LYME BORRELIOSIS AND OTHER IXODID TICK-BORNE DISEASES – A EUROPEAN PERSPECTIVE

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IXODID TICKS IN EUROPE, THEIR GEOGRAPHIC DISTRIBUTION AND ECOLOGICAL REQUIREMENTS

Ticks of the *Ixodes ricinus* complex are vectors of *Borrelia burgdorferi* and several other bacterial and viral infectious agents. In Europe the main tick vector is *Ixodes ricinus*, commonly called the sheep tick or castor bean tick, and in Asia it is *Ixodes persulcatus*, the taiga tick. There is an area of overlap in the range of these species in parts of eastern Europe including the Baltic republics and western regions of Russia. (Map from EUCALB). *Ixodes ricinus* is widely distributed, from countries on the western seaboard eastwards to Russia and it overlaps with *I. persulcatus* in western Russia, the Baltic republics and eastern Europe.

**FIGURE 1** Distribution of *Ixodes ricinus* complex ticks *(Courtesy of Professor J Gray and Mr B Kaye)*
Ixodes ricinus ticks have three active stages in their life-cycle, usually over two to three years, and they take a single blood meal at each stage lasting from about three to seven days. (European Union Concerted Action on Lyme Borreliosis [EUCALB] website, 2010) There is a high mortality throughout the process, with few ticks surviving to complete the life-cycle from an initial egg batch of about 2,000. The essential habitat requirements for tick survival are high humidity to maintain water balance and presence of suitable animal species as feeding hosts. Ticks survive only in areas where there is good vegetation cover, with a mat of decaying vegetation (leaf litter etc) that will maintain a relative humidity of 80-85% during the driest periods, providing protection against desiccation during the long interstadial development periods. Immature ticks can feed on a wide variety of mammalian and ground-feeding avian hosts which may be reservoir-competent for Borrelia burgdorferi and other potential human pathogens, whereas adult female ticks usually feed successfully only on large mammals such as deer, sheep, cattle and horses, underlining the importance of these hosts to the reproductive stage of the tick life-cycle.

These essential requirements are optimally provided in mixed deciduous woodland. They can also occur in coniferous forests provided that there is enough vegetation litter and a moist microclimate. Some heathland, moorland and pastureland habitats of regions with mild, damp climates, such as the British Isles, also provide suitable conditions and in these environments large animals are likely to be feeding hosts for all three stages of the tick life-cycle. (Gray JS, 1991; Gray JS, 1998) Many areas in southern Europe, are too hot and dry for survival of Ixodes
Areas experiencing repeated droughts or episodes of severe flooding are also less favourable for tick survival.

There is evidence for changing distribution of ixodid tick populations in some parts of Europe, which may be related to changing climate, and are demonstrated most significantly at the geographic distribution limits of *Ixodes ricinus*. Few ticks are found in altitudes greater than about 1100 metres, but this altitude limit has risen significantly over 30 years from an earlier maximal altitude of 700 metres, as shown by well-documented studies in the Czech Republic and Switzerland. (Lindgren E, Jaenson T, 2006) An extension in the northerly distribution of ixodid ticks into higher latitudes has occurred in Scandinavia in the past 20 years, associated with less severe winter temperatures and a greater number of days with temperature >10°C.

More generally in Europe, changing climate has led to milder and shorter winters in many regions with earlier onset of spring (on average two weeks earlier than seen before the 1980s) and longer autumns, leading to earlier start of tick feeding activity and potentially greater tick survival. Conversely, conditions will become less favourable for ixodid ticks if areas of hotter, more arid conditions expand in southern Europe. Tick survival and activity is also affected by more localized and short-term weather conditions. Other ecological aspects include changes in biodiversity. The possible effects of these and other factors on *Ixodes ricinus* and Lyme borreliosis in Europe were addressed in a World Health Organisation publication in 2006, and this continues to be an important area of research. (Lindgren E, Jaenson T. 2006) The summary of findings and recommendations of a 2007 workshop on environmental change and infectious diseases disease burden in Europe, organised by the European Centre for Disease Control (ECDC) is another valuable data source, as is a review published in 2009. (ECDC 2007; Gray JS et al 2009)

Other factors contributing to increased tick populations include changes in land use, including agriculture and forestry practice, with reforestation projects and pine monoculture being replaced by mixed forestry in many parts of Europe. Increased deer population densities are reported from many areas, which can also promote tick reproductive success, as deer are the most important feeding hosts for ticks’ reproductive lifecycle stage. Wider geographic range of deer caused by deer population pressure has also resulted in expansion of tick populations into new areas in many parts of Europe. A report from the WHO Regional Office for Europe published in 2004 provided a comprehensive survey of vector-borne diseases, including tick-transmitted infections, and ecological, environmental and human behavioural factors influencing their incidence. (WHO 2004)
CRITICAL RESEARCH NEEDS IN TICK-BORNE DISEASE
Human factors must be considered when assessing risks of tick-transmitted infections. These include residential, occupational and recreational factors. People living and working in tick habitats are at obvious risk of acquiring tick-transmitted infections. Housing developments in previously semi-rural and rural environments can expose new populations to these risks, as has been seen in many parts of Europe. Occupational risks include forestry and game management, and there have been significant changes in these industries in the past fifty years. Recreational aspects are important, with the high and increasing popularity of outdoor activities in tick-permissive environments, which can expose participants with little previous awareness of ticks to risk of tick-transmitted infections. Epidemiological studies from various European countries suggest that recreational activities, including those undertaken on vacation in other countries, are major factors for acquisition of Lyme borreliosis. This can have important economic implications for tourism in endemic areas.
Human beings can be incidental hosts for all three stages of the *Ixodes ricinus* lifecycle, although in practice the nymphal stage feed is the most likely to result in transmission of *Borrelia burgdorferi*, which is the most common tick-transmitted infection, as the organism rarely infects larval ticks transovarially. Few ticks survive to adulthood and in general only female adults take significant feeds. Because of their larger size adults are more likely to be noticed and removed earlier in the feeding period than earlier-stage ticks. The immature stages of *Ixodes persulcatus* appear not to feed readily on human beings and most transmission of infectious agents from this *Ixodes* species results from adult feeds. (Korenburg E et al, 2001) Human behavioural factors related to tick-transmitted diseases are addressed in more detail in a later section.

Ixodid feeding activity is affected by several factors, including diapause (dormancy) mechanisms, day-length, temperature and availability of hosts. (EUCALB website) These latter features produce some variations throughout Europe. In general *Ixodes ricinus* ticks feed between March and October, peaking in May to July, with a smaller secondary peak in the early autumn, but in countries with mild winters there can be a low level of feeding activity and potential risk of infection on warmer winter days. *Ixodes persulcatus* appears to have a similar level of activity in spring and early summer but is rarely active in autumn. Tick feeding seasonality affects the epidemiology of tick-transmitted infections, with peak incidence of tick-borne encephalitis in the late spring and early summer months. Lyme borreliosis presentations peak slightly later, reflecting the longer incubation period.

**FIGURE 4** Stylized Seasonal Activity of *I. ricinus* in different habitats: Larvae

*Courtesy of Dr Jeremy Gray*

- **solid line = spring population**
- **broken line = autumn population**
- **a = exposed meadow**
- **b = dense hill vegetation or secondary deciduous woodland**
- **c = highly sheltered woodland**
- **d = spring-derived but autumn-feeding**
FIGURE 5 Stylized Seasonal Activity of *I. ricinus* in different habitats: Nymphs

Stylised seasonal activity of *I. ricinus* in different habitats

- solid line = spring population
- broken line = autumn population
- a = exposed meadow;
- b = dense hill vegetation or secondary deciduous woodland;
- c = highly sheltered woodland;
- d = spring-derived but autumn-feeding.

FIGURE 6 Stylized Seasonal Activity of *I. ricinus* in different habitats: Adults

Stylised seasonal activity of *I. ricinus* in different habitats

- solid line = spring population
- broken line = autumn population
- a = exposed meadow;
- b = dense hill vegetation or secondary deciduous woodland;
- c = highly sheltered woodland;
- d = spring-derived but autumn-feeding.
Ixodid Ticks and Micro-Organism Carriage

A variety of micro-organisms have been identified in *Ixodes ricinus* and *I. persulcatus* ticks but organism carriage or DNA positivity must be distinguished from vector-competence. To establish vector-competence a tick must be capable of maintaining organisms obtained at an earlier feed or transovarially and able to transmit them during a subsequent feed.

*Ixodes ricinus* and *I. persulcatus* are known to be vector-competent for the flavivirus agents of tick-borne encephalitis virus and louping-ill. The latter is a well-recognised pathogen of sheep, cattle, goats and grouse, and also causes rare cases of human disease in the UK and Ireland. Several other viruses have been identified in *Ixodes ricinus*, including Tribec, Tettnang and Eyach viruses, but the public health importance of these agents seems to be very limited. (WHO 2004)

Tick-borne encephalitis is focally endemic in many parts of western, central and eastern Europe and in southern Scandinavia. It is mandatorily notifiable in most states in which it occurs and between 2,000 and 3,000 cases are reported annually from European countries, including the Baltic states. It has an estimated mortality of between 0.5% and 2%, and significant long-term morbidity following meningoencephalitis, especially in older people. There is evidence of increased range and incidence into higher latitudes and altitudes in some regions, which may in part be due to changing climate, but other biological and human behavioural factors also play significant roles. Studies suggest that in the long-term the incidence may decrease in the more southerly regions as climate change alters tick seasonal dynamics, disrupting synchrony of larval and nymph co-feeding on rodent reservoirs. Co-feeding appears to be an important factor in maintaining enzootic cycles of TBE. (Randolph SE. 2001; ECDC Workshop 2007) An effective TBE vaccine is available.

*Ixodes ricinus* is vector-competent for *Borrelia burgdorferi* sensu lato, the causes of Lyme borreliosis, which is by far the most common tick-transmitted disease in Europe. European Lyme borreliosis will be described in more detail later. It is also a vector for *Borrelia miyamotoi*, a member of the relapsing fever group of borreliae, which was first identified in Japan in 1995 and has been found in tick populations in many parts of Russia and Europe. At present it is unclear if this organism has human pathogenic potential. (Karan LS et al. 2007)

*Anaplasma phagocytophilum* and rickettsioses, including *Rickettsia helvetica* are also tick-transmitted. *Anaplasma phagocytophilum* is widely distributed in Europe and livestock infections are common, causing a significant financial burden. (Bown KJ et al. 2009) Fewer than 100 human cases have been reported since the first European case was identified in Slovenia in 1997. Seroprevalence studies have found antibodies in 1.5-21% of forestry workers and other tick exposed people in northern and central Europe but significant systemic disease appears to be very uncommon. (Parola P et al. 2005).

*Rickettsia helvetica* was first isolated from *Ixodes ricinus* ticks in Switzerland in 1979 and subsequently identified in many European countries. Few human cases of clinical disease have been serologically confirmed, presenting with relatively mild, self-limited illnesses with headache and myalgias and less frequently with a rash and/or an eschar. A condition characterised by eschar, usually on the scalp accompanied by regional lymphadenopathy following bites from *Dermacentor spp* ticks have been documented from France and Hungary, where the condition has been termed tick-borne lymphadenopathy (TIBOLA). It is caused by *R*
slovaca, which is widely distributed in Dermacenter spp ticks in Europe, and was first isolated in 1968. With molecular methods allowing more sensitive detection and refinement of speciation the range of rickettsial agents associated with Ixodes spp and other tick species is likely to increase, as extensively reviewed in 2005. (Parola P et al. 2005b)

Ixodes ricinus can also transmit Babesia spp, which are intraerythrocytic protozoa. Babesia divergens is the cause of redwater fever in cattle and also causes occasional cases of human disease, which can be overwhelming in asplenic or otherwise immunocompromised patients. About 40 cases have been reported in Europe in the past ten years, and numbers are likely to rise with increased awareness of tick-transmitted infections and rising numbers of potentially susceptible individuals. A few cases of Babesia venatorum (previously termed Babesia-EU1) infection have been reported in splenectomised patients, causing less severe clinical presentations than those seen with B. divergens. This organism and several Babesia divergens-like strains have been identified in European deer. A human case of European-acquired Babesia microti infection was reported in 2007 and the species has been identified in ticks and animal reservoirs in several regions of Europe. Data on babesial infection has recently been reviewed and it is likely that other babesial species and reservoir hosts will be identified. (Gray JS et al. 2010)

Francisella tularensis, the agent of tularemia, and Coxiella burnetii, which causes Q fever, can also be transmitted to human beings through tick bites, but other transmission routes are more important for these organisms. The authors of the 2004 WHO report on vector-borne diseases in Europe concluded that a large outbreak of tularemia in Kosovo that occurred in 1999-2000 was associated with food or water contamination from a rodent source, related to post-war disruption and poor living conditions. Another outbreak in north-central Sweden in 1981 was thought to be related mainly to transmission by mosquitoes. Sporadic cases of tick-transmitted tularemia are well-documented, particularly in eastern Europe and Scandinavia, highlighting the importance of raising public and healthcare worker awareness of this risk to enable early recognition and treatment. Tick transmission may be significant in maintaining enzootic infection cycles of both tularemia and Q fever.

There is debate about the vector-competence of ixodid ticks for Bartonella spp including Bartonella henselae, the agent of catscratch disease. Bartonellae are common mammalian haemoparasites and there is increasing appreciation of the range of clinical presentations found in human infections. Bartonella DNA has been identified in many tick species, including Ixodes ricinus. This is not surprising, given the significant prevalence of Bartonella spp infection in rodent tick-feeding hosts, but transmission to human beings from I. ricinus or I. scapularis has not been proven. Only one study, published in 1996, showed successful culture of a Bartonella species from an I. ricinus tick, suggesting that the organisms may not easily remain viable in ticks. The issue was recently reviewed in some detail and on currently available evidence it appears that Ixodes spp ticks are very unlikely to be significant vectors of bartonellosis. (Telford III SR, Wormser GP, 2010; Angelakis E et al. 2010)

**Borrelia Burgdorferi Sensu Lato in Europe**

At least fifteen genospecies of Borrelia burgdorferi sensu lato have been identified and three cause the bulk of Lyme borreliosis in Europe: Borrelia afzelii, Borrelia garinii and Borrelia burgdorferi sensu stricto. (EUCALB website) Borrelia garinii OspA serotype 4 has
recently been designated *B. bavariensis*. Another genospecies, *B. spielmanii*, has been isolated occasionally from erythema migrans lesions but seems to cause little systemic ill-effects. *Borrelia valaisiana* appears to be non-pathogenic and is found in many parts of Europe, including the UK and Ireland, where it has been identified as the major infecting borrelia in some tick populations. This may account in part for the lower incidence of Lyme borreliosis in these countries by comparison to other European regions, where most infected ticks carry more pathogenic genospecies. *Borrelia lusitaniae* is rare, with foci mainly on the Iberian peninsula, and there have been only a few reports of associated human disease.

Many mammalian species are reservoir-competent for *Borrelia burgdorferi* sensu lato. The most important are rodents, particularly *Apodemus spp* mice, voles and squirrels. *Borrelia afzelii* is strongly associated with these species, and *B. bavariensis* (previously *B. garinii* OspA serotype 4) with *Apodemus spp*. Birds, particularly ground-feeding species such as thrushes, blackbirds and pheasants are potential reservoirs of *B. valaisiana* and *B. garinii*. Some lizard species appear to be reservoir-competent for *B. lusitaniae*. (EUCALB website): Ungulates (deer, sheep, goats, cattle and pigs) are crucially involved in the eco-epidemiology of Lyme borreliosis as maintenance hosts for ticks, but they are not significant as reservoir hosts.

The mammalian and avian reservoir host differences for *B. afzelii* and *B. garinii* are thought to be linked to differences in sensitivity of these genospecies to the host serum complement. Borrelial complement regulator-acquiring surface proteins (CRASPs) bind host immune regulators that protect spirochaetes from complement lysis. The CRASP repertoire of *B. garinii* protects the spirochaete from avian complement lysis, whereas the CRASPs of *B. afzelii* and *B. bavariensis* protect these organisms from lysis by rodent sera. (Piesman J, Schwan TG. 2010)

Geographic distribution of different European genospecies has some effect on incidence and distribution of various clinical presentations of Lyme borreliosis in different parts of the continent. A useful meta-analysis based on publications between 1986 and 2003 summarised tick infection rates and genospecies identified in studies from 24 European countries. (Rauter C, Hartung T. 2005) All pathogenic genospecies can cause erythema migrans *B. burgdorferi* sensu stricto is arthritogenic and causes disease presentations similar to those found in the USA, but is the least common of the major pathogenic genospecies in Europe. Lyme arthritis is a less frequent European complication than neuroborreliosis, predominantly caused by *B. garinii*, the most neurotropic genospecies which is widespread, particularly in western Europe. The most common genospecies in central and eastern European countries and Scandinavia is *B. afzelii*, which causes erythema migrans lesions that are less rapidly progressive and have less evidence of inflammatory response than those caused by *B. burgdorferi* sensu stricto or *B. garinii*. They are also less likely to have extracutaneous manifestations, but can cause some cases of neuroborreliosis and acrodermatitis chronica atrophicans, an indolent gradually progressive skin condition which may persist for years if left untreated.

**Epidemiology of Lyme Borreliosis in Europe**

There is no centralised reporting or surveillance system for Lyme borreliosis or tick-borne encephalitis in Europe. A ECDC-funded initiative is underway to collate all currently available data on Lyme borreliosis and will report during 2011. It aims to provide a pan-European assessment of the epidemiological patterns, laboratory diagnostic and reporting criteria
and overall impact of Lyme borreliosis on human populations throughout the EU and EFTA countries. Data is also being sought from the current EU Candidate countries and from a number of European Neighbourhood Policy countries. A similar programme is underway for TBE.

Epidemiological evidence for Lyme borreliosis is available piecemeal from numerous sources, including national or regional mandatory notification schemes in a few countries, surveillance schemes in some endemic regions, primary care surveys, seroprevalence studies and reporting systems based on laboratory-confirmed cases. About 85,000 cases are reported annually in Europe but this is a considerable underestimate, both because of inconsistent case reporting mechanisms and under-recognition of disease manifestations, particularly erythema migrans. (Lindgren and Jaenson 2006) In 2002 it was estimated that at least 60,000 cases are likely to occur annually in Germany alone, giving an approximate incidence rate of 75/100,000 in that country. (Mehnert WH, Krause G. 2005) Reviewing data from various sources it is likely that there are over 200,000 cases annually in Europe, with a bimodal age incidence, peaking in the 5-15 and 45-65 age groups.

Overall national figures have only limited value, especially in the larger, more industrialised countries where most of the population is urban-dwelling, as they do not indicate regional and sub-regional variations in risk, which can be very marked. Regional and local data analysis is important for the appropriate targeting of public health and clinical interventions.

**Mandatory Notification Schemes**

Few countries have mandatory notification schemes for Lyme borreliosis. Erythema migrans and other manifestations of the disease are mandatorily notifiable in Slovenia, with a reported incidence rate in 2005 of 206/100,000. (Smith R, Takkinen J. 2006) Notifications are incomplete, especially for erythema migrans, but data related to disseminated and late complications are more accurate because most Slovenian patients with these presentations are managed within a few research-orientated institutions. Neuroborreliosis has been notifiable in Denmark since 1994; with an annual average of 83 cases (1.5/100,000), ranging from 41 in 2002 to 104 in 2006. (Christiansen AH, Molbak K. 2005) Cases of disseminated and late borreliosis have been notifiable in Norway since 1995. Annual incidence of neuroborreliosis varied from 75 to 200 cases in the ten years 1995-2004 (average 3/100,000), with a marked increase of nearly 100 cases between 2003 and 2004. (Nygard K et al. 2005) As neurological complications are the most significant manifestations of disseminated and late Lyme borreliosis in Europe data on neuroborreliosis obtained from the Slovenian, Danish and Norwegian notification schemes can give useful information on epidemiological trends in widely geographically separated areas of Europe. Pan-European monitoring methods for neuroborreliosis would be a welcome epidemiological initiative.

**Regional Clinical Surveillance and Prospective Studies**

In some countries case surveillance is regionally focussed on areas of known high endemicity, e.g. Alsace and Limousin in France and in six eastern states of Germany. (Institut Veille Sanitaire; 2008; Mehnert WH, Krause G. 2005; Fulop B, Poggensee G. 2008) A French national primary care-based prospective study estimated an overall national incidence rate of 9.4/100,000, (Letrilliart L et al. 2005), whereas data from the Alsace study suggested a regional rate of 180-232/100,000, which varied from 30 to 511/100,000 between individual cantons in the
region. Erythema migrans was the only manifestation of disease in 90% of the cases; a further 5% had evidence of neuroborreliosis. Similar detailed and useful study reports are available for several other regions of France from L’Institut Veille Sanitaire. A prospective study performed in the Wurzburg region of Germany in 1996 followed an extensive awareness campaign and reported an incidence rate of 111/100,000 (313 cases). Erythema migrans was the only manifestation in 89% of cases. (Huppertz HI et al. 1999) It is notable that in these and other recent prospective studies erythema migrans was the presenting feature in around 90% of cases. In an earlier primary care-based prospective study performed in southern Sweden in 1992-1993 the overall annual incidence was 69/100,000 (1471 cases) and ranged focally from 26 to 160/100,000.(Berglund J et al. 1995) Erythema migrans was the presenting feature in 77% of patients; 16% had neuroborreliosis and 7% had arthritis. Prospective community-based studies can provide longer-term benefits in addition to their epidemiological value, through raising awareness of the condition, its clinical features, management and prevention within primary and secondary care health care providers and the general community.

Laboratory Based Surveillance

Some countries use laboratory-based surveillance, and erythema migrans cases are certainly under-reported in these schemes. Variability in test requesting patterns and diagnostic methods limit the validity of direct comparisons of laboratory-based surveillance findings between countries. Nevertheless, some useful demographic, geographic and seasonality data can be obtained and year-on-year data compared in stable data collection systems, especially in schemes that approach referring clinicians and patients for additional clinical and tick exposure risk information such as the enhanced surveillance system in England and Wales, where the great majority of specialised laboratory tests for Lyme borreliosis are performed in a single reference facility. Annual incidence of laboratory-confirmed cases rose from 268 (0.5/100,000) in 2001 to 812 (1.52/100,000) in 2008, with a rate of 15/100,000 in one focal area. Neuroborreliosis accounts for between 10% and 20% of laboratory-confirmed cases each year and appears to be a useful sentinel for year-on-year comparison. It has been estimated that there may be 2,000-3,000 cases of Lyme borreliosis annually in the UK. (Health Protection Agency, 2010)

Seroprevalence studies have been performed in many parts of Europe. Population groups studied include healthy blood donors and people whose residence, occupation or recreational interests place them at higher risk of acquiring Borrelia burgdorferi infection. The overall picture shows a trend of increasing seroprevalence from west to east in Europe, which is consistent with findings from prospective studies and other surveillance methods. The findings from some of these studies and the prospective studies also suggest significant incidence of asymptomatic infections.

Clinical Presentations of Lyme Borreliosis in Europe

Clinical case definitions for use in Europe were published in 1996 by the European Union Concerted Action on Lyme Borreliosis (EUCALB).(Stanek G et al. 1996) A recent review by the EUCALB group has affirmed the robust nature of the 1996 definitions, as more recently published evidence has necessitated only minor additions to the definitions, which were updated in 2010.(Stanek G et al. 2010) The 1996 case definitions have been cited in various diagnosis and treatment guidelines and recommendations from European specialist societies and national
groups, summarised in a presentation at the 2010 European Conference on Clinical Microbiology and Infectious Diseases (ECCMID). (O’Connell S. 2010)

The EUCALB case definitions acknowledge similarities between the major manifestations of Lyme borreliosis and North America, including erythema migrans, early neuroborreliosis and Lyme arthritis. They also recognise the broader spectrum of clinical presentations seen in Europe, eg borrelial lymphocytoma, acrodermatitis chronica atrophicans and late encephalomyelitis, all of which are rarely reported in association with American-acquired infections. The 2010 case definitions also describe rare ocular manifestations, including conjunctivitis, uveitis and papillitis and discuss objective and subjective long-term sequelae of *Borrelia burgdorferi* infection. They describe the requirement for laboratory supporting evidence for the diagnosis of all manifestations other than erythema migrans. A brief resume of the principal features is given here.

**Erythema Migrans in Europe**

The variety of pathogenic borrelial genospecies in Europe can cause some variation in presentations of erythema migrans. For example a rash caused by *B. afzelii* usually expands more slowly and is more likely to have central clearing than one caused by *B. burgdorferi sensu stricto*, and less likely to be accompanied by significant systemic symptoms. (Strle F et al 1999) Erythema migrans caused by *B. garinii* is usually more homogeneous and less annular than that caused by *B. afzelii*, and it is more frequently accompanied by systemic symptoms. Overall the clinical picture of *B. garinii* infection suggests greater acute pathogenicity than caused by *B. afzelii*.

**Other Skin Manifestations of European Lyme Borreliosis**

Borrelial lymphocytoma is an uncommon early manifestation, presenting as a bluish-red nodule or plaque, usually on the earlobe, ear helix, nipple or scrotum, occurring more frequently in children than adults. It has a distinctive histological appearance, with an intense B- lymphocytic infiltrate and has occasionally been misdiagnosed as cutaneous B-cell lymphoma.

Acrodermatitis chronica atrophicans is an uncommon later manifestation of active infection, which is usually seen in older adults, predominantly women. It presents with bluish-red discolouration, usually on the extensor surfaces of one or more limbs. There can be doughy swelling and atrophic changes developing later. Local involvement of peripheral nerves can cause an axonal polyneuropathy, usually presenting with predominantly mild sensory symptoms. *B. afzelii* causes the great majority of ACA presentations, which occur more frequently in Scandinavia and central Europe than in the west of the continent.

**Neuroborreliosis in Europe**

Neuroborreliosis is the most common complication of European Lyme borreliosis and most cases appear to be caused by *B. garinii*, which is the most neurotropic of the pathogenic genospecies. The European Federation of Neurological Societies (EFNS) recently published guidelines for diagnosis and treatment, giving detailed descriptions of presentations in adults and children. (Mygland A et al. 2010) About 95% of European neuroborreliosis cases present acutely, usually within twelve weeks of infection, and early neuroborreliosis is often self-
limiting. The most common manifestation in adults is a painful meningoradiculitis (Garin-Bujadoux-Bannwarth syndrome). Pain may be very severe and paresis can affect muscles innervated by the facial (unilateral or bilateral) or other cranial nerves and those of the trunk and limbs. In children the most common presentations of acute neuroborreliosis are facial palsy, which may be an isolated clinical feature, other cranial nerve palsies and lymphocytic meningitis, and headache can be a prominent feature. Painful radiculopathy is very uncommon in children.

Although the differences between presentations of European and American Lyme neuroborreliosis have been stressed over the years, they may have been overemphasised in the case of early neuroborreliosis. (Halperin J, 2008) This is also supported by clinical experience in the UK, where between 10 and 20% of patients with serologically confirmed Lyme borreliosis acquired infections abroad, in mainland Europe or USA. Clinicians in the UK have noted marked similarities in acute neurological presentations of patients with USA- acquired infection and those acquired in the UK and other parts of Europe.

Less than 5% of European neuroborreliosis patients present with late neuroborreliosis, with duration of symptoms from six months to several years. (Mygland et al, 2010) This condition is likely to have a chronic course if left untreated and can affect the central and peripheral nervous systems.

Central nervous system manifestations of late neuroborreliosis include encephalitis or encephalomyelitis with tetraspastic syndrome, spastic-ataxic gait disorder and disturbed micturition, which may lead to misdiagnosis with other conditions such as multiple sclerosis if the possibility of neuroborreliosis is overlooked. Clinical awareness of this possibility is crucial, as antibiotic treatment will arrest progression. The degree of clinical recovery following microbiological cure depends on the severity of tissue damage. Recovery may be slow, especially in older patients, and can be incomplete, particularly in those who had been severely affected prior to treatment.

Peripheral nervous system manifestations include radiculopathy and mononeuropathy. Occasional patients, mainly in the older age groups, present with radiculopathy of gradual onset, progressing over many months and resulting in severe debilitating pain. This most commonly affects a lower limb and can be misdiagnosed as nerve entrapment conditions such as sciatica. The patient may not be aware of, or may have forgotten an earlier tick bite or erythema migrans. It is important that clinicians are aware of this condition, as antibiotic treatment usually brings rapid reduction in pain. It has been suggested that this more slowly evolving manifestation of radiculopathy may be related to direct spread of borreliae from the inoculation site along nerves to the nerve roots. (Rupprecht TA et al. 2008) A polyneuropathy can also occur in association with acrodermatitis chronica atrophicans, which is an uncommon late manifestation of cutaneous B afzelii infection.

**Lyme Arthritis in Europe**

Lyme arthritis is less prominent a feature of Lyme borreliosis in Europe than in the USA although myalgias and arthralgias frequently occur in early disease Borrelia burgdorferi sensu stricto, which is less prevalent in Europe than B afzelii or B garinii, appears to be the predominant cause of Lyme arthritis, which occurs most frequently in areas of Europe where this genospecies is most prevalent. The clinical and laboratory findings and outcomes are similar to
Diagnostic Tests for Lyme Borreliosis in Europe

The EUCALB case definitions, EFNS guidelines for neuroborreliosis and other European guidelines and consensus documents recommend that laboratory support should be sought for the clinical diagnosis of all manifestations of Lyme borreliosis other than erythema migrans, as clinical features of later stage presentations are not unique to Borrelia burgdorferi infection. (Stanek G et al. 2010; Mygland A et al. 2010; O’Connell S, 2010) In all cases the clinical presentation and tick exposure risk should be carefully evaluated and tests performed only on patients in whom there is a significant likelihood of Lyme borreliosis, i.e. the pre-test likelihood of infection should be evaluated. In recent years there has been a tendency for “tests for Lyme disease” to be included as part of a broad serological investigation panel for patients with a wide range of clinical presentations, without adequate consideration of its appropriateness in the individual patient’s case. Indiscriminate testing without significant clinical indications can lead to misleading results, as the positive predictive value in such circumstances is low.

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) published guidelines for the laboratory diagnosis of bacterial tick borne diseases in Europe, including Lyme borreliosis, in 2004. (Brouqui P et al, 2004) The German Society of Hygiene published recommendations for test use and performance in 2000. (Wilske B et al, 2000). These have been widely used in Europe. Testing for Lyme borreliosis in Europe as recommended by these authorities has many similarities to standard practices recommended in North America and a recent publication gives an excellent overview of the issues in European and American infections. (Wang G et al, 2010)

Antibody detection is the most widely available and useful method and there have been significant improvements in both sensitivity and specificity of tests in recent years, particularly with developments in recombinant antigens derived from the major pathogenic genospecies. Direct testing methods using culture or DNA detection have more limited practical value, similar to the situation in North America. (Wilske B et al, 2007; Aguero-Rosenfeld M et al, 2005)

The greater heterogeneity of pathogenic genospecies in Europe must be considered when evaluating test methods. In the case of DNA detection, borrelial DNA targets should be capable of detecting all pathogenic genospecies. A variety of target sequences are currently used in Europe, including those based on OspA, flagellin, 16s RNA and 5S-23S rRNA gene intergenic spacer region. Sensitivity of the method is similar to that of culture on tissues (about 70% overall for erythema migrans and as high as 90% for ACA). In neuroborreliosis only about 10-30% of DNA detection tests on CSF are positive, and highest rates are obtained on samples taken within the first two weeks of a clinical presentation. It is considerably more sensitive than culture for synovial tissue and fluid, for which culture has rarely been successful. (Wilske et al 2007) Borrelial DNA detection in blood culture samples from European erythema migrans patients has a lower yield than those taken in American-acquired infections, most likely because B afzelii, the most common infecting organism, has a lower frequency of haematogenous dissemination than B burgdorferi sensu stricto and because smaller sample volumes have been used in European studies.
Several factors are significant in relation to antibody testing for European Lyme borreliosis. These include genospecies variation and also variations within genospecies; heterogeneity of immunodominant epitopes, speed of immune response development to individual infecting genospecies and duration of infection prior to testing. Generally *Borrelia burgdorferi* sensu stricto seems to cause the most acute infection presentations of the three major infecting genospecies, and immune response development is brisker than that seen in most *B. afzelii* infections, which have slower development of rash, and lower incidence of significant systemic symptoms. The immune response to early *B garinii* infection also seems to be detectable earlier than that of *B afzelii* in many cases.

Patients with prolonged infection prior to antibody testing usually exhibit a broad expansion of immune response. Patients with ACA and late neuroborreliosis are usually strongly seropositive, with reactions on IgG immunoblot to many borrelial antigens, similar to findings in European and American patients with well-established Lyme arthritis. In response to concerns regarding seronegativity in patients with late stage infection the EUCLAB case definition revision group reviewed published case reports of suspected seronegative late Lyme borreliosis. They concluded: “The diagnosis of so called ‘seronegative chronic Lyme disease’ in supposed long-standing infections is highly unsatisfactory, requiring further clinical and laboratory investigations. Seronegative late LB, if it occurs at all, is extremely rare and there have been only two reported cases of apparently seronegative ACA and one of seronegative Lyme arthritis in immunocompetent patients. There are no reliable reports of seronegative late-stage Lyme neuroborreliosis.” (Stanek G et al. CMI 2010)

Most European countries follow a two-tier antibody testing approach, similar to the American system, with a first-stage test using a sensitive screening immunoassay and second tier test to assess specificity, usually immunoblot. There is increasing interest in using more highly specific immunoassays such as those based on C6 synthetic peptide or recombinant VlsE antigens rather than immunoblots as second tests. (Nyman D et al, 2006) Assessments of this simpler approach are underway in several European centres. It would be a significant gain if this approach were found to be equivalent or superior to the traditional second-tier immunoblot system, as it involves less complex laboratory procedures and interpretation is objective, although immunoblots would still be necessary in some situations. Development of a highly sensitive and specific single tier system remains the ultimate aim.

Developments in antibody test formats, incorporating recombinant antigens (including homologous proteins from different genospecies) in immunoassays and immunoblots have increased the sensitivity of new-generation tests. These are now widely used in European laboratories and some are available on automated test platforms. Some specificity problems remain, particularly with IgM tests, including immunoblots, and false-positive IgM results frequently lead to misdiagnosis if not critically evaluated in the light of the patient’s clinical presentation. Generally IgM test use should be restricted to patients with short duration of illness and later samples tested if there is diagnostic uncertainty as to the specificity of an IgM result.

European criteria for IgG immunoblot positivity require fewer reactions to be present than the CDC criteria (ie two or three out of eight to ten candidate bands compared to five of ten in the CDC criteria) but European candidate bands exclude less specific antigens such as p41 and p60 that are included in the CDC candidates. The European criteria also reflect the slightly slower evolution of antibody response generally seen in European infections. Experts emphasise the need for strict attention to performance and interpretation of reaction (cut-off) controls, to
avoid inappropriate scoring of very weak non-specific reactions, which is a frequent cause of
false-positive immunoblots and potential misdiagnosis. High background seropositivity (between
5% and 20% in many European endemic regions) can also cause confusion and potential
misdiagnosis if the clinical significance of a result is not carefully assessed in the circumstances
of the patient’s history and clinical findings. (Wilske B et al, 2007; Stanek G et al, 2010.)

The formal diagnosis of neuroborreliosis in Europe requires CSF evaluation, including
white cell count and assessment of intrathecal antibody synthesis, including CSF/serum antibody
index, although in practice many clinicians do not perform CSF sampling routinely in patients
whose history and clinical examination are strongly indicative of neuroborreliosis and have
positive serum antibody tests. In very acute presentations of neuroborreliosis some patients may
have antibodies in CSF before seroconversion in peripheral blood. (Stanek G et al, 2010;
Mygland A et al 2010) Lymphocytic pleiocytosis is almost always present in both early and late
neuroborreliosis and many patients have raised protein and oligoclonal IgG bands. Patients with
ACA-associated neuropathy often have normal CSF as this is essentially a localised peripheral
manifestation.

Tests That Are Not Recommended For Diagnosis of Lyme Borreliosis In Europe

The EUCALB case definitions, the EFNS guidelines and numerous other European
guidelines and consensus documents do not recommend certain tests that have been marketed as
Lyme-diagnostic tests. These include live microscopy of blood, urinary borrelial antigen or PCR
tests, unvalidated antibody test methods, immunoblots interpreted using poorly specific criteria,
lymphocyte transformation (LTT) tests and CD57 lymphocyte subpopulation typing, as they lack

Outcome data of Treated Infections

Several recent publications have reviewed outcome data in adults and children treated for
various manifestations of Lyme borreliosis. A Norwegian population-based study prospectively
enrolled all children in with suspected neuroborreliosis between 1996 and 2006. (Oymar K,
Tveitnes D, 2009) All 143 children received antibiotic treatment (mainly two weeks of
ceftriaxone). Following treatment four children had minor residual facial palsy; the remainder
had recovered completely. This valuable paper gives an excellent illustration of clinical
presentations of paediatric neuroborreliosis and the associated laboratory findings, with high
rates of seropositivity and CSF pleiocytosis at presentation.

A recent Swedish prospective paediatric neuroborreliosis study of 177 children also
enrolled a healthy control group. (Skogman BH et al 2008) Outcomes were evaluated at six
months after treatment and were good, with no evidence of progressive or recurrent
abnormalities. About 10% of the children had some residual facial weakness, but no other
objective findings were present. Non-specific symptoms such as headache and fatigue were
reported less frequently by patients than controls. Antibiotic choice (doxycycline or ceftriaxone)
did not affect outcomes.

A Slovenian prospective study comparing outcomes of treatment with doxycycline or
cefuroxime axetil for erythema migrans in 285 adults also enrolled a healthy control
Outcomes were good, with no significant differences between the treatment groups, and the incidence of non-specific symptoms at six and twelve months follow-up did not exceed those of the control group. Both of these studies illustrate a significant rate of non-specific symptoms such as headache and fatigue in non-infected healthy control populations.

A Norwegian prospective double-blind study compared outcomes of oral doxycycline or parenteral ceftriaxone treatment in adults with neuroborreliosis. Patients with early neuroborreliosis, defined as pre-treatment duration of less than six months, were followed up for one year. Out of 85 patients 41 had remaining complaints (14 with objective findings, 27 with subjective symptoms). Remaining complaints were associated with longer (>6 weeks) pre-treatment duration, higher CSF cell count and female gender. Objective findings, but not subjective symptoms, were associated with pre-treatment duration of > 6 weeks, underlying the importance of early diagnosis and treatment. There were no differences in outcomes between the antibiotic treatment groups.

**Persisting Symptoms Following Treated Lyme Borreliosis**

Further work is required to understand the incidence, causes and best management of persisting symptoms following appropriately treated infection. The two European trials described above that incorporated healthy non-infected controls showed significant incidence of non-specific symptoms in the control groups, and it would be helpful if further studies on patients with a broader range of Lyme borreliosis presentations incorporated healthy control subjects, in order to provide a better assessment of the true incidence of post-treatment non-specific symptoms that are attributable to Lyme borreliosis.

Persisting symptoms are well-documented following other systemic infections, and risk seems to correlate with severity of symptoms during the acute events. Studies in patients with continuing symptoms following Lyme borreliosis have not shown evidence of persisting infection nor of sustained benefit from extended antibiotic treatment. Further research is required to review possible causes including immunological mechanisms. A recent publication of a study on samples from patients with persisting symptoms provided some intriguing data on heightened reactivity of anti-neural antibodies in patients with persisting symptoms compared to healthy post-Lyme borreliosis healthy and normal healthy controls, suggesting the possibility of a differential immune system response in post Lyme-syndrome patients.

**Nonstandard Medical Practices In Europe Associated With Lyme Borreliosis**

Some European patients have been diagnosed with Lyme borreliosis or chronic Lyme disease on the basis of poorly specific clinical criteria and non-standard laboratory tests, including live blood microscopy, lymphocyte transformation tests or inadequately validated antibody tests including unorthodox immunoblot criteria. False-positive IgM tests (including immunoblots) appear to be a particularly significant problem leading to misdiagnosis. Unorthodox treatment modalities include multiple or very prolonged courses of oral or parenteral antibiotics and parasitic agents. Some patients have received other agents including arsenicals. Misdiagnosis and inappropriate treatment can cause significant harm to patients, both from potential adverse effects and loss of opportunity for correct diagnosis and appropriate management.
Prevention of Lyme Borreliosis and Other Tick-Transmitted Infections

No vaccine for Lyme borreliosis is currently available in Europe and none is likely to be available in the near future. Antibiotic prophylaxis following tick bites is not routinely recommended, although some European guidelines and consensus documents suggest post-exposure antibiotics could be used under certain very restricted circumstances, for example in immunodeficient individuals. (SPILF 2007)

An effective vaccine is available for tick-borne encephalitis. A very active immunisation and tick awareness programme in Austria resulted in a marked decline in TBE incidence from a peak of 677 cases in 1979, just prior to the vaccine’s introduction, to 41 in 1999. (WHO 2004) The vaccine is recommended for residents of TBE-endemic regions throughout Europe and for visitors whose outdoor activities expose them to risk of tick bite. It is now widely promoted in travellers’ health clinics and through outdoor-recreation interest groups and media outlets. The vaccine’s efficacy should not be allowed to distract users from the continuing need for tick bite avoidance strategies, particularly to prevent Lyme borreliosis, which is far more prevalent and widespread in distribution than TBE in Europe.

Primary prevention of tick-borne infections entails awareness of ticks and their potential for transmitting a variety of infections, most commonly Lyme borreliosis, so public education is an important measure. Many countries’ public health authorities and special interest groups such as sporting associations and voluntary groups have annual publicity campaigns, often timed to coincide with the start of the tick-feeding season. Raising the health professionals’ awareness of tickborne infections, their prevention, recognition and management is essential to minimise risk of missed diagnosis or inadequate treatment. ECDC recently issued an educational toolkit on tickborne diseases, with modules for adults, children and healthcare professionals. The toolkit is designed to be modifiable by national public health authorities as appropriate for local circumstances. (ECDC 2010)

People should avoid tick infested areas if possible, but if this is not practicable they should take personal measures to reduce tick bite risk. These include minimising the amount of exposed skin, using DEET-containing insect repellents and frequently checking for attached ticks. People such as forestry workers who have frequent and potentially heavy contact with ticks should consider wearing permethrin-treated clothing.

Lyme borreliosis is unlikely to be transmitted within the first hours of a blood meal, so early removal of attached ticks is a valuable protection measure. There is some experimental evidence in animals to suggest that Borrelia afzelii can be transmitted at a relatively early stage of an I ricinus feed, with a steadily rising risk from about 24 hours of attachment. (Kahl O et al, 1998; Crippa M et al, 2002) Although this differs from the North American situation, where there is a longer lag phase before Ixodes scapularis transmits Borrelia burgdorferi sensu stricto, a thorough search for attached ticks at the end of each day in a tick-infested area remains a very valuable protective measure against Lyme borreliosis in Europe.

Environmental aspects of related to tickborne disease prevention have been considered by a number of European authorities, including the European Centre for Disease Control and the World Health Organisation Regional Office for Europe. (Lindgren E, Jaenson T, 2006; ECDC 2007) Possible measures included widespread use of acaricides, removal of deer populations and controlled burning of tick-permissive vegetation. None are regarded as feasible or acceptable for
large-scale use. Modification of local vegetation by landscaping and removal of leaf litter and undergrowth in gardens and parks may be helpful in reducing tick and host animal abundance in residential settings. Personal protection against tick bites remains the most important measure.

**Health Promotion in Relation to Tick-Borne Infections; Presentation of Evidence-Based Medicine And Science to Patients, Support Groups and the Wider Public**

The ECDC educational toolkit is a welcome initiative, particularly if it is taken up by national and regional public health authorities or stimulates more locally based activities, particularly in populations with low awareness of ticks. A UK Rural Economy and Land Use (RELU) multidisciplinary research project included a study of educational needs of residents, workers and visitors for prevention of tickborne infection in Lyme-endemic areas that are heavily used for recreational purposes. It also surveyed Lyme borreliosis awareness and knowledge amongst Lyme borreliosis patients and health professionals in urban and rural practice. The project is due to report in 2011. Preliminary feedback has been useful in assessing the differing educational needs of diverse groups and was presented at the Health Protection Agency Conference 2010. (Marcu A et al. 2010)

Much information on tickborne infections available from media sources, including the Internet is of variable quality, ranging from highly accurate, valuable content for raising awareness and disease prevention, to poor quality and misleading, a recent example being the promotion of Lyme borreliosis as an inducer of autism. There is a need to develop methods of presenting the best scientific evidence on conditions such as Lyme borreliosis to the general public, reaching out in ways that are accessible but not condescending to readers and viewers without a scientific background. An important example causing misunderstanding is a frequently-quoted statement that “tests for Lyme are highly inaccurate”, alluding to statistics for antibody positivity in early infection, but implying that these figures are correct for all stages of disease. The work of organisations such as Sense About Science, building understanding and trust between scientists, clinicians and the media and public may be useful in helping to model new approaches to this important aspect of tickborne diseases. (Sense About Science 2010)

A proactive approach has already been taken by the ALSUntangled group of clinician/scientists, an international scientific effort to help people with amyotrophic lateral sclerosis investigate alternative and off-label therapies. They reviewed claims of a causal link between ALS and Lyme borreliosis and published a report on Lyme disease testing and treatment in 2009, concluding that there was no convincing evidence to support such a link. (ALSUntangled group 2009)

**Possible Directions for Future Research Related To Lyme Borreliosis and Other Tick-Transmitted Infections**

Many further developments are required in the broad range of basic sciences associated with tickborne diseases, including biology of ticks, feeding hosts, infecting agents and ecosystems, in addition to greater understanding of human disease processes. Many areas of basic research are beyond the scope of this paper and are addressed by others, but some important issues already discussed here can be summarised.
Ecology and Epidemiology in Europe

- Multidisciplinary work on ecological changes affecting tick populations and their distribution, reservoir hosts and human interaction with the environment is ongoing and greater co-ordination of effort should be encouraged.
- More systematic epidemiological data collection on tickborne diseases is necessary and preliminary work funded by ECDC, due to report in 2011 should lay a firm base for future improvements.

Diagnostic Tests for Lyme Borreliosis

- Diagnostic tests have improved significantly, particularly through developments in recombinant and synthetic peptide based antigens, but testing algorithms and the two-tier testing approach have not been reviewed to take account of these changes. There is an urgent need to for a Europe-wide (and inter-continental) assessment, with a view to minimising the need for immunoblot tests. The experience of Scandinavian workers would be particularly helpful, as immunoblots are less widely used in that region, without apparent harm. The value of currently available IgM tests should be carefully scrutinised, as experience of many laboratory workers and clinicians suggests that their potential for misleading results may outweigh their benefit.
- Further developments in antibody tests, aiming for increased sensitivity without loss of specificity would be most welcome, although this may be difficult to achieve because of the relatively slow development of antibody response to \( \text{B. burgdorferi} \) by comparison to many other infectious agents.
- A reference repository of large volumes of sera with well-defined clinical provenance should be created, for use in development and evaluation of new diagnostic tests and to allow comparison with currently available laboratory assays.
- Research into development of laboratory markers of response to treatment would be valuable.
- There is an urgent need for educational efforts to encourage clinicians in the appropriate use of laboratory tests, particularly in the assessment of pre-test probability of disease likelihood.
- Diagnostic tests for other tickborne infections should be developed further.

Persisting Symptoms Following Treatment of Lyme Borreliosis

- Further research is urgently required into the incidence and possible mechanisms of persistent post-infection symptoms, which can occur following many systemic infections, including Lyme borreliosis. Lyme borreliosis could be a useful model for studying mechanisms of post-infection syndromes, from a host immune response perspective as well as pathogen aspects. This could be a focus for an international research collaboration.
- Development of optimal management strategies for patients affected by persisting symptoms following infections should be a priority.

Prevention

- Vaccine development against Lyme borreliosis in Europe is an active area of research.
• A broader approach to education about ticks, infection risks and tick bite prevention should be encouraged.

Communication Issues

• Further multidisciplinary work is urgently required in this area, as outlined above. This should include patients, support groups and members of the general public in addition to the wide range of professionals working in the field of Lyme borreliosis and other tickborne infections. The diverse needs of different communities should be taken into consideration in assessing needs.

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