4 Tick-borne Infections (Including Zoonoses) in Europe and the Mediterranean Basin*

Frank Koenen,[1] Ilaria Pascucci,[2] Thomas G.T. Jaenson,[3] Maxime Madder,[4] Rita de Sousa,[5] Agustín Estrada-Peña,[6] Róbert Farkas[7] and Mo Salman[8]

[1]Veterinary and Agrochemical Research Centre, CODA-CERVA, Brussels, Belgium; [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy; [3]Uppsala University, Sweden; [4]Institute of Tropical Medicine, Antwerp, Belgium and University of Pretoria, South Africa; [5]National Institute of Health Doutor Ricardo Jorge, IP (INSA), Águas de Moura, Portugal; [6]University of Zaragoza, Spain; [7]Szent István University, Budapest, Hungary; and [8]Colorado State University, USA

4.1 African Swine Fever

African swine fever (ASF) is a viral swine disease caused by the African swine fever virus (ASFV), an icosahedral complex DNA virus that is a unique member of the *Asfarviridae* family. It affects only porcine species, those of all breeds and ages. The disease was first described in Kenya by Montgomery in 1921 when the virus spread from infected warthogs (*Phacochoerus aethiopicus*) to domestic pigs (*Sus scrofa*) and resulted in a 100% case fatality rate. The disease is currently present in Africa, mainly in countries located south of the Sahara, and in most cases it is endemic. In Europe, ASF is still endemic in Sardinia. More recently, in 2007, ASFV spread to the Transcaucasian countries (TCC) and the Russian Federation (RF) (Sánchez-Vizcaíno, 2006).

Pigs are the only domestic animal species that are naturally infected by ASFV. Wild boar have also been identified as susceptible to ASFV infection, with clinical signs and case fatality rates similar to those observed in domestic pigs in Spain, Portugal and Sardinia (Italy) and, experimentally, in feral pigs in Florida (Sánchez-Vizcaíno, 2006). Wild boar and feral pigs can transmit the virus directly to domestic swine. In Africa, it has been observed that ASFV induces an inapparent infection in three species of wild pigs (warthogs, bush pigs and red river hogs), while the role played by the giant forest hog has not yet been clarified (Jori and Bastos, 2009).

At present, no treatment or effective vaccine against ASFV is available. Since 1963, when the first live attenuated vaccine was used in Portugal, many efforts have been made in this area, but with unsatisfactory results. As no vaccine for ASFV currently exists, the prevention of this disease in

* Frank Koenen wrote Section 4.1; Ilaria Pascucci, Section 4.2; Thomas G.T. Jaenson, Sections 4.3, 4.7, 4.11.2; Maxime Madder, Sections 4.4, 4.5; Rita de Sousa, Section 4.6; Agustín Estrada-Peña, Section 4.8; Róbert Farkas, Sections 4.9, 4.10, 4.11.3, 4.11.4; Mo Salman, Section 4.11.1.

disease-free areas depends on preventing the introduction of the virus (Sánchez-Vizcaíno, 2006).

ASFV is spread among domestic pigs via the oral–nasal route. However, it has also been demonstrated that the virus can be infectious by a number of other routes, including tick bites, experimental inoculation via cutaneous scarification, and by the intramuscular, intravenous, subcutaneous and intraperitoneal routes. The infection usually commences in the monocytes and macrophages of the tonsils and mandibular lymph nodes. From there it spreads through the draining lymph nodes and blood to the target organs (lymph nodes, bone marrow, spleen, lung, liver and kidney) which are the principal sites of secondary replication.

The clinical signs of ASF can resemble a variety of other swine haemorrhagic diseases and can easily be confused with classical swine fever (hog cholera) and erysipelas. Laboratory tests are necessary to confirm the diagnosis. ASF can also present different clinical signs that depend primarily upon the virulence of the virus, infectious dose and mode of infection, with a range of clinical forms varying from acute to subclinical and chronic.

Some species of soft ticks have proved to be ASFV reservoirs and vectors, such as *Ornithodoros moubata* and *O. porcinus* in Africa and *O. erraticus* in the Iberian Peninsula (Spain and Portugal). In *O. moubata*, transovarial and trans-stadial ASFV transmission have been described; in *O. erraticus*, only trans-stadial transmission has been demonstrated. Other soft tick species that are widely distributed in North and South America have been identified as harbouring and transmitting ASFV, and in the experimental setting, *O. savignyi*, present in Africa, has been shown to transmit ASFV to domestic pigs (see Table 2.1).

In Africa, ASFV is maintained by a cycle of infection between wild pigs and soft ticks. In some of these wild pigs, ASFV infection is characterized by low levels of virus in the tissues and low or undetectable levels of viraemia; however, these levels of virus are sufficient to infect soft tick vectors and for tick transmission of the virus to domestic pigs. This cycle of the virus makes ASF very difficult to eradicate in Africa. In Sardinia, where ASF is still present, wild boars are as susceptible as domestic pigs. No ticks from the *O. erraticus* complex have been found in Sardinia.

Experience of past outbreaks of ASF outside Africa has shown that the introduction of ASFV into a non-infected pig population within a free region is most often linked to entry through international ports or airports. The infected material is typically garbage containing uncooked pork that is used for pig feeding (Sánchez-Vizcaíno, 2006). Once ASFV is established in domestic swine, infected animals are the most important source of virus dissemination to susceptible pigs. In Europe, ASFV was introduced for the first time in 1957 into Portugal through waste from international flights. Although this first outbreak was rapidly eradicated, in 1960 the virus entered Europe again in Lisbon (Portugal) and spread through the rest of Portugal and Spain, where ASFV remained endemic until 1995. During this period, some other outbreaks occurred in other European countries, affecting Andorra (1975), Belgium (1985), France (1964, 1967 and 1974), Malta (1978), the Netherlands (1986) and Italy (1967, 1969 and 1993), including the island of Sardinia, where ASF has remained endemic since 1978. All of these virus introductions were also linked to swill feeding.

In Europe, several epidemiological paths are known to be able to maintain ASFV in domestic pig populations and this complicates the control of the disease. The main routes of transmission are: swill, domestic pig and wild boar interactions, and pig–tick interactions (Sánchez-Vizcaíno, 2006). In 2010, a scientific opinion issued by the EFSA (European Food Safety Authority) Panel on Animal Health and Animal Welfare (2010a) on ASF contained an assessment of the risk of introduction of this virus into the European Union (EU), especially from the Caucasus (Wieland *et al.*, 2011). Another scientific report from the EFSA Panel on Animal Health and Animal Welfare (2010c) discusses the role of tick vectors in the epidemiology

of Crimean-Congo haemorrhagic fever (CCHF) and ASF in Eurasia, and contains geographic distribution maps of the tick vector and ASFV.

4.1.1 Virus–tick interaction

Several factors can influence the vector competence of soft tick species for ASFV. Some authors consider ASFV and the *Ornithodoros* tick as co-evolving organisms. Actually, noticeable telomeric similarities in the genomes of ASFV and *Borrelia* (the latter shares the same *Ornithodoros* tick host in Africa and is considered to be an original pathogen of soft ticks) suggest that ASFV is also a primary organism of *Ornithodoros* ticks and that it coadapts to its tick hosts (Hinnebusch and Barbour, 1991). This hypothesis could explain the noticeable discrepancies concerning infection success rates that have been reported in several past surveys. For example, De Tray (1963) reported consistent establishment of the virus isolate 'Uganda' in specimens of the *O. moubata* group (34/35 were infected) whereas another isolate, 'Tengani', caused persistent infection only in a small proportion of ticks (2/46 were infected). More recently, Kleiboeker *et al*. (1999) compared oral and intra-haemocoelic experimental infections of *Ornithodoros* ticks collected from warthog burrows in Kruger National Park and the Northern Transvaal region of South Africa, as well as infections of ticks from Masai Mara Reserve in Kenya. These researchers used three different viruses from South Africa, Malawi and Zimbabwe, all originally isolated from ticks collected in the field. The oral infection using the isolate from Malawi was self-limiting (decline of virus titres and number of ticks containing virus), while the others persisted. According to Kleiboeker *et al*. (1999), the cytopathology caused by the Malawi strain in infected ticks suggested the non-adaptation of the isolate to express specific genes that allow the production of large quantities of progeny virus without damaging the host cell. The reason why this virus was originally isolated from ticks could be the large opportunity for those ticks to feed on infected pigs with high viraemic titres during an ASF outbreak and the leakage of midgut contents into the haemocoel without tick mortality, instead of real adaptation of this virus isolate to the tick host (Kleiboeker *et al.*, 1999). Dixon and Wilkinson (1988) suggested that virus replication in ticks and warthogs may require additional host-specific genes that are not necessary for multiplication in domestic pigs, and that the introduction of virus from tick/warthog sources into domestic pig populations would remove the selection pressure for maintaining these genes. However, no more information is currently available on specific determinants for tick/warthog hosts, as was previously suggested. In addition, it is unknown whether ASFV is able to come back from the domestic to the sylvatic cycle, although some authors suggest that recombination processes during co-infections in ticks may exist (Plowright, 1977; Dixon and Wilkinson, 1988).

4.1.2 Genetic diversification of ASFV

By sequencing the C-terminal end of the *p72* gene, Bastos *et al*. (2003) and Lubisi *et al*. (2005) observed higher genetic variations in genotypes directly isolated from *Ornithodoros* ticks and warthogs, or in genotypes circulating in East and southern Africa where the sylvatic cycle plays a crucial part in the epidemiology of ASF. Some other genotypes were only found in domestic pigs and presented low genetic divergence (Lubisi *et al*., 2005). In Madagascar, using concatenated sequences of the *p22* and *p32* genes, Michaud (Michaud *et al*., 2007) detected relatively high genetic divergence between Malagasy virus isolates collected on domestic pigs from 1998 and 2003, compared with that observed on West African and European isolates since the 1970s. In Madagascar, it has been suspected that the introduced virus was adapted to local bush pigs and *Ornithodoros* ticks, leading to its accelerated diversification. Such diversification phenomena as reported in several African

countries has been previously analysed by Dixon and Wilkinson (1988) on Zambian virus isolates from *Ornithodoros* ticks. A considerable genetic diversity was observed between virus isolates from ticks collected from the same regions and even from the same warthog burrows. This diversity resulted from peculiar point mutations all along the length of the genome, instead of insertions/deletions in the region close to the left-hand terminus of the genome usually observed for host selection (Dixon and Wilkinson, 1988). In this case, ticks would be able to enhance the diversification of ASFV and the emergence of new virulent isolates to domestic pigs. However, no information is yet available on the location and the expression of this genetic diversification. In addition, this process does not seem compatible with the persistence of ASFV in Iberian *Ornithodoros* ticks and the observed genetic homogeneity of ASFV in Europe.

4.2 Crimean-Congo Haemorrhagic Fever

CCHF is a tick-borne zoonotic infection that has public health concern in several regions of the world including Africa, the Middle East, the Balkans region, Greece, Turkey and western Asia. The infection is caused by a virus belonging to the genus *Nairovirus* (family *Bunyaviridae*) and is transmitted by several species of hard (ixodid) ticks, particularly by those belonging to the *Hyalomma* genus (Horak *et al*., 2001).

Phylogenetic analyses performed on S-, M- and L-RNA segments of the Crimean-Congo haemorrhagic fever virus (CCHFV) showed virus strains grouped in seven different clades (Deyde *et al*., 2006). Three clades are distributed in Africa, two in Europe and the other two in Asia. Despite the potential for dispersal of the virus in Africa and Eurasia, it appears that circulation of the virus is largely confined to within two specific regions in these continents, and corresponds to the distribution and dispersal of tick vectors of the virus (Burt and Swanepoel, 2005; Paweska, 2007). It has also been concluded that viral strains have a latitudinal relationship in which there is not much interchange of the strains between different latitudes (i.e. Africa and Europe).

The virus may be maintained in tick populations during inter-epizootic periods through several mechanisms, such as transstadial and transovarial transmission, and non-viraemic transmission of ticks aggregated on the same host (co-feeding). Outbreaks usually take place during the peak activity periods of *Hyalomma* ticks, coinciding with the hot and dry season (Swanepoel, 2006).

There are a large number of potential vertebrate host reservoirs for CCHFV, reflecting the diverse feeding preferences of the immature and adult tick vectors. Antibodies against the virus have been detected in domestic and wild animals, including hares, hedgehogs, rodents, bats, and large mammals such as giraffes and rhinoceroses. The most important source of virus transmission is immature ticks of the *Hyalomma* genus which have fed on the blood of viraemic small vertebrates. Once infected, the tick remains infected throughout its life (transstadial transmission), and the mature tick may transmit the virus to large vertebrates such as livestock. Domestic ruminants such as cattle, sheep and goats will have viraemia for approximately a week after becoming infected. They may be the source of infection for humans during slaughtering or veterinary procedures such as castration. In Appendix 1, Table 1.24 contains data on the serological surveillance of CCHF in domestic animals. The level of viraemia in birds is usually low and unnoticeable, even though migratory birds may play role in the epidemiology of the virus by disseminating infected *Hyalomma* ticks. Tick-infested birds migrating from Russia across the Black Sea were suggested as a link to introduction of the virus in Turkey (Karti *et al*., 2004). Nevertheless, many social and environmental factors affect CCHF occurrence in Turkey. The epidemiological features of the disease are still under discussion.

The most common cause of infection in humans is a bite from an infected tick. Infection can also occur through direct contact

with blood or tissues from infected humans or livestock. The highly pathogenic nature of the virus occasionally results in serious nosocomial outbreaks (Swanepoel, 1995; Aradaib *et al*., 2010; Elata *et al*., 2011; Naderi *et al*., 2011). Clinical infection in humans is initially manifested as an acute febrile illness that can be followed by a fatal haemorrhagic syndrome with case-fatality rates of up to 50% (Swanepoel *et al*., 1987). Misdiagnosis is frequently due to the nonspecific clinical signs (Fisgin *et al*., 2010). The diagnosis is based on serology or viral RNA detection by molecular techniques. During human infection, viral genomes are present in saliva and urine with viral loads similar to those in blood (Bodur *et al*., 2010). Two animal models have been recently established by using genetically defective mice in which the interferon response had been altered (Bente *et al*., 2010; Bereczky *et al*., 2010). These animal models will be beneficial for the development of treatments and vaccines (Keshtkar-Jahromi *et al*., 2011).

Outbreaks in South Africa arose among slaughterhouse operators during the slaughter of ostriches which were heavily infested with ticks. The infection occurred when the infected ticks on the carcasses were squashed during skinning (Swanepoel, 1998). Nevertheless, meat from butchered animals does not pose a risk; in this substrate the CCHFV is quickly inactivated by a drop in pH, as occurs during the maturation process that the meat undergoes after slaughter. A scientific report from the EFSA Panel and Animal Health and Welfare (2010c) addresses the role of tick vectors in the epidemiology of CCHF and ASF in Eurasia.

4.3 Tick-borne Encephalitis Group

4.3.1 Tick-borne encephalitis

Tick-borne encephalitis (TBE) is one of the most important and serious human infections occurring in Europe and many parts of Asia. The aetiological agent, tick-borne encephalitis virus (TBEV), is a member of the genus *Flavivirus* of the family *Flaviviridae*. TBEV is believed to cause at least 11,000 human cases of encephalitis in Russia and more than 3000 cases in the rest of Europe annually (Donoso Mantke *et al*., 2008). Related viruses are louping ill virus (LIV), Langat virus (LGTV) and Powassan virus (POWV), which may also cause human encephalitis, and Omsk haemorrhagic fever virus (OHFV), Kyasanur Forest disease virus (KFDV) and Alkhurma virus (ALKV), which cause serious haemorrhagic fevers rather than encephalitis (Gritsun *et al*., 2003).

Three subtypes of the TBEV are recognized: the Western or Central European subtype, including the Kumlinge virus on Åland in Finland; the Siberian subtype; and the Far Eastern subtype. Recently, the Siberian subtype was recognized as a human pathogen in western Finland where populations of the vector, *Ixodes persulcatus*, were also recorded.

I. ricinus is the main tick vector involved in TBEV infections in Europe. All its stages can attack humans although the nymphs are the most important ones as vectors of the virus to humans. This is due to several factors: the unfed larvae are usually not infected but may become infected while taking their first blood meal on a viraemic host or, which is more important, by co-feeding with infective nymphs; the nymphs are far more abundant in nature than the adult ticks; and the colourful adult females are relatively large (3.5 mm) compared with the dull-coloured smaller (1.5 mm) nymphs and, therefore, are more easily detected and removed when encountered on the human body. In TBE foci in central and northern Europe, the infection prevalence of TBEV in nymphs ranges around 0.1–0.5%, with that in adults about 0.3–6.0%. The infection prevalence in adult females of *I. persulcatus*, which is the main vector stage for the Eastern TBEV, tends to be much higher (up to 40%) than in *I. ricinus* (Labuda and Nuttall, 2008). Co-circulations of both Western TBEV and Eastern TBEV occur in some foci in the Baltic States, where the distributions of the two tick species overlap. The Far Eastern subtype has been discovered not only in Siberia but also in some European localities (Chausov *et al*., 2010). In Estonia, all three human-pathogenic subtypes of TBEV have been

found in the same areas (Golovljova *et al*., 2004). Apart from the two main vector species, *I. ricinus* and *I. persulcatus*, several other tick species, including *I. hexagonus*, *I. arboricola*, *Haemaphysalis* (*Ha*.) *concinna, Ha. inermis* and *Ha. punctata* are competent but secondary vectors (Labuda and Nuttall, 2008).

Until not long ago, it was believed that viraemic small rodents, particularly the bank vole, *Clethrionomys glareolus*, and the field mouse, *Apodemus flavicollis*, and insectivores were the principal reservoirs of TBEV that infected the vectors. However, the viraemia in these rodents is usually of short duration (2 days). For the maintenance of TBEV in *I. ricinus* populations, co-feeding transmission between infective nymphs and susceptible larvae feeding very close to and on the same small rodent is now considered to be much more important than transmission via viraemic small mammals (Labuda and Nuttall, 2008).

Many cases of TBE in humans are unrecognized and without clinical signs or symptoms. In some cases, however, the clinical syndrome of TBE disease is severe, with a life-threatening neurological syndrome and high case fatality (5 to 35%) in its Eastern form, mainly in Russia. In contrast, the case fatality in Western Europe, mainly in Central and Northern Europe, is usually comparatively low (approximately 1%) with nearly all deaths confined to patients above 60 years of age. Patients infected with the Siberian subtype may suffer from a milder but often more chronic disease compared with the disease caused by the Far Eastern subtype.

The incidence of TBE usually fluctuates from year to year, but an increased incidence has been noted in some countries (Danielova *et al*., 2006; Lindquist and Vapalahti, 2008), and new TBE foci seem to have appeared, especially in the last decade. This is presumably the result of a complex interaction of factors such as the changing climate affecting the vector both directly and indirectly by affecting the plant and host communities, sociopolitical changes and technological factors, e.g., better diagnostic methods and increased awareness (Donoso Mantke *et al*., 2008; Telford and Goethert, 2008).

4.3.2 Louping ill

Louping ill virus (LIV), also known as ovine encephalitis/encephalomyelitis virus, is closely related to TBEV and is the only member of this virus complex present in the British Isles, where the vector is *I. ricinus* (Reid, 1988). Louping ill (LI) is endemic in sheep-farming areas of northern England, Scotland, Wales, Ireland and Norway. Several tick hosts, such as the red grouse, willow grouse, field vole and deer become viraemic when infected with LIV. The viraemia, however, is usually too low to be infective to feeding tick larvae. In contrast to these wild hosts, sheep and red grouse consistently develop viraemia sufficient to infect tick larvae and amplify the virus. Occasionally, horses, cattle and goats develop viraemias sufficiently high to be infective to tick larvae (Reid, 1988). Mountain hares, *Lepus timidus*, may be maintenance hosts for LIV by non-viraemic transmission between co-feeding ticks, as experimentally demonstrated by Jones *et al*. (1997). One of the main assumptions for the transmission of a vector-borne pathogen is usually that feeding by the vector is the sole or main route of host infection. Gilbert *et al*. (2004), however, demonstrated experimentally a transmission route whereby an important tick host, the red grouse (*Lagopus lagopus scoticus*), became infected with LIV after eating infected *I. ricinus* ticks. These authors estimated from field observations conducted in Scotland that this mode of infection could account for 73–98% of all virus infections in wild red grouse in their first season. Certainly, this way of transmission has potential implications for the understanding of other vector-borne pathogens in which hosts may ingest vectors through foraging or grooming.

LI is principally a disease of sheep and red grouse, and less commonly of cattle, other domesticated animals and birds. Dogs, and particularly sheepdogs and hunting dogs in endemic areas, are occasionally infected. Clinical signs include fever, ataxia, trembling, salivation, coma and death. The virus can cause severe encephalitis in humans; about 35 cases have been recorded. Most of these cases were due to accidents while handling the virus in the laboratory

(Labuda and Nuttal, 2008). Definitive diagnosis is based upon the isolation and identification of the virus (Reid, 1988; Lobetti, 2007). LIV infection in sheep is exacerbated by co-infection with *Anaplasma phagocytophilum* (Reid, 1988).

Experimental and trans-stadial transmission have been reported in *Rhipicephalus* (*Rh.*) *appendiculatus* and *Hyalomma* (*Hy.*) *anatolicum*, but there is no evidence that they are natural vectors or that any other tick species except *I. ricinus* play any significant role in the epidemiology of the disease (Reid, 1988).

Although *I. ricinus* is the primary vector and virus reservoir, because of the trans-stadial – but presumably not transovarial – transmission, the vector efficiency of this species is relatively restricted. Even when virions are acquired by the feeding larvae, only a few of the nymphs become infected. Like the prevalence of TBEV in the *I. ricinus* population, the prevalence of LIV in *I. ricinus* is also low.

4.3.3 Other viruses related to TBE

Infections of domesticated animals similar to TBE and LI also occur in other European countries (Spain, Bulgaria, Greece and Turkey). Greek goat encephalitis virus (GGEV), which was isolated from the brain of a newborn goat with neurological symptoms, is currently classified in the TBEV group. The vector of GGEV has not yet been specifically identified but it is considered as likely to be *I. ricinus*. A study during 2003–2006 in goat and sheep farming rural areas of northern Greece suggested the presence of TBEV in two pools of *I. ricinus* ticks. Sequence analysis showed that the virus was GGEV. These virus-positive ticks were detected in regions where a high prevalence of TBE antibodies was present in humans. TBEV is considered not to be endemic in Greece, so most probably the seroprevalence of TBE antibodies in humans is due to cross-reactivity to GGEV (Grard *et al*., 2007). The Turkish subtype (Turkish sheep encephalitis virus) is more closely related to LIV and should be reclassified.

4.4 Anaplasmoses

Alphaproteobacteria of the order *Rickettsiales* are obligate intracellular organisms with a wide range of eukaryotic hosts. There are two well-characterized families: *Anaplasmataceae* and *Rickettsiaceae*. In the family *Anaplasmataceae*, four genera have been identified: *Anaplasma*, *Ehrlichia*, *Wolbachia* and *Neorickettsia*. Several species of the genus *Anaplasma* pose severe threats to livestock and human health. The main *Anaplasma* species responsible for animal infections or zoonosis in Europe and the Mediterranean basin are listed in Table 4.1. Anaplasmosis was formerly known as gall sickness, a disease of ruminants caused by intraerythrocytic bacteria. As a result of a taxonomic reorganization of the order *Rickettsiales* (Dumler *et al*., 2001), some species of the genus *Ehrlichia* (*E. equi*, *E. phagocytophila* and *Ehrlichia* spp. causing human granulocytic ehrlichiosis – HGE) were renamed as *A. phagocytophilum* in the genus *Anaplasma*. This species is the aetiological agent of human and animal granulocytic anaplasmosis. Similarly, *E. bovis* and *E. platys* are now known as *A. bovis* and *A. platys*. These latter, recently added, species all invade blood cells other than erythrocytes in their respective mammalian hosts.

Infection of domestic and wild animals and humans with these organisms may lead to a clinical disease collectively called anaplasmosis that manifests as a febrile systemic illness with haematological abnormalities and lymphadenopathy (Rikihisa, 2006).

4.4.1 *Anaplasma phagocytophilum*

A. phagocytophilum, formerly known as *E. phagocytophila* and *E. equi*, and the agent of HGE, is a commonly found bacterium causing tick-borne fever (TBF) in sheep; pasture fever in cattle and wild ruminants (deer, bison and wild goats); human granulocytic anaplasmosis in humans; equine granulocytic anaplasmosis in horses; and canine granulocytic anaplasmosis in dogs (Strle, 2004; Rymaszewska and Grenda, 2008). It was first recognized in Scotland in 1932 and is now identified in most other European

Table 4.1. Anaplasmoses in Europe and in the Mediterranean basin transmitted by hard ticks.

Anaplasma spp.	Disease	Host range	Tick involved (in Europe and the Mediterranean basin)
A. phagocytophilum	Tick-borne fever (TBF)	Sheep	*I. ricinus, Ha. punctata,*
	Pasture fever	Cattle, wild ruminants	*I. persulcatus,*
	Human granulocytic anaplasmosis	Humans	*I. trianguliceps,* *Rh. sanguineus*
	Equine granulocytic anaplasmosis	Horses, llamas, rodents	
	Canine granulocytic anaplasmosis	Dogs	*I. ricinus, Ha. punctata,* *I. persulcatus,* *I. trianguliceps,* *Rh. sanguineus*
A. marginale	Bovine anaplasmosis	Ruminants	*I. ricinus, I. persulcatus,* *Rh. sanguineus,* *Rh. bursa, Rh. annulatus*
A. centrale	Bovine anaplasmosis	Cattle	*I. ricinus, I. persulcatus,* *Rh. sanguineus,* *Rh. bursa, Rh. annulatus*
A. bovis	Bovine mononuclear or agranulocytic anaplasmosis	Cattle, small mammals	*Hy. excavatum,* *Rh. sanguineus,* *Rh. turanicus*
A. ovis	Ovine anaplasmosis	Goats, sheep, cattle	*Rh. bursa,* *Rh. sanguineus*
A. platys	Canine infectious cyclic thrombocytopenia	Dogs	*Rh. sanguineus,* *Rh. turanicus*

A., *Anaplasma*; *Ha.*, *Haemaphysalis*; *Hy.*, *Hyalomma*; *I.*, *Ixodes*; *Rh.*, *Rhipicephalus*.

countries. Apart from domestic ruminants, free-living ruminants such as feral goats, and red, fallow and roe deer tested positive for *A. phagocytophilum*. Not only a tick-ruminant cycle, but also a rodent-tick cycle, is believed to maintain the TBF variants. The wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*A. flavicollis*), field vole (*Microtus agrestis*) and bank vole (*Myodes glareolus*) are found to be competent reservoirs of infection (Barandika *et al*., 2007). The bacterium infects granulocytic leucocytes (neutrophils, eosinophils and basophils), monocytes and tissue macrophages.

TBF in sheep and pasture fever in cattle are characterized by fever, neutropenia, lymphopenia, thrombocytopenia and general immunosuppression (Woldehiwet, 2006). Human granulocytic anaplasmosis is a multisystemic disease that occurs more in adults than in children, especially in persons above the age of 60 years. The disease is characterized by acute fever, headache, myalgia, nausea and lethargy, similar to symptoms of the common flu. In particular, immunocompromised patients are at high risk. Meningoencephalitis, respiratory distress, shock and opportunistic infections are occasional complications. In Europe, no fatal cases have been reported, but the mortality rate in the USA has been shown to be between 7 and 10% (reviewed by Bakken and Dumler, 2008 and Rymaszewska and Grenda, 2008). Equine granulocytic anaplasmosis occurs in horses as their natural host but also in llamas and rodents. It is generally a benign disease in these animals, yet fulminating cases have been described. Mortality is low, and the disease is always acute, never chronic.

The main vector of *A. phagocytophilum* is *I. ricinus* (Strle, 2004), and the prevalence of infection varies among regions and with the development stage of the tick (Stuen, 2007). For example, in unfed nymphs the infection rate varied between 0.25 and 25% (Walker *et al*., 2001). The survival of the parasite is

believed to be over a year while ticks are awaiting a new host. Only trans-stadial transmission occurs.

The transmission of *A. phagocytophilum* has also been associated with other tick species, such as *Ha. punctata*, in areas of the UK where *I. ricinus* was not present (MacLeod, 1936). It has also been linked with *I. persulcatus, I. trianguliceps* and *Rh. sanguineus* (Alekseev *et al*., 1998; Ogden *et al*., 1998; Alberti *et al*., 2005). The role of the latter species as a vector is not yet determined, as only one *Rh. sanguineus* was found to be positive; this tick was removed from a dog showing clinical signs of tick-borne disease (TBD).

4.4.2 *Anaplasma marginale*

Bovine anaplasmosis, caused by *A. marginale*, was formerly known as gall sickness, and is a disease that affects domestic and wild ruminants (water buffalo, bison, African antelopes and mule deer). The disease is characterized by fever, anaemia, weight loss, reduction of milk production and in pregnant females, abortion; it may lead to death (Rymaszewska and Grenda, 2008; Kocan *et al*., 2010). *A. marginale* is present in tropical and subtropical regions although this bacterial species is frequently detected in Europe (Sicily, Hungary and Spain) (de la Fuente *et al*., 2005; Naranjo *et al*., 2006; Hornok *et al*., 2007b; Torina *et al*., 2007, 2008). It is an obligate intracellular species invading erythrocytes mostly in ruminants, both domestic and wild, i.e. calves, water buffalo, bison, African antelopes and mule deer.

In cattle, the disease in cattle causes considerable losses to dairy and beef industries worldwide. Trans-stadial transmission of the bacteria is effected by ticks of approximately 20 species; in Europe this mainly involves *I. ricinus*, *I. persulcatus*, *Rh. sanguineus*, *Rh. bursa* and *Rh.* (*Boophilus*) *annulatus* (Kocan *et al*., 2004). Calves under the age of 6 months have innate resistance and will not develop clinical anaplasmosis, no matter the immune status of the mothers. Thereafter, the risk for serious diseases increases with age, unless sufficient contact in the first months of life allowed for the development of immunity. Hence, cattle reared in endemic regions develop a naturally acquired immunity, quite often without passing through a stage of clinical disease, as endemic stability means that all calves need to come into contact with the disease, reservoirs and stable vector populations.

Wild ruminants (antelopes, buffalo, deer, eland) can function as reservoirs of *A. marginale* and the infection can be maintained in game reserves (deer-to-deer transmission) without bovine intervention being necessary (Potgieter and Stoltsz, 2004).

It appears that *A. marginale* is often introduced into a herd by ticks, but subsequently mechanical transmission (transmission by insects or by veterinary interventions) may become more important. Mechanical transmission occurs via the contaminated mouthparts of biting flies, but can only be achieved within a few minutes after the initial bite, although the pathogen can remain viable and infective in arthropods for several days after ingestion (Ewing, 1981; Hornok *et al*., 2008). Horseflies (*Tabanus* spp.) and stable flies (*Stomoxys* spp.), and to a lesser extent mosquitoes (*Psorophora* spp.), transmit *A. marginale* and also *A. centrale*.

4.4.3 *Anaplasma centrale*

A. centrale is considered as a separate species or subspecies of *A. marginale*, and is also an intraerythrocytic tick-borne pathogen that causes mild infections in cattle. A cross-immunity between the two bacteria exists, and because of its mild virulence this naturally attenuated strain has been used for more than 100 years in live-blood vaccines to protect cattle from the more virulent *A. marginale* (Potgieter and Stoltsz, 2004). These vaccines are mainly used in Africa, Australia, Latin America and Israel (Rymaszewska and Grenda, 2008).

Not much is known about the epidemiology of *A. centrale*; only few strains have been characterized. In Europe, the pathogen has been detected mainly in Italy (George *et al*., 2001; Carelli *et al*., 2008).

The first case of bovine anaplasmosis caused by *A. centrale* in Europe was reported in 2008 in Italy (Carelli *et al*., 2008). The case involved a naturally occurring infection in a dairy cow, and the clinical signs were typical of acute anaplasmosis caused by *A. marginale*. Molecular analysis of the Italian strains linked these to the African *A. centrale* strains (Carelli *et al*., 2008).

Recently, a complete genome sequence of *A. centrale* was compared with that of virulent *A. marginale* (Herndon *et al*., 2010) with the aim of identifying possible outer membrane protein candidates for the development of a safer inactivated vaccine.

4.4.4 *Anaplasma bovis*

A. bovis is the aetiological agent of bovine mononuclear or agranulocytic anaplasmosis, a disease occurring mainly in cattle and small mammals (Goethert and Telford, 2003). Goats appear to be resistant. *A. bovis* infects the monocytes of the peripheral blood and the macrophages of the reticuloendothelial system. Infection may occur with limited or no clinical signs. The disease is characterized by weakness, weight loss, fever, enlargement of the prescapular lymph nodes, paleness of the mucous membranes and mucous nasal secretion (Uilenberg, 1997). This disease has been reported in Italy (Georges *et al*., 2001) and Israel (Harrus *et al*., 2011), but is most commonly present in South America, West, Central and southern Africa, and the Indian subcontinent. The transmission of the disease is transstadial by known vectors: *Amblyomma* (*Am*.) *variegatum*, *Rh. appendiculatus* and *Hy. excavatum* (Coetzer and Tustin, 2004). The pathogen has recently been detected in Israel in unfed *Rh. sanguineus* and *Rh. turanicus* adults collected from vegetation (Harrus *et al*., 2011).

4.4.5 *Anaplasma ovis*

A. ovis mainly infects wild ruminants and small ruminants like sheep and goats, and is prevalent worldwide (Rymaszewska and Grenda, 2008). In Europe, it has been detected in Italy, Hungary and Turkey (de la Fuente *et al*., 2002, 2005; Christova *et al*., 2003; Hornok *et al*., 2007b; Aktas *et al*., 2009). This bacterium also infects erythrocytes, but in general anaplasmosis due to *A. ovis* in small ruminants is a benign infection with low morbidity and mortality. Goats are normally more susceptible than sheep or cattle. The biological vector of *A. ovis* in the Mediterranean basin is *Rh. bursa* (Friedhoff, 1997), but possibly also *Rh. sanguineus*. *A. ovis* was identified molecularly in the salivary glands of two *Rh. sanguineus* ticks collected from sheep in Turkey (Aktas *et al*., 2009), although the authors also suggest that *D. marginatus* could play a role in the transmission because of the high prevalence of ticks found in the affected flock. In contrast to *A. marginale*, vaccination with *A. centrale* does not protect against *A. ovis*.

4.4.6 *Anaplasma platys*

Canine anaplasmosis or canine infectious cyclic thrombocytopenia (CICT) is caused by *A. platys*. The bacterium multiplies in platelets, but infected dogs may remain asymptomatic (Harvey *et al*., 1978). Clinical signs are usually not observed, apart from occasional haemorrhages after trauma or surgery. The infection has been detected worldwide, and in Europe cases have been reported in Spain, France, Greece and Italy (Sainz *et al*., 1999; Sparagano *et al*., 2003; Mylonakis *et al*., 2004; Alberti and Sparagano, 2006; Torina *et al*., 2008; Yabsley *et al*., 2008). Serological tests may cross-react with other *Anaplasma* and *in vivo* tests may be inaccurate because of low bacteraemias (de la Fuente *et al*., 2006).

Cases of importation of both the infectious agent and the vector have been reported in dogs visiting the Mediterranean region (Heyman *et al*., 2007; Nijhof *et al*., 2007). The implicated vector seems to be *Rh. sanguineus* (Inokuma *et al*., 2000; Sanogo *et al*., 2003; Sparagano *et al*., 2003), a cosmopolitan tick species that also transmits *E. canis*, although ticks of *Rh. sanguineus* fed on experimentally infected dogs were not able to infect naive dogs in the adult stage (Simpson *et al*., 1991).

Similarly, as mentioned for *A. bovis*, unfed *Rh. turanicus* adults collected from the vegetation also have been shown to harbour *A. platys* (Harrus *et al*., 2011).

4.5 Ehrlichioses

4.5.1 *Ehrlichia canis*

E. canis is a tick-transmitted obligate intracellular Gram-negative bacterium; in dogs it infects monocytes and causes classical canine monocytic ehrlichiosis. It resides as a colony within a membrane-lined intracellular vacuole or morula. Canine monocytic ehrlichiosis is also known by other names, such as tracker dog disease, tropical canine pancytopenia, canine haemorrhagic fever and canine typhus. The disease has been known since 1935 as a disease of dogs and other canids (jackal, wolf, fox, coyote) (Donatein and Lestoquard, 1935), but its importance was not seriously considered until 1968 when an epizootic occurred in Vietnam among military working dogs.

The bacterium is transmitted transstadially by the kennel tick (or brown dog tick), *Rh. sanguineus*, and is widespread in tropical and temperate areas of the world (from 50°N to 35°S). Its distribution has expanded with the distribution of its vector and is maintained in nature by persistent infections of wild and domestic canids (Groves *et al*., 1975). In Europe, *E. canis* is restricted to the Mediterranean and Balkan countries (Hornok *et al*., 2010), although several cases of canine ehrlichiosis have been detected in more northern regions in dogs with a history of travelling (Hirsch and Pantchev, 2008).

The disease has three clinicopathological phases: acute, subclinical and chronic (Skotarczak, 2003). German Shepherd dogs and their crosses are particularly prone to more severe signs of disease, and infections in this breed are associated with a poorer prognosis (Raoult and Parola, 2007).

Several cases of human infections with *E. canis* have been reported (Maeda *et al*., 1987; Sambri *et al*., 2004; Perez *et al*., 2006). Clinical signs of canine monocytic ehrlichiosis in humans are very similar to those of human monocytic ehrlichiosis, a zoonosis present in the USA and caused by the closely related *E. chaffeensis* (Perez *et al*., 2006).

4.5.2 *Ehrlichia ruminantium*

E. ruminantium, previously known as *Cowdria ruminantium*, causes heartwater or cowdriosis (other names are black gall sickness, mad gall sickness, infectious exudative pericarditis or malignant rickettsiosis of ruminants). Heartwater is an infectious tick-transmitted disease of ruminants, i.e. bovines, sheep, goats and various wild species. Transmission is related to the *Amblyomma* vector. In Africa, at least ten species are capable of transmission; the most important are *Am. variegatum* and *Am. hebraeum*, and the adults of both species parasitize cattle. The most widely distributed *Amblyomma* species in Africa is *Am. variegatum*, and this even spreads outside the continent. *Am. hebraeum* is the most important vector in southern Africa.

Heartwater occurs in sub-Saharan Africa and in several African islands, the islands in the Indian Ocean and several Caribbean islands, i.e. Guadeloupe and Antigua. In continental America, it has not been observed, in spite of the presence of potential vectors (Coetzer and Tustin, 2004). The control of *Am. variegatum* in the Caribbean has been a daunting task. Although the ticks had been eradicated for a number of years, the islands became infested again after the import of immature stages on migrating cattle egrets (Corn *et al*., 1993).

Am. variegatum sporadically occurs in the Mediterranean basin, most probably imported on migratory birds (Papadopoulos *et al*., 1996). According to a predictive GIS (geographical information system) model using temperature and land use, the survival of *Am. variegatum* and *Am. hebraeum* in this region would be best suited to Sardinia, Sicily and the south-western part of the Italian peninsula (Pascucci *et al*., 2007). This poses a possible risk for the introduction of *E. ruminantium* into the Mediterranean region.

4.6 Rickettsioses

Rickettsial diseases are among the oldest known arthropod-borne diseases affecting human health throughout the ages. These diseases exist primarily in endemic and enzootic foci and occasionally give rise to sporadic or seasonal outbreaks, causing illness and death worldwide (Maxey, 1899). Evidence suggests that rickettsiae and *Rickettsia*-like organisms have evolved and survived as obligate intracellular bacteria, cultivating long and well-established relationships with arthropods (lice, mites, fleas and ticks) and vertebrate hosts (Azad and Beard, 1998). The vectors of rickettsiae do include lice, mites and fleas, but most *Rickettsia* species are associated with ticks, which are both their vectors and reservoirs. Ticks become infected with rickettsiae mainly by feeding on rickettsaemic host animals, particularly young rodents, and by the passage of rickettsiae via eggs to the progeny of infected female ticks. Trans-stadial persistence from larvae to nymphs and to adults is also important for maintaining the infection so that transovarial transmission is successful (Burgdorfer and Brinton, 1975). Geographic distribution and activity of infected ticks are important determinants in the epidemiology of tick-borne rickettsioses. Humans are accidental hosts that become infected when ticks containing virulent rickettsiae in their salivary glands take a blood meal and inject the rickettsiae into the feeding site. Currently, there are 12 tick-transmitted species of rickettsiae that are known to cause disease in man. Eight are present in Europe and the Mediterranean basin, and the majority have been described in the last 25 years. This boom in new or rediscovery of *Rickettsia* species in recent years is undoubtedly related to the development of cell culture systems and the advent of molecular genetics technology. Likewise, the detection and identification of long-known pathogens in patients from new and distinct geographic regions has refuelled interest in these agents. These emerging infections often have diverse presentations that underscore the need for increased awareness among clinicians.

4.6.1 Rickettsiae as bacteria

The genus *Rickettsia* is classified in the *Alphaproteobacteria* subdivision, order *Rickettsiales* (which also includes the families *Anaplasmataceae* and *Holosporaceae*), family *Rickettsiaceae* (Dumler *et al*., 2001). This family includes the genus *Rickettsia* along with *Orientia* (Tamura *et al*., 1995).

Rickettsiae are coccobacillary Gram-negative, obligate intracellular bacteria that reside free in the cytosol and occasionally in the nucleus of host cells (Walker, 2006). This lifestyle within a highly specialized niche, the eukaryotic cell, has given rise to unique adaptations such as the reduction of bacterial metabolism and the exploitation of host metabolites. The ability to transport substrates that are present in the host cell cytosol but rarely available in the extracellular milieu is likely to have contributed to the evolution of the small-sized rickettsial genome (1.11–1.12 Mb). Many of the *de novo* biosynthetic pathways characteristic of free-living bacteria are no longer present in rickettsiae (Andersson and Kurland, 1998).

The rickettsiae are traditionally divided into two antigenically distinct groups based on their lipopolysaccharide (LPS): the typhus group (TG) that includes two species, *R. prowazekii*, the agent of epidemic typhus transmitted by the human body louse (*Pediculus humanus corporis*), and *R. typhi*, the agent of murine typhus transmitted by the rat flea; and the spotted fever group (SFG) that includes the majority of rickettsial species, which are transmitted by different arthropod vectors.

Different phylogenetic studies using the sequencing of 16S rDNA (Roux and Raoult, 1995) and diverse rickettsial genes – *glt*A (Roux *et al*., 1997), *omp*A (Fournier *et al*., 1998), *omp*B (Roux and Raoult, 2000), *sca*2 (Sekeyova *et al*., 2001), *sca*4 (Ngwamidiba *et al*., 2005) and *sca*1 (Ngwamidiba *et al*., 2006) – have shown distinct groups of *Rickettsia* species, but some authors still believe that it is difficult to define the SFG species. This is because some species of *Rickettsia* are so closely related that they could be considered to be strains of a single species (Walker, 2007). For example, the sequence analysis of 16S rDNA for *R. conorii*,

R. sibirica, *R. africae*, *R. parkeri* and many others has shown less than 0.5% of divergence between them (Roux and Raoult, 1995).

The diagnosis of rickettsial disease is most often confirmed by serological tests because culture, rickettsial DNA detection by PCR and other methods require specialized laboratories, and also because serum is usually the most commonly available sample. Indirect immunofluorescent assay remains the gold standard serological test and is the mostly widely used technique for diagnosis of rickettsial diseases (Brouqui *et al*., 2004). However, owing to the presence of shared protein and LPS antigens among spotted fever group rickettsiae, the use of serological methods to distinguish between infections due to closely related rickettsiae is extremely difficult. Western blot (WB) and cross adsorption (CA) immunoassays can sometimes assist in the differentiation of SFG or TG rickettsiae; however, interpretation should be undertaken cautiously because some strains are so closely related that they cannot be distinguished (Teysseire and Raoult, 1992). The advent of molecular methods based on PCR has though enabled the development of specific and rapid tools for the detection and identification of *Rickettsia* species. It should be noted that the sensitivity of PCR in skin biopsy (eschar) is higher than in blood. Culture of the agent is the ultimate criterion to confirm the diagnosis and to identify the species of *Rickettsia* from the patient's blood (Gouriet *et al*., 2005; de Sousa *et al*., 2008b). This technique can reach a good level of success if the sample is collected and preserved under appropriate conditions until isolation procedures begin.

The main *Rickettsia* species responsible for animal infections or zoonoses in Europe and the Mediterranean basin are listed in Table 4.2.

4.6.2 *Rickettsia conorii*

R. conorii, the causative agent of Mediterranean spotted fever (MSF), is the most frequently isolated rickettsia and has

Table 4.2. Rickettsioses in Europe and in the Mediterranean basin transmitted by hard ticks.

Rickettsia spp.	Disease	Tick involved (based on publications review)
R. conorii Malish	Mediterranean spotted fever (MSF)	*Rh. sanguineus, Rh. turanicus*
R. conorii Israeli spotted fever strain	Israeli spotted fever (ISF)	
R. conorii Astrakhan strain	Astrakhan fever (AF)	*Rh. pumilio, Rh. sanguineus*
R. sibirica mongolitimonae strain	Lymphangitis-associated rickettsiosis (LAR)	*Hy. anatolicum, Hy. excavatum, Rh. pusillus*
R. slovaca	Tick-borne lymphadenopathy (TIBOLA)/*Dermacentor*-borne necrosis erythema lymphadenopathy (DEBONEL)	*D. marginatus, D. reticulatus, Ha. inermis, Ha. punctata*
R. massiliae	Unnamed	*Rh. sanguineus, Rh. turanicus*
R. aeschlimannii	Unnamed	*D. reticulatus, Ha. punctata, Ha. inermis, Hy. detritum (syn. Hy. scupense), Hy. marginatum, Rh. bursa, Rh. sanguineus, Rh. turanicus*
R. helvetica	Unnamed	*D. reticulatus, I. hexagonus, I. ricinus, I. ventalloi*
R. monacensis	Unnamed	*Ha. punctata, I. ricinus*

D., *Dermacentor*; *Ha.*, *Haemaphysalis*; *Hy.*, *Hyalomma*; *I.*, *Ixodes*; *Rh.*, *Rhipicephalus*.

the widest geographical distribution of the SFG rickettsial species. *Rh. sanguineus*, commonly known as the brown dog tick, is the main vector and reservoir for *R. conorii* strains in the Mediterranean area, former USSR, northern Africa and India (Rehacek and Tarasevich, 1988). In some situations, mainly in anthropozoonotic and domestic cycles, dogs are the main host for feeding of all the stages of *Rh. sanguineus* (Gilot, 1984). Dogs are transient reservoirs because they have a short-lived rickettsaemia after infection, but do not seem to be a reservoir for *R. conorii*. One study performed in Portugal also showed that dogs can present with febrile illness related to infection with *R. conorii* Malish and *R. conorii* Israeli spotted fever strains (Alexandre *et al*., 2011).

MSF was described for the first time by Conor and Brusch in Tunisia in 1910, and is widely distributed in the Old World, being endemic in southern Europe, Africa, the Middle East, India and Pakistan. Case reports and isolations of *R. conorii* from patients in the Mediterranean area continue to be described, including recent cases in Croatia (Sardelic *et al*., 2003), Turkey (Kuloglu *et al*., 2004) and Greece (Psaroulaki *et al*., 2005a). Sporadic cases have also occurred in non-endemic countries in northern and central Europe, such as Belgium (Lambert *et al*., 1984), Switzerland (Chamot *et al*., 1987), Sweden (Vene, 1989) and Germany (McDonald *et al*., 1988), as well as in Japan (Yoshikawa, *et al*., 2005), the USA (Anderson *et al*., 1981) and the UK (Chai *et al*., 2008). Most of these cases were attributed to the introduction of imported vectors or travellers who acquired the infection in endemic areas (Walker, 2003; Menn *et al*., 2010).

In some countries, the incidence of MSF is unknown, and most of the knowledge is based on the human seroprevalence of antibodies to SFG rickettsiae. Inasmuch as the reporting of cases of MSF is not obligatory in all endemic countries, it is difficult to compare the incidence of the disease in different regions. However, it seems that in the last few decades there has been an increase in reported MSF cases in Portugal, Italy, Spain, France and Israel (Walker and Fishbein, 1991); this is likely to be due to climate changes influencing tick activity. In Portugal, the national incidence rate during the period 1989–2003 was $8.9/10^5$ inhabitants per year. During that same period the incidence of MSF in the Bragança and Beja districts of Portugal reached rates of $56.8/10^5$ inhabitants and $47.4/10^5$ inhabitants, respectively (de Sousa *et al*., 2003; de Sousa and Bacellar, 2004). A study by de Sousa *et al*. (2006a), showed that the average winter temperatures in Portugal over the last 10 years have been the warmest on record and this, coupled with low rainfall, possibly played a role in doubling the number of confirmed MSF cases during 2000–2005.

In Italy, during a 5 year period from 1998 to 2002, a national incidence rate of $1.6/10^5$ inhabitants was reported. However, the same period of time in Sicily, accounting for 51.4% of all clinical cases, had an incidence rate of $9.3/10^5$ inhabitants (Ciceroni *et al*., 2006). In France, a prospective study in the south of Corsica showed a higher incidence of $48/10^5$ inhabitants compared with other regions (Raoult *et al*., 1985). Otero *et al*. (1982), estimated that in Israel the annual incidence of MSF is $6.2/10^5$ inhabitants and that the highest incidences of the disease occur in the western coastal area and the southern Negev Desert.

MSF is seasonal, and in most of European countries the cases are encountered in late spring and summer (81–88%), peaking in July and August when immature stages of the tick predominate. As the larvae and nymphs of the associated ticks are small and their attachment to the body during feeding is painless, they are more difficult to detect and so more likely to transmit MSF organisms (Raoult and Roux, 1997).

The disease is characterized by a generalized endothelial infection of the microvasculature and the main clinical features are due to the injury of blood vessels. The histopathological phenomenon of vasculitis can involve all the organs, not only the skin, and it has particularly serious manifestations when the lungs and brain are affected (Valbuena and Walker, 2009). The incubation period ranges from 3 to 7 days after the tick bite, but it can be longer. The onset of MSF is generally abrupt and the disease is characterized by

fever, a maculopapular rash involving the entire body, including the palms and soles, and the presence of an eschar at the site of the tick bite. Occasionally, the eschar is not found and is seen rarely in multiples. Recently, clinical signs and symptoms were reported in a group of 71 Portuguese patients with confirmed diagnosis either by PCR or isolation of *R. conorii* Malish. The main clinical signs were fever (94%), maculopapular rash (94%), eschar (60%), myalgias (84%), headache (78%), asthenia (95%) and anorexia (73%). Purpuric or petechial rashes were present in 6% of patients with severe forms; this was indicative of a bad prognosis (de Sousa *et al*., 2008b).

R. conorii has always been considered to produce a less severe disease than *R. rickettsii*. However, severe forms of MSF have been reported in 6% of patients, and a case fatality rate of 1.4–13% was reported for hospitalized patients in France, Israel, Spain, Algeria (Oran) and Portugal (Ruiz-Beltran *et al*., 1985; Walker *et al*., 1987; Amaro *et al*., 2003; de Sousa *et al*., 2008b). Moreover, in southern Portugal the fatality rate in 1997 reached 32.3% in hospitalized patients (de Sousa *et al*., 2003). The co-morbidity condition of diabetes mellitus was identified as the risk factor for a fatal outcome. Statistical analysis of a representative sample of Portuguese patients with MSF also showed that alcoholism increases the risk for a fatal outcome. Other underlying conditions, such as heart failure, increasing age and glucose-6-phosphate dehydrogenase (G6PD) deficiency can be also be implicated in severe illness (Walker, 1990; Regev-Yochay *et al*., 2000).

It is still unclear whether the more severe and fatal cases are simply related to host factors or whether there are differences in the virulence of the bacteria causing the disease.

4.6.3 *Rickettsia conorii* Israeli spotted fever strain

Israeli spotted fever (ISF) was described for the first time in 1946 in Israel, and some years later the aetiological agent was isolated from *Rh. sanguineus* ticks and from a patient (Goldwasser *et al*., 1974). The disease that initially seemed restricted to that area is actually widespread in the Mediterranean basin, and has been detected in *Rh. sanguineus* collected in Portugal and Sicily (Giammanco *et al*., 2003; de Sousa *et al*., 2007). Several clinical cases were described in those same countries, and more recently patients have also been reported in Tunisia and Libya (Bacellar *et al*., 1999; Giammanco *et al*., 2005; de Sousa *et al*., 2008b; Znazen *et al*., 2011). In general, clinical and laboratory data are similar to those found in patients infected with *R. conorii* strains. However, studies in Portuguese and Sicilian patients infected with the *R. conorii* ISF strain showed that only 39% and 40% of patients had eschars; this is less frequent than what is reported by patients infected with the *R. conorii* Malish strain (de Sousa *et al*., 2005; Giammanco *et al*., 2005). In Israel, eschars have been described in only 4% of cases, and some clinical series reported the total absence of an eschar (Gross and Yagupsky, 1987; Wolach *et al*., 1989). A prospective study conducted in Portugal during 1994–2006 identified and compared two groups of patients infected by Malish and ISF strains, confirmed either by isolation or DNA detection by PCR (de Sousa *et al*., 2008b). Although the comparison of the clinical manifestations of MSF caused by different strains revealed a tremendous overlap, some differences were found. Patients infected with ISF had a recognized tick bite, but significantly fewer reported presence of an eschar compared with patients infected by the Malish strain. Also, significantly higher percentages of ISF patients had nausea, vomiting and increased levels of total bilirubin, γ-glutamyl transferase and alkaline phosphatase. The most important and statistically significant finding documented in the study was that the ISF strain was associated with a higher number of fatal cases. Severe forms and fatal cases were also described in patients in Sicily and Tunisia, and also in travellers that visited endemic areas (de Sousa *et al*., 2003, 2008b; Giammanco *et al*., 2005; Boillat *et al*., 2008; Chai *et al*., 2008). The differences in virulence between strains are not yet understood and comparative analysis of genomes will probably lead to better comprehension.

4.6.4 *Rickettsia conorii* Astrakhan strain

Astrakhan fever (AF) was first reported in the 1970s in patients living in rural areas of Astrakhan, a region of Russia located on the Caspian Sea (Tarasevich and Mediannikov, 2006). The appearance of the clinical cases coincided with the construction of a petrochemical complex that was releasing enormous quantities of CO_2 into the atmosphere. It seems that the CO_2 attracted ticks to this area and somehow increased the probability of exposure of the population to the vector (Tarasevich and Mediannikov, 2006). Later, the disease was serologically diagnosed, and the *R. conorii* Astrakhan strain was isolated from humans and *Rh. pumilio* ticks (Tarasevich *et al*., 1991; Eremeeva *et al*., 1994). Since 1983, more than 2000 cases of the disease have been registered in a small area of the Astrakhan region (Tarasevich *et al*., 1991). Infection caused by the *R. conorii* Astrakhan strain showed similar clinical manifestations to those of the other strains of *R. conorii*; however, eschars have been reported in only 23% of patients (Tarasevich *et al*., 1991). *R. conorii* Astrakhan strain has recently been isolated from a patient in Chad and from *Rh. sanguineus* ticks in Kosovo (Fournier *et al*., 2003a,b).

4.6.5 *Rickettsia sibirica mongolitimonae* strain

R. sibirica mongolitimonae strain – initially named strain HA-91 – was originally isolated from a *Hy. asiaticum* tick collected in the Alashian region of Inner Mongolia in 1991 (Yu *et al*., 1993). In Europe and the Mediterranean basin, the *R. sibirica mongolitimonae* strain was detected in *Hy. anatolicum* from Greece, *Hyalomma* sp. from Israel and *Rh. pusillus* from Portugal and Spain (Psaroulaki *et al*., 2005b; de Sousa *et al*., 2006a; Toledo *et al*., 2009b). In 1996, the first human case of infection caused by this *Rickettsia* strain was described in southern France. The new strain was isolated from the blood and skin of a patient admitted in March to the Hospital La Timone in Marseille (Raoult *et al*., 1996). The patient had no travel history, and disease manifestations similar to MSF, but the unusual aspect of the case was its occurrence in March when MSF is rarely reported. Subsequently, other human cases were described in France, and the diagnosis was confirmed by rickettsial isolation and/or PCR detection of the agent in eschars (Fournier *et al*., 2005). Clinical cases with isolation or DNA detection of the agent were also reported in Greece, Portugal and Spain, and in French travellers who visited Algeria and Egypt (Fournier *et al*., 2005; Psaroulaki *et al*., 2005b; de Sousa *et al*., 2006b; Aguirrebengoa *et al*., 2008; Scolovschi *et al*., 2010). In France, most of the reported cases caused by the *R. sibirica mongolitimonae* strain have occurred in the spring; this is in contrast with the majority of Portuguese cases that occurred in summer during the MSF season. The occurrence of these cases in different months could be related to the differences in seasonal activity and population dynamics of the different vectors. The clinical presentation of *R. sibirica mongolitimonae* infection has included fever, a rare or diffuse maculopapular rash and eschars; 50% of patients also have a lymphangitis expanding from the inoculation eschar to the draining lymph node (de Sousa *et al*., 2008a). The latter feature has led to the name given to this disease: lymphangitis-associated rickettsiosis (LAR) (Fournier *et al*., 2005).

4.6.6 *Rickettsia slovaca*

R. slovaca was first isolated in 1968 from a *Dermacentor marginatus* tick in Slovakia (Rehacek, 1984). In subsequent years, suspected cases of infection caused by *R. slovaca* were reported in patients from Hungary and Slovakia (Mittermayer *et al*., 1980; Rehacek, 1984; Raoult *et al*., 2002). However, the first proven case of *R. slovaca* infection was reported only in 1997 in a French patient who presented with an eschar on the scalp and enlarged cervical lymph nodes after receiving a bite from a *Dermacentor* tick (Raoult *et al*., 1997). The lymphadenopathy present in most of the patients that have been seen infected

with *R. slovaca* has led to this clinical syndrome being named tick-borne lymphadenopathy (TIBOLA). Spanish researchers have also coined the name *Dermacentor*-borne necrosis erythema lymphadenopathy (DEBONEL) (Lakos, 1997; Raoult *et al*., 1997). *R. slovaca* has been identified in *D. marginatus* and *D. reticulatus* in most European countries. The prevalence rates of *R. slovaca* infection that have been found in *Dermacentor* ticks in Europe range from 21% in Hungary to higher rates in Spain (40.6%), Portugal (41.5%) and Switzerland (45.4%) (Beati *et al*., 1994; Bacellar *et al*., 1995; Lakos and Raoult, 1999; Oteo *et al*., 2006; Parola *et al*., 2009; Milhano *et al*., 2010).

The number of *R. slovaca* infections in Europe is still under-evaluated; the majority of patients have been reported from France, Hungary, Spain, Slovakia and Italy (Lakos, 2002; Raoult *et al*., 2002; Ibarra *et al*., 2006; Selmi *et al*., 2008; Parola *et al*., 2009). The epidemiological and clinical findings on *R. slovaca* infections among patients in France, Hungary, Spain and Italy showed that the infection occurred mainly during the colder months of the year, mostly from October to April, in accordance with the density and activity of *Dermacentor* ticks. It seems that children and women had a higher risk of infection, and patients were more frequently bitten on the scalp (68–100%). Fever is present in 12–67% of the patients; rash is rare (14–23%) compared with other rickettsioses. The enlargement of lymph nodes has been reported in almost all of the patients (74–100%) (Lakos, 2002; Raoult *et al*., 2002; Ibarra *et al*., 2006; Selmi *et al*., 2008, Parola *et al*., 2009). No complications were observed, but 21–52% of patients developed a localized alopecia at the site of the tick bite and around 37% suffered from persistent asthenia. Only 50% of the patients develop detectable antibodies, which may reflect the fact that this disease is a localized infection.

R. raoultii (formerly genotypes RpA4, DnS14, DnS28), the other aetiological agent of TIBOLA/DEBONEL, was described for the first time in *Rh. pumilio* collected in the Astrakhan region and in *D. nutallii* in Siberia. Later, this *Rickettsia* was detected in *Dermacentor* ticks in other European countries (Rydkina *et al*., 1999). The pathogenicity of the species has been suggested by the amplification of its DNA from the blood and skin biopsy samples of patients with a clinical picture of *R. slovaca*-like infection. This *Rickettsia* species was also found in *Dermacentor* removed from Spanish and French patients with cases of TIBOLA/DEBONEL (Ibarra *et al*., 2006; Parola *et al*., 2009). Nevertheless, *R. slovaca* remains the main aetiological agent responsible for the majority of TIBOLA/DEBONEL cases.

4.6.7 *Rickettsia massiliae*

R. massiliae was isolated in 1992 from ticks in France near Marseille (Beati and Raoult, 1993). Subsequently, this rickettsia has been detected by molecular methods and isolated in several countries in Europe (Scolovschi *et al*., 2010). *R. massiliae* has been found mainly in ticks from the *Rhipicephalus* genus: *Rh. sanguineus* and *Rh. turanicus*. The first human case was described in a Sicilian patient who was admitted at Palermo hospital in 1985. However, it was not until 2005 that the isolate discovered 20 years before was characterized and identified as *R. massiliae* (Vitale *et al*., 2006). The patient presented with an eschar on his right ankle, and a maculopapular rash involving the palms and soles, revealing similar manifestations to those found with other rickettsioses. He recovered completely after receiving tetracycline. Recently, another case of *R. massiliae* infection was diagnosed in a patient from Argentina. The diagnosis was confirmed by PCR in a Spanish laboratory (García-García *et al*., 2010).

4.6.8 *Rickettsia aeschlimannii*

R. aeschlimannii was first characterized in 1997 from *Hy. marginatum* ticks from Morocco (Beati *et al*., 1997). Later, this rickettsia was also described from *Hy. marginatum* ticks from Portugal, France, Spain, Croatia, Algeria, Italy and Egypt (Beati *et al*., 1997; Bacellar, 1999; Punda-Polic *et al*., 2002; Fernández-Soto *et al*., 2003). However, until now the two described human cases caused by *R. aeschlimannii* were

only diagnosed in Africa. The first case was in a French traveller who had visited Morocco, and the second case was detected in a South African patient who was bitten by a *Rh. appendiculatus* tick (Pretorius and Birtles, 2002). Symptoms exhibited by the patients were similar to those of MSF, and infection by *R. aeschlimannii* was confirmed by PCR amplification of rickettsial DNA from serum and skin biopsy (Pretorius and Birtles, 2002; Raoult *et al*., 2002). Recent studies in Algeria, Corsica, Greece, Spain, Germany and the European part of Russia also revealed that other tick species can harbour *R. aeschlimannii*, such as: *Hy. anatolicum*, *Hy. detritum* (syn. *Hy. scupense*), *Hy. rufipes*, *D. reticulatus*, *I. ricinus, Ha. punctata, Ha. inermis, Rh. bursa, Rh. sanguineus* and *Rh. turanicus* (Fernández-Soto *et al*., 2003, Matsumoto *et al*., 2004; Psaroulaki *et al*., 2006; Mokrani *et al*., 2008; Bitam *et al*., 2009; Shypnov *et al*., 2009).

4.6.9 *Rickettsia helvetica*

R. helvetica was detected for the first time in Swiss *I. ricinus* in 1979, and confirmed as a new member of the SFG *Rickettsia* in 1993 (Beati *et al*., 1993). *R. helvetica* has been detected and isolated from *I. ricinus* in many European countries, including Austria, Bulgaria, Denmark, Eastern Ukraine, France, Germany, Hungary, Italy, Moldova, the Netherlands, Poland, Portugal, Slovenia, Spain, Sweden and the UK (Bacellar *et al*. 1995; Parola *et al*., 1998; Nilsson *et al*., 1999; Beninati *et al*., 2002; Christova *et al*., 2003; Prosenc *et al*., 2003; Fernández-Soto *et al*., 2004; Sréter-Lancz *et al*., 2005; Nijhof *et al*., 2007; Skarphedinsson *et al*., 2007; Blaschitz *et al*., 2008; Chmielewski *et al*., 2009; Movila *et al*., 2009; Pluta *et al*., 2010; Tijsse-Klassen *et al*., 2011). In Portugal and Spain, the organism has been also detected in *I. ventalloi* parasitizing birds (Santos-Silva *et al*. 2006; Movila *et al*., 2011). The prevalence of *R. helvetica* in ticks from different countries has been found to range from 2.8% in Poland to 91.4% in the south of Germany (Chmielewski *et al*., 2009; Silaghi *et al*., 2011). Detection of co-infections with *R. helvetica* and *Borrelia burgdorferi s.l.* in the same vector (*I. ricinus*) has gained attention for possible exacerbation of the illness arising from *R. helvetica* infection or Lyme borreliosis (Fernández- Soto *et al*., 2004; Milhano *et al*., 2010).

R. helvetica infection has been progressively becoming a clinical entity of its own. It was reported for the first time in 1999 in Sweden in two patients with fatal perimyocarditis, and it was recently reported in two Swedish patients with meningitis and septicaemia (Nilsson *et al*., 1999). Serological associations with *R. helvetica* infections have been reported in patients in Europe (France, Italy, Switzerland) and Asia (Thailand), but additional evaluation and isolation of the bacterium from clinical samples are needed to confirm the pathogenicity of *R. helvetica* (Baumann *et al*., 2003; Fournier *et al*. 2004; Ciceroni *et al*., 2006; Nilsson *et al*. 2010). In 2002, an association of *R. helvetica* with sarcoidosis was proposed; however, the validity of this association has been questioned and later serological studies did not reveal the presence of anti-rickettsial antibodies in a group of Scandinavian sarcoidosis patients (Nilsson *et al*., 2002; Planck *et al*., 2004).

4.6.10 *Rickettsia monacensis*

R. monacensis was isolated and characterized from *I. ricinus* for the first time in Germany. Since then, most European countries have reported the presence of this agent, essentially based on molecular detection (Simser *et al*., 2002). More recently, this *Rickettsia* species has also been isolated from *I. ricinus* in Portugal, and it was shown that *R. monacensis* was easily propagated and isolated in Vero cell lines at 28°C in conditions different from that for the isolate from Germany (Milhano *et al*., 2010). *R. monacensis* was recently associated with febrile disease in humans in northern Spain (Jado *et al*., 2007).

Table 1.25 in Appendix 1 elaborates on the geographical distribution of *Rickettsia* spp. and their reported diseases in Europe and in the Mediterranean basin. This table was generated independently of the main systematic literature review that is described in the introduction.

4.7 Lyme Borrelioses

Lyme borreliosis (LB) or Lyme disease is the most common tick-borne disease of humans in the northern hemisphere. It is a complex of several different zoonotic infections of which the aetiological agents are transmitted by hard ticks. At least 18 species or genospecies of spirochaetes in the *B. burgdorferi s.l.* complex have so far been described. Several are pathogenic to humans and domestic animals. They include *B. burgdorferi*, which is predominant in North America but also present in Eurasia, and *B. afzelii* and *B. garinii*, which are predominant in Eurasia. They are transmitted by tick species of the genus *Ixodes*, mainly *I. ricinus* in Europe, *I. persulcatus* in Eurasia, *I. pacificus* in the western USA and *I. scapularis* in the eastern USA. In Europe, at least another four *Borrelia* species, i.e. *B. bavariensis*, *B. valaisiana*, *B. spielmanii* and *B. lusitaniae* sometimes infect humans and may cause human LB (Richter *et al*., 2004; Piesman and Gern, 2008; Rudenko *et al*., 2011). In Europe, three tick species are considered to be vectors of LB spirochaetes, i.e. *Ixodes ricinus, I. hexagonus* and *I. uriae* (Piesman and Gern, 2008). Although the level of infection in the adult ticks in European populations of *I. ricinus* is higher (mean 17.4%; range 3–58%) than in the nymphs (mean 10.8%; range 2–43%), the nymphs are usually more important than the female adult ticks for transmission of the pathogens to humans (Hubálek and Halouzka, 1998). Larvae are rarely infected (mean 1.9%; range 0–11%; Hubálek and Halouzka, 1998). In *I. persulcatus*, however, the nymphs rarely feed on humans, so in this case it is the adult female ticks that are responsible for nearly all human infections with LB spirochaetes.

The enzootic cycle in general involves *Ixodes* spp. larvae and nymphs which become infected when feeding on infective wild bacteraemic mammals, particularly insectivores (shrews, hedgehogs), rodents (mice, voles, rats and squirrels) or hares. Certain bird species also serve as vertebrate reservoirs to the spirochaetes. Co-feeding transmission has been demonstrated to occur when sheep serve as a *Borrelia* reservoir (Ogden *et al*., 1997).

It is important to distinguish between vertebrate hosts for the ticks and vertebrate reservoirs of the spirochaetes. Cervids appear to be refractory to the infection and usually do not serve as *Borrelia* reservoirs, but are extremely important hosts to *I. ricinus* females (Jaenson and Tälleklint, 1992). Many species of *Borrelia* may circulate in the same ecosystem, with the result that a single tick can be infected with two or more species of *Borrelia* – and with the TBE virus and other species of human-pathogenic bacteria. Throughout Europe, 13% of *Borrelia* infections in *I. ricinus* are mixed infections (Rauter and Hartung, 2005; Piesman and Gern, 2008). Multiple infection of a tick may occur because the host on which the tick was feeding had a multiple infection or because the tick had fed two or more times on hosts infected with different *Borrelia* spp. In Europe, *B. garinii* and *B. valaisiana* are predominant in the mixed infections, followed by mixed *B. garinii/B. afzelii* infection (Piesman and Gern, 2008). *B. afzelii* is mainly associated with rodents, while some serotypes of *B. garinii* and all serotypes of *B. valaisiana* are associated with birds. *B. lusitaniae* is associated with lizards in the Mediterranean countries, and often infects vector ticks more frequently than do the other genospecies in the complex (Richter and Matuschka, 2006).

LB is prevalent in most parts of Europe, but its prevalence is lower in southern Europe, such as in Portugal and Italy than in the former USSR to Japan, Mongolia and north-western China (Rauter and Hartung, 2005). The infection also occurs in some specific locations in North Africa. In North America, nearly all human LB cases are confined to the north-eastern USA, but the infection also occurs in other parts, including California, but with a lower prevalence than the north-eastern part of the country. LB is becoming increasingly prevalent in southern Canada and has also been reported from South America, including Mexico and Brazil. Climate change (not synonymous with global warming in the author's opinion, as climate change is a broad concept that includes all potential intentional and non-intentional changes in weather conditions) and an increasing abundance of deer could

be associated with the spread of LB in Northern Europe (Gray *et al*., 2009).

Human LB infections sometimes cause clinical disease, which can range from a relatively short influenza-like illness often accompanied by excruciating (nocturnal) pain, to a severe syndrome with neurological involvement, including meningitis, chronic severe arthritis and/or myocarditis. For instance, it has been estimated that in Sweden alone about 10,000 people annually contract the infection, resulting in clinical disease (Berglund, 2004). Among domesticated animals, clinical symptoms associated with LB have been reported in dogs, cattle and horses. Antibody titres against *B. burgdorferi s.l*. in dogs, cats and livestock can be high, but it is often difficult to establish a cause-and-effect relationship between exposure to the spirochaetes and clinical signs.

4.8 Recurrent (Relapsing) Fever

Relapsing fever is an infection caused by several spirochaetes of the genus *Borrelia* (Cutler, 2006). Relapsing fever borrelioses are characterized by recurrent febrile episodes and spirochaetaemia. Other than the louse-borne relapsing fever caused by *B. recurrentis* and transmitted by the body louse *Pediculus humanus*, endemic tick-borne relapsing fever is a zoonotic disease transmitted worldwide by soft tick species of the genus *Ornithodoros*. Within each region, specific relationships usually exist between the *Ornithodoros* vector species, *Borrelia* species and their distribution areas. Reservoir hosts are usually wild rodents. *Ornithodoros* ticks are included in the family Argasidae. They live close to their host, although the time spent on the host is relatively short. After each blood meal they are found in their habitats, typically in cracks and crevices of rodent burrows, but also in human shelters or just below the soil surface. Ticks become infected during a blood meal on a vertebrate with spirochaetaemia. Spirochaetes then invade all tissues of the tick, including ovaries (responsible for transmission between generations), salivary glands and excretory organs. Vertebrates and humans become infected during a blood meal through contamination of the feeding site by salivary and/or coxal secretions of the tick (Parola and Raoult, 2001).

B. hispanica is found in Spain, Portugal, Cyprus, Greece and North Africa. It has been isolated in *O. erraticus*, a tick commonly found in south-western Europe. This tick species usually lives in the burrows of wild rodents, its natural host. In Spain and Portugal; however, it has adapted to bite domestic pigs that are kept in continuous grazing and sometimes overnight in large burrows or inside old buildings, and this tick species has adapted to live in these habitats (Estrada-Peña and Jongejan, 1999). Humans may be bitten, and hence relapsing fever was sporadically reported in countries such as Spain during the 20th century, probably with an underestimated incidence (Sánchez-Yebra *et al*., 1997). The disease caused by *B. hispanica* is one of the less severe in the relapsing fever group, and presents with neurological signs in less than 5% of cases (Cadavid and Barbour, 1998). In 1996, a new *Borrelia* species was isolated in southern Spain from three patients with relapsing fever and from *O. erraticus* ticks found in nearby areas (Anda *et al*., 1996). The reservoir of this bacterium is still unknown and further records of the pathogen are unavailable. Although this new *Borrelia* species has not yet been cultured, molecular analyses have shown that it is closely related to *B. hispanica*, *B. duttoni* (an African species not present in Europe) and *B. crocidurae*.

On the borders of Europe, several other relapsing borrelioses are present. *B. persica*, the agent of Persian relapsing fever, is found in Israel, Syria, Egypt, Iran and Central Asia. It is transmitted by *O. tholozani* (Rodhain, 1998). This tick commonly lives in localities where livestock are housed, for example man-made shelters, caves and rocky overhangs (Estrada-Peña and Jongejan, 1999). The disease is sometimes severe (Cadavid and Barbour, 1998). *B. caucasica*, present in the Caucasus and Iraq, is transmitted by *O. verrucosus*, another argasid parasite of rodents. *B. latyschevii* is transmitted by *O. tartakovskyi* in Central Asia, the former USSR and Iran (Estrada-Peña and Jongejan, 1999; Rebaudet and Parola, 2006).

4.9 Piroplasmoses

4.9.1 Babesioses

Babesioses are caused by naturally tick-transmitted and generally host-specific intra-erythrocytic protozoan parasites of the genus *Babesia* (phylum Apicomplexa, order Piroplasmida). Babesiae, which are the second most common blood-borne parasites of mammals after the trypanosomes, are capable of infecting a wide variety of vertebrate mammalian species, including humans. More than 100 species have been identified, and these are traditionally divided on the basis of their morphology into the small and large babesiae (Telford *et al*., 1993; Homer *et al*., 2000). Molecular analysis suggests that the host range of many *Babesia* species is less restricted than previously believed and that still-unrecognized species may cause zoonotic infections in a variety of animals and humans (Gray and Weiss, 2008).

To date, only ixodid ticks have been identified as vectors for *Babesia* species. Some *Babesia* species can infect more than one genus of ticks; others can infect only ticks from the genus *Ixodes*. Several tick vectors can carry more than one *Babesia* species. The vectors become infected when ingesting the infected blood cells from a vertebrate reservoir that is competent in maintaining the *Babesia* organisms in an infectious state. Certain species of *Babesia*, such as *B. divergens* and *B. canis*, are transmitted transovarially to the next generation because they invade the female tick's ovaries. These species may persist in several generations of ticks, even without new infections. Some other *Babesia* species (e.g. *B. microti*) are only transmitted trans-stadially. All species of *Babesia* are naturally transmitted by the bite of infected female ticks. It has been demonstrated that male ticks may transmit *Babesia* species, although the epidemiological importance of male ticks in transmission has yet to be established.

Infection is initiated by inoculation of the sporozoites with the saliva of the vector tick into the bloodstream of the host. Transmission only occurs a few days after the tick has attached, because maturation of the sporozoites in the salivary glands of the vector is stimulated by feeding. *Babesia* species directly invade red blood cells, where their asexual multiplication most often results in two, sometimes four, daughter cells; these then leave the host cell and each enters another red cell (Homer *et al*., 2000; Uilenberg, 2006). Animals and humans can also acquire the infection through the transfusion of contaminated blood products.

The two major factors involved in the pathogenesis of babesiosis are the release of pharmacologically active agents and intravascular haemolysis. The relative importance of each varies with the species of *Babesia*. The clinical features of babesiosis vary substantially from asymptomatic to life threatening, depending on the condition of the host and the parasite involved. During the acute babesial infection, the host may become severely ill as a result of host-mediated immunopathological mechanisms and erythrocyte lysis. Typically, the infected host can suffer high fever, anaemia and hyperbilirubinuria, possibly followed by alterations in the kidneys and other organs. All mammalian hosts examined have been able to develop immunity to *Babesia* species in which both humoral and cellular factors are involved. In endemic areas, all or almost all individuals of the host population are infected when they are young, with no or minimal clinical disease. The introduction of susceptible animals into endemic regions could lead to the recrudescence of babesiosis (Telford *et al*., 1993; Homer *et al*., 2000; Uilenberg, 2001; Hunfeld *et al*., 2008).

Babesioses are well-recognized diseases of veterinary importance in Europe and North Africa. One of the most important and widespread *Babesia* species affecting cattle in temperate Europe is *B. divergens*, and this species probably occurs wherever the vector *I. ricinus* is present, which includes North Africa (Bouattour and Darghouth, 1996). *B. major*, another species in Europe, gives a much milder infection that is transmitted by *Ha. punctata* in western Europe. A low or non-pathogenic bovine *Babesia* species, *B. occultans*, was detected for the first time in unfed *Hy. marginatum* ticks collected in three bioclimatic regions of Tunisia. It was supposed that this species may have a wide distribution in the Mediterranean region, and not only in

sub-Saharan Africa as previously described (Ros-García *et al.,* 2011). The taxonomy and even the geographical distribution of babesial parasites of small ruminants are not quite settled. The main species are *B. motasi* and *B. ovis*, which are transmitted by *Ha. punctata* and *Rh. bursa*, respectively. *B. motasi* in northwestern Europe produces a clinically milder disease than it does in the Mediterranean basin, and there are serological differences as well (Uilenberg, 2006). Babesiosis in the horse is mentioned with theileriosis (see Section 4.9.2).

On the basis of differences in vector specificity, geographical distribution, pathogenicity, antigenic properties and molecular investigations, the species *B. canis* of dogs has been subdivided into three subspecies, namely *B. c. canis*, *B. c. vogeli* and *B. c. rossi* (Kjemtrup *et al*., 2000). These subspecies are currently considered to be separate species. *Babesia c. canis*, transmitted by *D. reticulatus* ticks, is the most common canine *Babesia* subspecies in temperate regions of Europe (Cacció *et al*., 2002; Irwin, 2009). *Babesia c. vogeli*, transmitted by *Rh. sanguineus*, has also been reported in continental Europe (Cacció *et al*., 2002; Duh *et al*., 2004; Cardoso *et al*., 2008). This subspecies is considered to be a mildly virulent subspecies, and commonly induces moderate clinical signs in dogs. Besides these large parasites, small babesiae such as *B. gibsoni*, transmitted by the brown dog tick, *Rh. sanguineus,* and the *B. microti*-like or 'Spanish isolate', with the proposed name of *Theileria annae*, have been reported from some European countries (Zahler *et al*., 2000; Camacho, 2006; Beck *et al*., 2009). Several studies have proved that *B. gibsoni* infection can be transmitted from dog to dog via bite wounds, saliva or ingested blood independently of the limitations of vector tick infestation (Jefferies *et al*., 2007).

Babesioses are an emerging zoonotic problem caused by several species of protozoans in the genus *Babesia* (Homer *et al*., 2000; Kjemtrup and Conrad, 2000; Herwaldt *et al*., 2003; Hunfeld *et al*., 2008).The first confirmed case of human babesiosis was diagnosed in a splenectomized Yugoslavian cattle farmer who died of a fatal *B. divergens* infection in 1956 (Skrabalo and Deanovic, 1957). To date, more than 60 cases of human babesiosis have been reported from Europe. At least 70% of the cases in Europe are associated with the cattle piroplasm, *B. divergens* (Genchi, 2007). In recent years, molecular studies have confirmed the responsibility of *B. divergens* in two cases of human babesiosis in the Canary Islands (Olmeda *et al*., 1997) and in Portugal (Centeno-Lima *et al*., 2003). A new European *B. divergens*-like organism (EU1), named *B. venatorum*, has been described from deer and from *I. ricinus* (Duh *et al*., 2005; Gray, 2006; Bonnet *et al*., 2007). This parasite species was involved in the first documented cases of human babesiosis in asplenic men in Italy, Austria and Germany (Herwaldt *et al*., 2003; Häselbarth *et al*., 2007). A human infection in a splenectomized patient, caused by a strain named *Babesia* EU3 that has high homology with *Babesia* EU1, was reported from Germany (Häselbarth *et al*., 2007). *B. microti* infection has also been reported in Europe (Hildebrandt *et al*., 2007). Sero-surveys suggest that a low percentage of Europeans (<3.4%) from several countries may be infected with *B. microti*. Most infected patients share splenectomy as a risk factor for acquiring the disease, but the rising number of HIV-positive individuals and the increasing population of immunocompromised patients may also serve to boost the number of human cases. The clinical course of human babesiosis varies according to the aetiological agent and ranges from subclinical infection to a severe disease with sudden onset. In many individuals, however, babesiosis is a mild, self-limited disease that requires only supportive therapy. Splenectomized or elderly patients infected with *B. microti* or *B. divergens* tend to develop severe and sometimes fatal illnesses (Homer *et al*. 2000; Gray, 2006).

4.9.2 Theilerioses

Theileria spp. (phylum Apicomplexa, order Piroplasmida) are tick-borne intracellular protozoan haemoparasites causing infection and often disease of veterinary and economic importance in livestock and wild animals in different regions of the world (Preston, 2001; Uilenberg, 2001). The genus *Theileria* differs

from *Babesia* in that *Theileria* first penetrates lymphocytes or macrophages and develops there, and then enters red blood cells where the parasites multiply, forming tetrads often in the shape of a Maltese cross (Uilenberg, 2006).

The clinical signs of theileriosis in animals differ from babesioses in the absence of haemoglobinuria and the occurrence of a less severe anaemia. Ticks can only transmit these haemoparasites trans-stadially. There is no transovarial transmission because theileriae do not passage the ovaries and the eggs of the vectors. The newly hatched larvae of the ticks are never infected. Nymphs and adults become infective only if they were infected in the previous developmental stage. The transmission of parasites takes place by the injection of infected saliva of ticks, but it only occurs a few days after the tick has attached; the parasites have first to mature before they become infective (Mehlhorn and Schein, 1984; Preston, 2001).

A mild disease of domestic cattle is called tropical or Mediterranean theileriosis; this is caused by *T. annulata*, which is distributed in many areas of the world, extending from southern Europe to southern Asia (Brown, 1990). *T. annulata* is transmitted by a number of *Hyalomma* species, which are found in large numbers in the Mediterranean region, especially in semi-arid areas (Viseras and García-Fernández, 1999). *Hy. anatolicum anatolicum*, *Hy. detritum detritum* and *Hy. dromedarii* are considered as the main vectors in the field. Other species, such as *Hy. anatolicum excavatum* and *Hy. marginatum marginatum* may also play a role in the epidemiology of the disease in the field (Estrada-Peña *et al*., 2004). *T. sergenti/buffeli/orientalis* causes a mild or asymptomatic disease in cattle known as bovine benign theileriosis (Uilenberg, 1981). *T. ovis* and *T. lestoquardi* (formerly *T. hirci*) are recognized as the species that can cause serious theileriosis in small ruminants, particularly sheep, where the disease occurs in the Old World (Schnittger *et al*., 2000; Preston, 2001).

Equine piroplasmosis (EP) is a tick-borne intraerythrocytic protozoal disease of equids (horses, donkeys, mules and zebras) caused by *B. caballi* and *Theileria* (syn. *Babesia) equi. B. c. canis* of dogs has been reported in horses, but no clinical signs attributable to this parasitic species were described (Criado-Fornelio *et al*., 2003; Hornok *et al*., 2007a). This economically important protozoan disease of horses has been reported in many countries, thus making this disease a cause of great concern in the global horse industry. For this reason, testing of horses for EP is mandatory for the international movement of horses, either for participation in international events or for export. Only horses seronegative for both *T. equi* and *B. caballi* are qualified for importation to some countries, such as the USA, Canada, Australia and Japan (Bruning, 1996; Knowles, 1996). Within Europe, equine theileriosis is more prevalent in Portugal (Bashiruddin *et al*., 1999), Spain (Camacho *et al*., 2005) and Italy (Moretti *et al*., 2009). The disease agent is mainly spread by competent ticks. To date, up to 12 species of ixodid ticks belonging to the genera *Hyalomma*, *Dermacentor* and *Rhipicephalus* have been identified as vectors of both *B. caballi* and *T. equi* (Bruning, 1996; Massaro *et al*., 2003). Transmission of *T. equi* appears only to occur trans-stadially (de Waal, 1992). The parasites are also spread by the transfer of blood from infected to naive equids through shared needles, improperly shared equipment, and blood or serum transfusions (de Waal and Van Heerden, 2004). Transplacental transmission of *T. equi* from carrier mares to asymptomatic foals was recently confirmed (Allsopp *et al*., 2007).

Clinical signs of infection with EP are not pathognomonic, especially in endemic areas, and vary from mild to severe. Acute and subacute cases are the most commonly observed. The mild form of the disease can cause equids to appear weak or show lack of appetite, while more severe cases may have fever, anaemia, jaundice, swollen abdomen, haemoglobinuria and bilirubinuria, and sometimes result in death (de Waal and Van Heerden, 2004). In the chronic phase, the horse can appear normal. In some cases of acute or chronic disease, mortality can reach up to 50% (de Waal, 1992). Infected animals that recover from acute or primary infection of *T. equi* remain lifelong carriers because anti-theilerial drugs suppress but do not eliminate the parasite.

The detection of apparently healthy carrier horses therefore remains a worldwide challenge for controlling the spread of the disease. Carrier mares may transmit the organism to their offspring and this may result in abortion or neonatal piroplasmosis. Some researchers suggest that foals may be born as carriers yet remain apparently healthy as colostral *T. equi* antibody may act to suppress parasitaemia in the newborn, reducing the incidence of clinical neonatal piroplasmosis (Allsopp *et al.*, 2007).

4.10 Hepatozoonosis

Hepatozoonosis is an arthropod-borne infection of both wild and domestic animals, including mammals, birds, reptiles and amphibians. It is caused by about 300 apicomplexan protozoal species from the genus *Hepatozoon* (Smith, 1996). The vertebrates are intermediate hosts, and several blood-sucking invertebrates, including hard ticks, are definitive hosts in which the sexual reproduction and sporogony of the protozoans occurs. Transmission of *H. canis* to the dog takes place by ingestion of a tick or parts of ticks containing *Hepatozoon* oocysts; this is because the protozoan is not disseminated within the tick but remains in the haemocoel. No salivary transfer of these parasites has been documented. In this respect, *Hepatozoon* differs from many other tick-borne protozoal and bacterial pathogens. The ticks become infected when they ingest infected neutrophils and gametocytes while feeding on the blood of a parasitaemic dog. Trans-stadial transmission of *H. canis* from the nymph to the adult stage in the tick vector has been recorded, whereas transovarial transmission could not be demonstrated (Baneth, 2006; Baneth *et al.*, 2007). Japanese scientists (Murata *et al.*, 1993) have reported that vertical transmission of *H. canis* occurred in puppies born from an infected dam and raised in a tick-free environment.

In the Old World, canine hepatozoonosis caused by *H. canis* is of veterinary importance. *H. canis* has been reported from the Mediterranean region of Europe (Spain, Portugal, Italy, Greece and France), Africa, the Middle East, the Far East and South America, where its main vector, the brown dog tick, *Rh. sanguineus*, is enzootic (Vincent-Johnson *et al.*, 1997; Baneth *et al.*, 2003). Recently, Italian scientists reported that *I. ricinus* might also be implicated in parasite transmission, thus explaining the occurrence of hepatozoonosis in areas considered *Rh. sanguineus* free (Gabrielli *et al.*, 2010). However, further studies are needed to confirm this hypothesis. Hepatozoonosis is of increasing importance in dogs in regions that have previously been considered free of the infection. *H. canis* is regularly introduced by dogs into north-west Europe after ingestion of infected *Rh. sanguineus* during visits to endemic regions (Holland, 2001). The first detection of *H. canis* in Slovakia (Majláthová *et al.*, 2007) and Italy (Gabrielli *et al.*, 2010) has been reported in naturally infected red fox (*Vulpes vulpes*). Prevalence data for dogs are still restricted to small areas and range from 0.9% in France (Criado-Fornelio *et al.*, 2009) to 71% in Croatia (Vojta *et al.*, 2009). According to a sero-survey of *H. canis* in Israel, this protozoal infection appears to be endemic in red fox populations and these may serve as reservoirs of the parasite for domestic dogs (Fishman *et al.*, 2004).

When the infected vector is ingested by the dog, *H. canis* mainly infects the haemolymphatic tissues and blood-forming organs, including the bone marrow, lymph nodes and spleen. The pathogenicity and clinical manifestations of hepatozoonosis vary according to the age of the host and the degree of infection. Canine hepatozoonosis is typically a mild clinical disease. However, clinical signs vary from an apparently subclinical infection with a low parasitaemia, to a life-threatening disease with lethargy, fever, cachexia and anaemia with a large number of circulating parasites. The grave and potentially fatal disease occurs mostly in young animals or in dogs suffering from a concurrent infection or immunosuppressive conditions. *H. canis* is commonly associated with co-infection with other diseases, in particular ehrlichiosis and leishmaniosis in endemic areas (Baneth, 2006).

4.11 Other Potentially Tick-borne Infections

The following infections are associated or suspected to be associated with ticks as one of the potential – but not the only – modes of transmission.

4.11.1 African horse sickness

African horse sickness (AHS) is caused by African horse sickness virus (AHSV), a member of the genus *Orbivirus*, subfamily *Sedoreovirinae* and the family *Reoviridae*. The virion is approximately 70 nm in diameter (Polson and Deeks, 1963), is non-enveloped and consists of a double-layered icosahedral capsid with 32 capsomeres (Pringle and Wickner, 2000). The double-capsid particles contain seven structural proteins (VP1–VP7), with the outer capsid made up of VP2 and VP5, and the inner capsid (the core) made up of VP3 and VP7 (as major proteins) and VP1, VP4 and VP6 (minor proteins) (Roy *et al*., 1994). Among the capsid proteins, VP2 has been shown to be the most variable, and responsible for most of the antigenic variation of the virus (Iwata *et al*., 1992; Mellor and Hamblin, 2004); hence VP2 is the major target of the host's neutralizing antibodies response (Burrage *et al*., 1993, Roy *et al*., 1994).

AHSV infects all equids, with horses being the most susceptible to clinical disease, which has a severe morbidity and high case fatality rate of 50–95%. Mules have a similar morbidity to horses, but with a lower case fatality rate of 50–70% (Coetzer and Guthrie, 2004). Donkeys and zebras are very resistant to the disease, and usually just develop subclinical infection (Theiler, 1921; Barnard, 1993; Coetzer and Guthrie, 2004). Donkeys in the Middle East can have a case fatality rate of up to 10%, and may be more susceptible to clinical disease than the South African donkey (Alexander, 1948; Hamblin *et al*., 1998; Coetzer and Guthrie, 2004). Zebras only show mild fever when experimentally infected with the virus (Erasmus *et al*., 1978; Coetzer and Guthrie, 2004); they maintain year-round infections and are considered a reservoir host for the virus in endemic regions (Davies and Otieno, 1977; Barnard, 1993). AHSV has also been isolated in blood samples 40 days post infection in zebras. In comparison, in experimentally challenged horses, viraemia usually lasts 4–8 days, but does not exceed 21 days (Barnard *et al*., 1994; Coetzer and Guthrie, 2004). Donkeys were considered to be potential reservoirs, but the absence of viral antigens after 14–19 days post infection makes them unlikely long-term hosts, although they may play a small role in spread of the virus. Theiler demonstrated that AHSV can be transmitted to dogs and cause a similar pathology to that found in horses (Theiler, 1906). Dogs, however, are likely to be dead-end hosts for the virus (Braverman and Chizov-Ginzburg, 1996). The most common way for a dog to become infected with the virus is through the consumption of uncooked meat from an infected horse carcass, as was the case in Pretoria in 1980 (Van Rensburg *et al*., 1981).

AHSV is considered to be endemic in north-eastern parts of South Africa, primarily Mpumalanga Province, but outbreaks regularly occur in other parts of South Africa (Lord *et al*., 2002; Coetzer and Guthrie, 2004). In addition, major outbreaks of the virus have recently occurred in regions with close proximity to the Mediterranean Sea, such as in the Middle East (1959–1961), North Africa (1965, 1989, 1991), Spain (1966, 1987–1990) and Portugal (1989) (Rodriguez *et al*., 1992; Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004).

Clinical disease develops in susceptible animals after an incubation of about 5–7 days, depending on virulence and dose of virus (Theiler, 1921; Coetzer and Guthrie, 2004). While AHSV causes severe morbidity and mortality in the majority of infected horses, the pathogenesis and clinical disease that develops differs among cases. This difference is not completely understood, but is a function of both host factors (genetics and immune status) and virus factors (dose, route of infection in experimental inoculations and virulence phenotype) (Burrage and Laegreid, 1994).

The insect *Culicoides imicola* is the principal vector responsible for AHSV transmission, and its importance in AHSV transmission

has been recognized for over 50 years. *C. imicola* has historically been found in Africa and South-east Asia (Meiswinkel *et al*., 2004). Nevertheless tick species in general are also capable of transmitting the virus (Meiswinkel *et al*., 2000; Mellor and Hamblin, 2004).

4.11.2 Bartonelloses

Bartonella infections are widespread in wild and domesticated mammals, and several new species have been described during the last few decades. These alphaproteobacteria infect erythrocytes and endothelial cells, leading to persistent infections of their mammalian hosts. As *Bartonella* species tend to infect the blood of their vertebrate hosts chronically, these microparasites can be ingested and potentially transmitted by blood-feeding arthropods. Confirmed vectors of *B. henselae* (the aetiological agent of cat-scratch disease), *B. bacilliformis*, *B. quintana*, *B. grahamii* and *B. taylorii* are *Ctenocephalides felis*, *Lutzomyia verrucarum*, *Pediculus humanus humanus* and *Ctenophthalmus nobilis*, respectively (Billeter *et al*., 2008). *Bartonella* bacteria have been detected – based mainly on PCR – in several tick species, including *I. ricinus*, *I. scapularis*, *I. persulcatus*, *D. reticulatus*, *Rh. sanguineus* and *Carios kelleyi* (Billeter *et al*., 2008). Some of the reasons why *Bartonella* species might be transmitted by ticks have been listed by Telford and Wormser (2010): other arthropods can transmit *Bartonella* species; the DNA of *Bartonella* species is often detected in ticks; human cases of bartonellosis preceded by tick bites are on record; and *Bartonella* species are commonly present in important hosts of *Ixodes* ticks, i.e. deer and rodents.

The mere detection of *Bartonella* DNA by PCR in blood-feeding arthropods is no evidence that these bacteria are viable and infective, or that these arthropods are competent vectors of *Bartonella* species. In fact, there is no conclusive evidence that any *Bartonella* species can, under natural circumstances, infect a vertebrate via tick bite, although a recent laboratory investigation using a membrane feeding technique suggested that *I. ricinus* is a competent vector for *B. henselae* (Cotté *et al*., 2008). Trans-stadial transmission of the bacteria, their multiplication within the tick's salivary glands after a second blood meal, and transmission of viable and infective *B. henselae* from ticks to blood were recorded. However, this study may not be relevant to establishing the vector competence of ticks for bartonellae as certain of its parameters were unnatural: the ticks were fed continuously on blood containing exceedingly high numbers of bacteria; and the strain of *B. henselae* used is highly adapted to laboratory conditions and grows easily *in vitro*. As suggested by Telford and Wormser (2010), a more reliable proof of vector competence would be to feed an uninfected *Ixodes* sp. on a *B. henselae*-infected cat and then, after the tick has hatched, determine whether the nymph can transmit *B. henselae* by bite to an uninfected cat. However, additional (epidemiological) data would be necessary to conclusively prove that ticks are of importance as natural vectors of bartonellae.

4.11.3 Q fever

The aetiological agent of Q fever is the rickettsial parasite, *Coxiella burnetii*. This is an obligate intracellular bacterium, although it can survive for months or years outside host cells in such media as water, dried or frozen tissues and soil. The organism has been described as a possible biological weapon (Madariaga *et al*., 2003). Ticks are one of a broad range of reservoirs for the organism. More than 40 species of ticks including *D. marginatus*, are naturally infected with *C. burnetii*. After feeding on bacteraemic hosts, nymphs or adults can transmit the pathogen trans-stadially, and females can pass it transovarially (Lang, 1990; Toledo *et al*., 2009a).

Q fever is a worldwide zoonosis affecting mammals (including domestic animals), birds and arthropods in most areas in the world, including Europe (Aitken *et al*., 1987; Lang, 1990). The most common reservoirs for human infections are cattle, sheep and goats. Dogs, cats, birds and reptiles are also susceptible to infection and may play a role in maintaining the infection in natural habitats. Although *C. burnetii* does not usually cause clinical disease in animals, it occasionally

induces reproductive disorders such as stillbirths, abortion and metritis in pregnant goats and sheep, but is rarely documented in dairy cows (Aitken *et al*., 1987; Lang, 1990). Infected animals develop high rickettsaemias and excrete large numbers of *C. burnetii* in their faeces for weeks, as well as shedding organisms via tissues and fluids.

The disease is epidemic, especially on farms or in farming communities when infected domestic animals are being handled, such as during wool shearing, lambing, calving and slaughtering. Therefore, farmers, abattoir workers, meat-packing workers, veterinarians and laboratory workers in contact with livestock are at high risk of infection. Humans can also be infected by inhalation of the organism or by the ingestion of infected milk and/or fresh dairy products. The organism may survive in contaminated milk and butter for up to 3 months. Close contact with infected animals is not required; infection may be from contaminated dust, straw or manure, as was reported for an outbreak of Q fever in an urban area in southern Wales, UK (Ayres *et al*., 1996). After an incubation period of about 20 days, Q fever is characterized by mainly limited flu-like illness, pneumonia or hepatitis. It may become chronic and manifests with fatigue syndrome, chronic hepatitis, endocarditis and other endovascular infections. The fatality rate is less than 1% in acute cases but may rise to 30% in chronic cases. A scientific opinion on Q fever has been published by the EFSA Panel on Animal Health and Animal Welfare (2010b).

4.11.4 Tularaemia

Tularaemia is a zoonotic disease caused by one of the most infectious bacteria, *Francisella tularensis*, a Gram-negative obligate intracellular agent (Ellis *et al*., 2002). This bacterial species causes great concern as a potential bioterrorism agent, and is listed among Class A biothreat agents (Dennis *et al*., 2001; Oyston *et al*., 2004). *F. tularensis* can be recovered from contaminated water, soil and vegetation. Four closely related subspecies of *F. tularensis* have been identified: *F. t. tularensis*, *F. t. holarctica*, *F. t. mediasiatica* and *F. t. novicida* (Forsman *et al*., 1994). Subspecies *F. t. tularensis* and *F. t. holarctica* cause most human illness. *F. t. holarctica* is found throughout much of Europe except for the UK, Ireland and Iceland. Tularaemia is typically a disease of northern and central Europe and the countries of the former Soviet Union (Ellis *et al*., 2002; Tarnvik *et al*., 2004). Natural infections with *F. tularensis* have been reported in over 250 animal species and in a range of vertebrates, including mammals, birds, amphibians and fish, as well as in certain invertebrates (Morner and Addison, 2001; Oyston *et al*., 2004; Santic *et al*., 2006). However, tularaemia is primarily a disease of the orders Lagomorpha (rabbits and hares) and Rodentia (Friend, 2006). The European brown hare *(Lepus europaeus)* is a common host of *F. tularensis* in central Europe, where it causes a public health problem (Morner and Addison, 2001; Strauss and Pohlmeyer, 2001; Pikula *et al*., 2004). In Scandinavia and Russia, tularaemia occurs frequently in mountain hares (*L. timidus*) (Morner and Addison, 2001).

Haematophagous arthropods such as deer and horse flies, ticks and mosquitoes are common vectors of *F. tularensis* (Petersen *et al*., 2009). Ticks are believed to be the most important arthropods for *F. tularensis* as both mechanical and biological vectors (Hopla and Hopla, 1994). *D. reticulatus* plays an important role in the maintenance and transmission of *F. tularensis* among small and medium-sized mammals in central Europe (Guryčová *et al*., 1995, 2001; Hubalek *et al*., 1996). Other ticks, such as *I. ricinus*, *I. persulcatus*, *D. marginatus*, *Rh. rossicus* and *Ha. concinna*, have also been found to be naturally infected with *F. tularensis* in Europe (Hopla and Hopla, 1994; Keim *et al*., 2007).

Tularaemia, in a range from subclinical infection to death, rarely occurs among domestic animals, and is most frequently observed in sheep during the lambing season, and in cats and dogs (Friend, 2006; O'Toole *et al*., 2008). Humans are highly susceptible to *F. tularensis*. Infections in humans are typically sporadic, but outbreaks do occur (Matyas *et al*., 2007). The disease is not contagious and is most often transmitted to humans by the bite of an infected arthropod vector, by direct contact with infected

animals (e.g. voles, mice, water rats, squirrels, rabbits and hares), by contact with infected animal tissues or fluids, by ingestion of contaminated water or food, or by inhaling infected materials (Dennis *et al*., 2001). Several emergences or re-emergences of tularaemia have recently been seen all over the world (Petersen and Schriefer, 2005): the highest incidences occurred in confined geographical areas of Finland and Sweden (Eliasson *et al*., 2002), and the disease also appeared in Spain (Perez-Castrillon *et al*., 2001), Kosovo (Reintjes *et al*., 2002) and Germany (Kaysser *et al*., 2008).

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