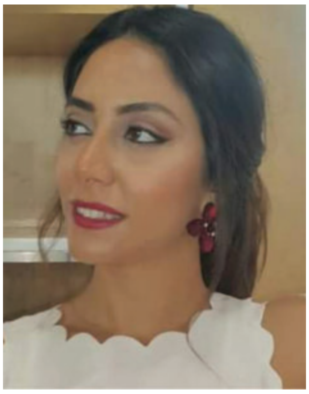
A concise overview on tick-borne human infections in Europe: a focus on Lyme borreliosis and tick-borne *Rickettsia* spp.



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Ticks are blood-feeding external parasites of mammals. Almost all ticks belong to one of two major families, the *Ixodidae* or hard ticks, and the *Argasidae* or soft ticks. Ticks are responsible of transmitting many diseases called 'tick-borne diseases'. *Borrelia* and *Rickettsia* spp., are the most important tick-transmitted bacterial pathogens circulating in Europe. In this review we will focus on the two tick-borne diseases caused by these bacterial pathogens, their vector, epidemiology, clinical diagnosis and symptoms.

Ticks are blood-feeding external parasites of mammals, birds and reptiles throughout the world. They belong to the order Ixodida. Almost all ticks belong to one of two major families, the *Ixodidae* or hard ticks, which are difficult to crush, and the *Argasidae* or soft ticks. The *Ixodidae* contain over 700 species of hard ticks with a scutum or hard shield, which is missing in the *Argasidae* family. The *Argasidae* contain about 200 species[1]; Table 1 shows the most important tick-borne diseases and their vectors.

Tick-borne diseases have been described throughout human history. A papyrus scroll dating back to the 16th century B.C. referred to what could be 'tick fever'[2]. At the end of the nineteenth century, Theobald Smith and Frederick Kilbourne were the first to demonstrate that ticks were responsible for transmitting diseases. Their experiment in cattle allowed them to conclude that the absence of ticks lead to the absence of Texas fever[3]. In this review we will focus on *Borrelia* and *Rickettsia* spp., the most important tick-transmitted bacterial pathogens circulating in Europe.

Lyme borreliosis (LB)

Causative agent, reservoir and vector

LB is an infectious disease caused by the extracellular bacterium *Borrelia burgdorferi* sensu lato (sl). This microorganism was determined to be the causative agent of LB by W. Burdogorfer in the early 1980s[4]. It is a spirochaete belonging to the order Spirochaetales, helically shaped, motile, 20–30 µm in length and 0.2–0.3 µm in width[4]. Three human pathogens can be distinguished within *B. burgdorferi* sl: *B. burgdorferi* sensu stricto (ss), *Borrelia afzelii* and *Borrelia garinii*. All of them are present in Europe[5].

Regarding the vector, *Ixodes* ticks transmit all species belonging to *B. burgdorferi* sl[6]. Eighty per cent of tick bites transmitting LB are caused by nymphal ticks. The most important reservoirs of *B. burgdorferi* sl in Europe are rodents, insectivores, hares and

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Table 1. Most important tick-borne diseases and their vectors in Europe.

Disease	Specific disease names	Agent	Vector
Anaplasmosis		*Anaplasma phagocytophilum*	*Ixodes* spp.
Lyme borreliosis		*Borrelia burgdorferi* *Borrelia afzelii* *Borrelia garinii*	*Ixodes* spp.
Tularemia		*Francisella tularensis*	*Dermacentor* spp. *Ixodes* spp.
Rickettsioses	Mediterranean spotted fever Lymphangitis-associated disease Scalp and enlarged neck lymphadenitis	*R. conorii* *R. helvetica* *R. monacensis* *R. sibirica mongolitimonae* *R. massiliae* *R. aeschlimannii* *R. slovaca* *R. raoultii* *R. hoogstraalii*	*Rh. sanguineus* *I. ricinus* *I. ricinus* *Hyalomma* spp./*Rhipicephalus pusillus* *Rh. sanguineus* *Hy. marginatum* *Dermacentor marginatus/ Dermacentor reticulatus* *Dermacentor marginatus/ Dermacentor reticulatus* *Haemaphysalis sulcata*
Babesiosis		*B. divergens* *B. venatorum* *B. microti*	*I. ricinus*
Tick-borne encephalitis		Tick-borne encephalitis virus	*I. ricinus* *I.scapularis* *I. persulactus*
Tick-borne relapsing fever		*Borrelia* spp. (*Borrelia miyamotoi*)	*Ixodes* spp.

several bird species[7]. Birds are more likely to carry *B. garinii*, so this microorganism may be carried over very long distances, especially in the case of migratory sea birds[8].

Epidemiology

LB is one of the most prevalent vector-borne diseases in Europe[9]. However, precise epidemiological data are not available for all European countries because the disease is notifiable in only a few countries[10,11]. The highest average incidence rates among the reporting countries were found in Belarus, Belgium, Croatia, Norway, the Russian Federation and Serbia (<5/100 000), Bulgaria, Finland, Hungary, Poland and Slovakia (<16/100 000), the Czech Republic, Estonia, and Lithuania (<36/100 000) and Slovenia (<130/100 000) (ecdc.europa.eu).

There are clear differences in LB incidence rates and clinical presentations across Europe[12]. Incidence rates in European countries vary from less than less than one per 100 000 inhabitants to about 350 per 100 000, with a mean annual number of notified cases in Europe exceeding 65 400[13]. Furthermore, the incidence rates of LB across Europe are influenced by geographical, environmental and climatic factors[14,15]. Studies on the future potential distribution of *I. ricinus* notably showed that this tick may emerge in European areas in which they are currently lacking, thus leading to an increased risk to human health[16].

Clinical symptoms and diagnosis

During LB, symptoms may vary depending on the stage of the disease. Early LB may be divided into early localised (1–4 weeks after the tick-bite) and early disseminated (3–10 weeks after the tick-bite) diseases, while late disseminated LB develops months to years later. In early LB, various clinical manifestations may be identified, notably the pathognomonic erythema migrans.

Erythema migrans (EM) is the most specific and frequent finding in patients with LB. European studies showed that it is present in 40 to 77% of LB patients[17]. Primary erythema migrans is a round or oval, expanding erythematous skin lesion that develops at the site of the infecting tick-bite[18]. Three to 30 days after the tick bite the skin lesion becomes apparent (most commonly 7–14 days). It is not associated with significant pruritis[19]. If the skin lesion disappears within a few days, it is not considered as an EM. Other

Table 2. Lyme borreliosis (LB) symptoms.

LB stage	Symptom name	Description	Epidemiology
Early disseminated	Early neurologic disease	Isolated meningitis Encephalopathy Radiculopathy Cranial neuropathy Mononeuropathy Multiplex lymphocytic meningitis Encephalomyelitis	Frequent in Europe
	Cardiac manifestation	Chest pain Palpitations Rhythm disorder	5% of LB cases
Late disseminated	Lyme arthritis	Long-lasting objective joint swelling (synovitis)	More frequent in the US than Europe
	Acrodermatitis chronica atrophicans	Red or bluish-red lesions	Rarely reported in the US Well recognised in Europe
	Borrelial Lymphocytoma	Bluish-red tumour-like skin infiltrate	–

secondary ring-shaped lesions may develop in certain cases and are named multiple EM. Less common LB symptoms are cited in Table 2.

The diagnosis of LB is difficult due to the unspecific nature of the majority of clinical symptoms, and makes laboratory support crucial. The culture of *Borrelia* spp. is time consuming and labor intensive, and is thus not used for the routine diagnosis[20]. In addition, in Europe, the microbiological diagnosis of LB must consider the heterogeneity of the agents depending on countries.

Serology is usually the routine method used to support clinical diagnosis[19,20]. However, serology suffers limitations. In particular, the antibody response in early LB may be weak or absent, especially in EM and early LNB[21]. Western blotting is used currently as a confirmatory assay in the serodiagnosis of LB, but is usually only employed following a positive screening assay. In addition, many biomolecular tests were developed in order to supplement western blot such as sensitive and specific Lyme Multiplex PCR-dot blot assay (LM-PCR assay) and nested polymerase chain reaction (nPCR)[21] applicable to blood and urine samples. However, PCR-based methods are not standardised.

Rickettsioses

Causative agents and vectors

Rickettsioses are worldwide zoonosis caused by obligate intracellular bacteria from the genera *Rickettsia* and *Orientia*. These bacteria belong to the alpha-*Proteobacteria* (Figure 1) and are transmitted by arthropods, mainly ticks, but also fleas, lice and mites[22]. These zoonoses are among the oldest known vector-borne diseases. In Europe, only *Rickettsia* spp. are etiological agents of rickettsioses[23]. Tick-borne rickettsioses (TBR) are the main rickettsial infections in Europe and will be developed in the next section.

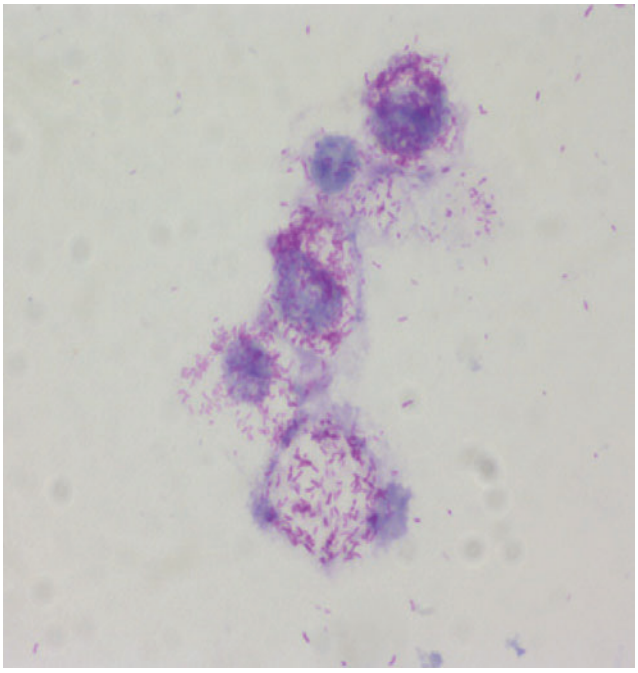

Figure 1. *Rickettsia conorii* cultivated on Vero cells.

Epidemiology

The prevalence of tick-borne rickettsioses depends on several parameters and most of them are directly related to the tick vectors: (i) the abundance of the tick itself, which is influenced by many factors, including climatic and ecological conditions; (ii) their affinity for humans; and (iii) the prevalence of rickettsia-infected ticks. In Europe, several *Rickettsia* species are responsible for tick-borne rickettsioses[24]. *Rickettsia conorii*, the main etiological agent of Mediterranean spotted fever (MSF), is the most important in terms of numbers of cases[24]. Its distribution is restricted to the Mediterranean area where it occurs in Spring and Summer. Other pathogenic *Rickettsia* species in Europe include *R. aeschlimannii, R. helvetica, R. hoogstraalii, R. massiliae, R. monacensis, R. raoultii, R. sibirica mongolitimonae,* and *R. slovaca* (Table 1, Figure 1).

Clinical symptoms and diagnosis

Typically, the clinical symptoms of tick-borne rickettsioses develop 6 to10 days after a tick-bite. Human rickettsioses are characterised by various combinations of symptoms, the most common being a triad consisting of a generalised maculopapular rash, an eschar at the inoculation site (Figure 2) and flu-like symptoms including high grade fever, myalgia, malaise, headache and nausea[25]. However, depending on species and the underlying patient's status, these major clinical signs vary greatly.

The diagnosis of rickettsial infections usually relies on epidemio-clinical data, and may be confirmed by laboratory testing[6]. In addition, the arthropods collected at the bite site or eschar may be useful for the diagnosis.

Serology is the most commonly used and available method worldwide due to its quick turnaround time, need for minimal sample preparation and to the serological cross-reactions observed among *Rickettsia* species that enable limiting the number of tested antigens[6]. Molecular testing is also well adapted for the diagnosis of tick-borne rickettsioses[26]. Molecular techniques overcome the drawback of seroconversion time, needed with serological testing[27]. PCR (either real-time or conventional) can be performed on whole blood, buffy coat or eschar material (crust, swabs, or biopsies)[6,28].

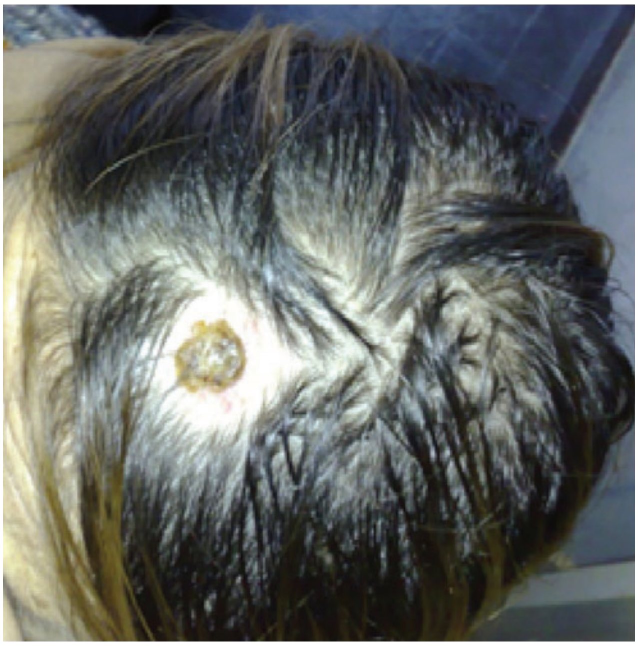

Figure 2. Inoculation eschar to the scalp of a patient with *R. slovaca* infection (SENLAT).

Rickettsial culture is also time consuming and should be performed in a BSL3 laboratory[29]. It is thus reserved to highly specialised laboratories. Matrix-assisted laser desorption/ionisation-time of flight Mass Spectrometry (MALDI-TOF MS) is a promising technique enabling both tick speciation and determining infection with *Rickettsiaceae*[30].

Conclusion

In Europe, the number of vector-borne disease is increasing in some regions. Ticks are notably expanding their range with climate changes. In addition, increased human travel and animal transport result in the epidemiology of tick-borne disease to be in a continuous dynamic change, thus leading to the emergence and/or spread of numerous tick-borne pathogens in Europe. Preventive measures that minimise tick-bite risk are one of the best ways to avoid contracting these diseases. Standardised diagnostic tools are crucial for treating and combating vector-borne diseases, especially when clinical symptoms are not specific. Finally, an increased interest should be given to tick-borne disease to avoid the small bite causing a big problem.

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Biographies

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