated at 1–2% (Watanabe and Takahashi 2008; Delsing and Kullberg 2008). Chronic Q fever infection persists for more than six months and concerns 5% of infected cases (Fournier et al. 1998). Endocarditis, chronic hepatitis, osteomyelitis, septic arthritis, interstitial lung disease, chronic fatigue syndrome, infection of aneurysm can occur (Porter et al. 2011). The prognosis of chronic infections is less favourable than for acute infections. Antibiotic treatment is less effective, and the disease is usually long, with mortality rates that can reach more than 50% (Watanabe and Takahashi 2008). In Europe, Q fever is widely distributed. The first outbreak of Q fever in Slovakia was recognized in 1954 among agricultural workers. The source of infection was a sheep flock imported from Romania. Shortly after, a local outbreak among textile plant workers occurred. Since then, many small epidemics have been reported from farms with imported animals as well as from factories processing imported wool and hides (Literak and Rehacek 1996). The largest epidemic with 113 recorded human cases was reported from Jedľové Kostol'any (W-central Slovakia) in 1993; the source of infection were goats imported from Bulgaria (Varga 1997). Serological monitoring of animals in Slovakia is done annually by veterinarians and the presence of antibodies against *C. burnetii* phase I and phase II in human sera is determined in samples provided by medical doctors in the laboratory at the Institute of Virology.

Between 2011–2020, only a few human Q fever cases were confirmed in Slovakia, with the highest incidence in 2020 (3 cases) (Fig. 4; <https://www.epis.sk>). Antibodies against *C. burnetii* were recorded in sera of 7.8% and 2.3% selected human patients and cattle, respectively (Avdičová et al. 2019).

**Fig. 4** Incidence of Q fever cases in Slovakia. Trend over 2011–2020. Modified from <https://www.epis.sk>



### *Francisella tularensis*

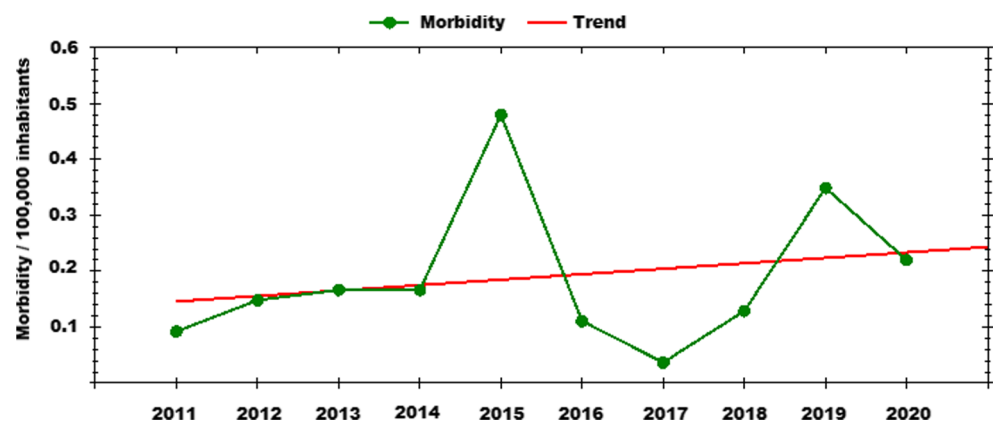
The causative agent of tularaemia, *Francisella tularensis*, is widely distributed in the northern hemisphere. Three subspecies of *F. tularensis* are known. By 1998, only information about the occurrence of *F. tularensis* subsp. *palaeartica* (*holarctica*) was available in Europe. *Francisella tularensis* subsp. *tularensis* was known from North America; it is a highly virulent agent for mammals and causes severe illness in humans. The first successful isolation of strains of *F. tularensis*, subsp. *tularensis* in Europe from fleas and mites parasitizing small mammals caught in W Slovakia was presented by Guryčová (1998). Ticks (*Dermacentor*, *Haemaphysalis*, *Ixodes* and other species), deer flies (*Chrysops* spp.) and other dipterans, as well as some mites and fleas infesting small mammals are able to transmit the bacteria (Guryčová 1998; Hubálek and Rudolf 2011) (Table 2). Humans can acquire the infection also through direct contact, e.g. during skinning of animals, by contamination of water with urine and faeces of infected rodents, with aerosol, or contaminated dust when working with hay and straw (Guryčová 1998; Hubálek and Rudolf 2011).

The first data on tularaemia in Slovakia come from Záhorie region (W Slovakia), from the territory of the former Bratislava region from 1936–1937 (Drbohlav 1937; Tománek 1937). The outbreak of the disease affected some districts of W Slovakia (Skalica, Senica, Malacky) adjacent to S Moravia, as well as the territory of lower Austria, where an epidemic occurred during that period. The source of infection were mainly wild hares and human contact with them (Vrta 1937). Sporadic human cases in the Záhorie area were reported again in the years 1946–1947. More detailed data on tularaemia in humans from this region have been recorded since 1950. Until 1956, morbidity did not exceed values of more than 0.5 cases per 100,000 inhabitants. Since 1956, there has been an increase in the number of human cases of tularaemia in W Slovakia,

while another large epidemic in the Bratislava region was recorded in 1959–1960 (Pučeková 1962). The source of infection in humans was contact with rabbits, wild rodents, and in people working in agriculture also contact with animal feed and litter. The trend of morbidity in Slovakia caused by tularaemia in the period 1960–1972 was characterized by periodic alternations of epidemic waves. The highest morbidity was registered in 1967, reaching 14.4 per 100,000 inhabitants. A minor outbreak was reported from E Slovakia in 1972, although previous epidemics occurred only in W and SW Slovakia. The overall trend of morbidity from tularaemia has been declining since the epidemics in the 1960s (Guryčová 2006), although a few outbreaks through activation of natural foci in W Slovakia have been recorded in 1995–1996 (Guryčová et al. 1999) and 2002 (Guryčová et al. 2010). Moreover, important changes in the epidemiology of the disease have been observed and the proportion of cases transmitted by other routes than through direct contact with infected hares has increased with over 10% cases transmitted by ticks and other arthropods (Guryčová et al. 1999; Guryčová 2006; Guryčová et al. 2010). During 2011–2020, the highest overall incidence of tularaemia was reported in 2015 (Fig. 5), with foci in the endemic regions of W Slovakia (<https://www.epis.sk>).

During the study of tularaemia outbreaks, Guryčová and Letkovský (1973) examined nearly 3,200 rodents (Rodentia), insectivores (Eulipotyphla) and lagomorphs (Lagomorpha) in W Slovakia between 1963–1969, and in addition a lower number of rodents and insectivores from central and E Slovakia (approximately 400 individuals). The authors reported isolations of *F. tularensis* strains from six rodent species (yellow-necked mouse *A. flavicollis*, the house mouse *Mus musculus* Linnaeus, 1758, the brown rat *Rattus norvegicus* (Berkenhout, 1769), bank vole *M. glareolus*, common vole *M. arvalis*, the European hamster *Cricetus cricetus* (Linnaeus, 1758)), three shrew species (the common shrew *S. araneus*, the lesser white-toothed shrew *Crocidura suaveolens* (Pallas, 1811)

**Fig. 5** Incidence of tularaemia cases in Slovakia. Trend over 2011–2020. Modified from <https://www.epis.sk>



the Eurasian water shrew *Neomys fodiens* (Pennant, 1771) and from the European hare. Guryčová and Letkovský (1973) also examined almost 6,000 ticks of four species from W Slovakia and isolated two *F. tularensis* strains only from *I. ricinus* nymphs and adults. Subsequent isolations of *F. tularensis* strains from small mammals, ticks, or other groups of ectoparasites were almost exclusively done in W Slovakia. In E Slovakia, monitoring of anti-tularaemia antibodies in various animal groups during 1997–2001 confirmed significant seropositivity in sheep, fallow deer, mouflon and dogs. Isolation of *F. tularensis* was successful only in one case, from *A. agrarius* at the locality Čerhov (district Trebišov, E Slovakia) (Guryčová et al. 2003).

In the period of four years (1977–1980), Guryčová et al. (1982) examined nearly 1500 mammals of thirteen species in the region of the Small Carpathians (W Slovakia). Fifteen strains of *F. tularensis* from *A. flavicollis*, *M. glareolus*, *M. subterraneus*, the southern water shrew *Neomys anomalus* Cabrera, 1907, *S. araneus* and the common weasel *Mustela nivalis* Linnaeus, 1766 were isolated. One strain was also isolated from the *I. trianguliceps* tick infesting *A. flavicollis*. In the surroundings of the Gbelce village (Hronská pahorkatina; district Nové Zámky, SW Slovakia), Kožuch et al. (1987) investigated more than 1800 *I. ricinus* (nymphs, males and females) during 1981–1985. Three strains of *F. tularensis* were isolated from pools of 979 *I. ricinus* nymphs. The authors also examined 279 small mammals of eight species. They successfully isolated one strain of *F. tularensis* from *M. arvalis*. Furthermore, Kožuch et al. (1995) examined 923 specimens of small mammals of seven species and reported the isolation of a *F. tularensis* strain from *M. glareolus* in W Slovakia (Záhorie region). This research confirmed the existence and active status of a mixed natural focus of TBE, tularaemia and haemorrhagic fever with renal syndrome in the studied area.

Výrosteková et al. (1991) investigated over 10,000 adult ticks of six species (*I. ricinus*, *D. reticulatus*, *D. marginatus*, *H. concinna*, *H. inermis*, *H. punctata*) and several hundreds of nymphs of these species for the presence of the etiological agent of tularaemia. Sampling of ticks by the flagging method was performed during 1984–1989 in different sites of SW Slovakia. Thirty strains of *F. tularensis* were isolated from pools of adult ticks, mostly from *D. reticulatus* (20 strains), but also from *I. ricinus* (8 strains) and *H. concinna* (2 strains). All isolates originated from W Slovakia, one from the area of Podunajské Biskupice near Bratislava, the second from near Šaštín – Stráže (Senica district). Three new foci of tularaemia were discovered – near Smolenice (Trnava district), Olichov (Nitra district) and Plášťovce (Levice district).

Results of tick monitoring for the presence of *F. tularensis* in the Danube basin (W Slovakia) and in the Slovak Karst (SE Slovakia) were presented in studies by Guryčová et al. (1995), Hubálek et al. (1990), and Výrosteková et al. (2001).

*Francisella tularensis* strains were successfully isolated from five tick species, *I. trianguliceps*, *I. ricinus*, *D. reticulatus*, *D. marginatus* and *H. concinna*. In addition, the mentioned authors presented summarizing studies on the role of ticks as epidemiological indicators of the activity of natural outbreaks of tularaemia in Slovakia. The research confirmed that the main areas with tularaemia foci were located in W and E Slovakia, mainly in floodplain areas with a common occurrence of three tick species - *D. reticulatus*, *H. concinna* and *I. ricinus*. *Dermacentor reticulatus*, having a one-year development cycle (Nosek 1972), is considered as an accelerator of the epizootic process of tularaemia. This tick species also represents the main vector of *F. tularensis* in tularaemia foci, which was confirmed by tens of isolates available from *D. reticulatus* males and females (Kmetý et al. 1990; Hubálek et al. 1990; Výrosteková et al. 1991; Guryčová et al. 1995; Guryčová 1998; Výrosteková et al. 2001]. On the other hand, two sympatrically occurring tick species in the floodplain areas, *I. ricinus* and *H. concinna*, have a multi-year cycle, allowing long-term persistence of the focus during latency of the disease. The experimentally proven transstadial transmission of *F. tularensis* strains in *I. ricinus* confirms this hypothesis (Výrosteková 1993; Výrosteková 1994). Several species of fleas and mites also significantly contribute to the persistence of tularaemia among small mammals and the circulation of the pathogen in a focus (Kmetý et al. 1987; Guryčová and Výrosteková 1989; Guryčová 1998). In submountain areas, *I. trianguliceps* probably plays a role of reservoir and vector of tularaemia agents among small mammals (Guryčová and Letkovský 1973; Guryčová and Výrosteková 1989). The vector role of the other tick species, namely *D. marginatus*, *H. inermis* and *H. punctata* in natural foci of tularaemia needs to be further studied as the examined samples have not been representative (Výrosteková et al. 1991; Guryčová 1998; Výrosteková et al. 2001].

Guryčová (1998) summarized almost 20-years of research of tularaemia outbreaks and noted that in total, 155 *F. tularensis* strains were isolated from different biological sources during the surveillance of tularaemia in Slovakia over the years 1978–1996. Of the total of 155 strains, 65 were isolated from small mammal tissues, 68 from ticks, four from mites (Mesostigmata) and 22 from fleas (Siphonaptera). The results of the comparative study revealed that all strains isolated from small mammals, ticks and five isolates from mites and fleas had biological properties characteristic of the strains of *F. tularensis* subsp. *palaearctica* (*holarctica*) biovar II. Biological properties of 17 strains isolated from mites and fleas in W Slovakia differed markedly, and were typical of the type strain of *F. tularensis* subsp. *tularensis*.

*Francisella*-like endosymbionts (FLEs) were also detected in 47.9% questing ticks in different sites of Slovakia, with the highest prevalence in *D. reticulatus* (79.9%), followed by *I. ricinus* (14.1%), while *H. inermis* were negative.

Sequencing of selected samples showed 98% identity with FLEs identified in *D. reticulatus* in France and *I. ricinus* from birds in Hungary (Špitalská et al. 2018).

### Babesia species

Babesiosis, a disease of veterinary and medical importance, is caused by intraerythrocytic parasites of the genus *Babesia* (Yabsley and Shock 2013). Human babesiosis is endemic in temperate zones of the northern hemisphere, including Central Europe (Vannier et al. 2015). *Babesia* spp. are transmitted by ticks of the family Ixodidae and humans represent accidental hosts. *Babesia* parasites undergo developmental changes in the ixodid tick (sexual phase - gamogony in the midgut, asexual proliferation - sporogony in salivary glands) and vertebrate host (asexual reproduction, merogony in erythrocytes) (Schnittger et al. 2012).

About 100 species of *Babesia* have been recognized worldwide, but only *B. divergens*, *B. microti* and *B. venatorum* have been associated with human disease in Europe, while the others cause infections in domesticated animals or wildlife (Yabsley and Shock 2013). Infections in free-living animals that serve as reservoirs of the parasites are mainly asymptomatic. Majority of the about 60 autochthonous European cases were attributed to *B. divergens* (Hildebrandt et al. 2013; Asensi et al. 2018) and only a few to *B. microti* and *B. venatorum* (Herwaldt et al. 2003; Hildebrandt et al. 2007). The disease manifestations can be severe, sometimes with fatal outcome in immunocompromised, asplenic or elderly persons. European zoonotic *Babesia* species and strains are transmitted by *I. ricinus*. Cattle are natural reservoirs of *B. divergens*, cervids (primarily roe deer) for *B. venatorum* and wild rodents for *B. microti* (Yabsley and Shock 2013). Transovarial transmission was confirmed for *B. divergens* and *B. venatorum* (Bonnet et al. 2007; Bonnet et al. 2009).

Previous studies on piroplasmids of Slovakia focused on *Babesia canis* transmitted by *D. reticulatus* and causing disease in dogs (Chandoga et al. 2002; Duh et al. 2006; Majláthová et al. 2011]. Human babesiosis is not listed among the notifiable diseases in Slovakia. Three cases have been reported since 1991 (<http://www.uvzsr.sk>, personal communication). As no published reports about the cases are available, it is not publicly known if they were autochthonous or imported and which *Babesia* spp. were the causative agents. Generally, the information on the occurrence of zoonotic *Babesia* spp. is scarce in Slovakia and is limited to a few recent studies. Natural infections of *I. ricinus* ticks and different rodent species with *B. microti* have been reported from SW and E Slovakia (Švehlová et al. 2014; Blaňarová et al. 2016; Hamšíková et al. 2016a) (Table 2). *Babesia microti* (Jena/Germany genotype) isolates from SW Slovakia

were identical to the zoonotic strain from Europe (Hamšíková et al. 2016a), whereas *B. microti* (both Jena, and Munich genotypes) were present in E Slovakia (Blaňarová et al. 2016), indicating that in some natural foci in Slovakia zoonotic and non-zoonotic strains may co-circulate. *Babesia venatorum* isolates were identical to the zoonotic strain from Europe and were detected in *I. ricinus* from SW (Švehlová et al. 2014; Hamšíková et al. 2016a) and E Slovakia (Víchová et al. 2013). The presence of *B. divergens* in *I. ricinus* from Slovakia has been indicated by using the RLB method, but has not been confirmed by sequencing (Koči et al. 2009). It is important to note that earlier reports on the occurrence of *B. divergens* before the sequence definition have to be considered with caution (Malandrin et al. 2010), because this species is highly similar to *B. capreoli* which is present in roe deer and is non-pathogenic to humans or cattle and has been detected in questing *I. ricinus* ticks also from SW Slovakia (Hamšíková et al. 2016a). In addition, presence of *B. odocoilei*, causing disease in cervids and occasionally in bovids in Europe, and *B. motasi* parasitizing sheep and goat and two novel yet undescribed *Babesia* species with unknown host range have recently been detected in *I. ricinus* and *H. concinna* ticks, respectively, in SW Slovakia (Hamšíková et al. 2016a; Kazimírová et al. 2018).

### Non-zoonotic microorganisms and parasites detected in ticks

In addition to viruses, bacteria and parasites that pose risk to humans, diverse microorganisms with non-confirmed zoonotic potential have been detected in ticks in Slovakia. Reports on detections of *Coxiella*-like and *Francisella*-like endosymbionts are mentioned above (Špitalská et al. 2018) (Table 2). *Arsenophonus nasoniae*, a male-killing endosymbiont of the *I. hookeri* wasp parasitising hard ticks has been detected in *I. ricinus* and isolated by using tick cell lines (Mediannikov et al. 2012). However, the presence of this bacterium in ticks was found to be associated with the presence of the hymenopteran parasitoid *I. hookeri* (Boháčsová et al. 2016). Previously, the parasitoid was repeatedly found in different hard tick species and represents a candidate for biological control of tick populations (Řeháček and Kocianová 1992; Řeháček 1998; Slovák 2003).

Results on the presence of *Spiroplasma ixodetis*-related helical mycoplasmas (class Mollicutes) in questing *I. ricinus* from SW Slovakia (Subramanian et al. 2012) and their isolation and cultivation in tick cell lines were also reported (Bell-Sakyi 2015). Current findings indicate that spiroplasmas are

natural endosymbionts of ticks and their potential pathogenicity to vertebrates is not known (Bell-Sakyi 2015).

*Candidatus* Cryptoplasma sp. REP belonging to the Anaplasmataceae family, was found to be associated with lizards and was also detected in questing and lizard-feeding *I. ricinus* ticks in Slovak Karst National Park in SE Slovakia (Kočíková et al. 2018). The endosymbiont *Wolbachia pipientis* was also detected in part of the examined ticks.

Asymptomatic infections caused by *Theileria* spp. are known from European free-living cervids and caprines, but none of the described *Theileria* spp. has been found to cause zoonotic disease (Yabsley and Shock 2013). Probably, *I. ricinus* and/or *Haemaphysalis* spp. are their vectors. In Slovakia, infections with *Theileria* spp. have been reported in cervids (red deer, roe deer, fallow deer) (Černý 1958; Kazimírová et al. 2018), in *I. ricinus* and *H. concinna* feeding on infected cervids, and rarely in questing *H. concinna* (Hamšíková et al. 2016a), suggesting that the latter tick species might be the natural vector of the detected *Theileria* spp. (Kazimírová et al. 2018). The *Theileria* spp. were found to be phylogenetically related with *Theileria capreoli*, but are probably represented by two closely related species associated with different cervids (Kazimírová et al. 2018).

*Hepatozoon canis* DNA was detected in questing *I. ricinus* ticks from SW Slovakia. Sequencing of the amplicons revealed 100% identity with European *H. canis* isolates from red foxes and dogs (Hamšíková et al. 2016b). The findings indicate the geographical spread of the parasite and a potential role of other tick species as vectors in areas where the competent vector, *R. sanguineus*, is not endemic.

Microsporidial parasites of arthropods, namely *Nosema slovaca* Weiser and Rehacek, 1975, were found to infect both *I. ricinus* and *D. reticulatus*, whereas the species *Unikaryon ixodis* (Weiser, 1957) was detected in *I. ricinus* only (Weiser et al. 1999).

Presence of trypanosomes was also detected in the haemolymph of unfed *I. ricinus* (Rehacek et al. 1974), but the species identity has not been confirmed and information about reservoirs, routes of transmission and pathogenicity are missing. Recently, a novel trypanosome designated as *Trypanosoma* sp. Bratislava1 from questing *I. ricinus* adults collected in SW Slovakia was isolated in tick cell culture and partially characterised (Luu et al. 2020). The analyses have suggested that it may be a new species related to species detected in ticks in South America and Asia, and to *Trypanosoma caninum* isolated from dogs in Brazil.

## Conclusions

In spite of the long tradition in the research of ecoepidemiology of TBD in Slovakia, still a lot of questions remain open and many aspects of the circulation and co-

circulation of tick-borne microorganisms need further exploration. As a result of global warming, changes in land use and urbanisation, and thanks to the development of advanced tools to analyse the tick microbiome, new microorganisms with unknown ecology and potential pathogenicity to humans and animals will probably be detected. Moreover, there is a risk of introduction and geographical spread of ticks and pathogens from the south. Proper diagnostic tools to identify neglected or unknown diseases are lacking and many cases of TBD probably remain undiagnosed or misdiagnosed. A long-term task for Slovak parasitologists and epidemiologists in connection with climate change, current processes of urbanization and synurbanisation of certain vertebrate groups (mainly birds and carnivores) as well as of invertebrates is the continuous monitoring of changes in the diversity of vertebrate hosts and abundance of ticks and their infection with pathogenic microorganisms, particularly in urban sites.

The genetic variability, geographic spread and transmission mechanisms of TBEV and *Borrelia* spirochaetes, including vector competence of ticks other than *I. ricinus* and reservoir competence of vertebrate hosts need further exploration. An important challenge for TBE is to find out why Slovakia is unique among European countries in terms of the relatively low number of total reported cases in contrast to the high proportion of alimentary infections. Since genospecific and intraspecific variability within *B. burgdorferi* s.l. affects its ecology and pathogenicity, the challenge for LB is to exactly identify the genotypes that cause human disease in close connection to the specific reservoir hosts to understand why in some areas the prevalence and heterogeneity of borrelia in questing ticks is really low (4%) as compared to areas where the prevalence of positive *I. ricinus* ticks exceeds 60%.

Anaplasmosis and rickettsioses are less prevalent than TBE and LB, but the occurrence of *A. phagocytophilum* and SFG rickettsiae in ticks and vertebrate hosts has been confirmed in many sites in Slovakia and new knowledge on the genetic variability of these bacteria and on the vector and reservoir competence of ticks has been gained. Even though the pathogenicity of *A. phagocytophilum* strains to humans in Europe seems to be much lower than in the USA or Asia, we assume that HGA cases are underestimated or misdiagnosed as acute LB or other infectious diseases. Monitoring of antibodies against *Rickettsia* spp. and *C. burnetii* is helpful in detecting cases of human infections and should be further developed. To confirm the role of individual tick species as vectors of *F. tularensis*, it will be necessary to investigate representative natural samples of *Haemaphysalis* spp., mainly of *H. inermis*, which is interesting from the ecological point of view and also from the point of view of phenology, that differs from the other tick species. In view of genetic typing of *F. tularensis* strains isolated and confirmed from various biological sources, it will be necessary to use not only cultivation methods, but also apply molecular methods, including next



generation sequencing. *Neoehrlichia mikurensis* as well as *Babesia* spp. can be a potential threat for immunocompromised patients. Thus the clinicians should consider them in differential diagnosis in patients with acute symptomatic and the history of tick bites. Moreover, confirmed coinfections of pathogens in ticks highlight the need of clinicians to consider mixed symptoms in diagnosis.

As a result of global changes there is a risk of introduction and spread of known tick-borne pathogens from endemic regions or emergence of new pathogens with unknown ecology and etiology. Slovakia should be prepared to identify these microorganisms and the risk areas and diagnose the related diseases by adopting modern and novel molecular and laboratory diagnostic tools and introduce proper preventive measures and treatment.

**Acknowledgements** The authors are grateful for the financial support of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences, Grants VEGA 2/0137/21, 2/0021/21, 2/0014/21 and of the Slovak Research and Development Agency, grants APVV-16-0463, APVV-16-0518, and APVV-18-0201.

## Declarations

**Conflict of interests** The authors declare no conflict of interests.

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