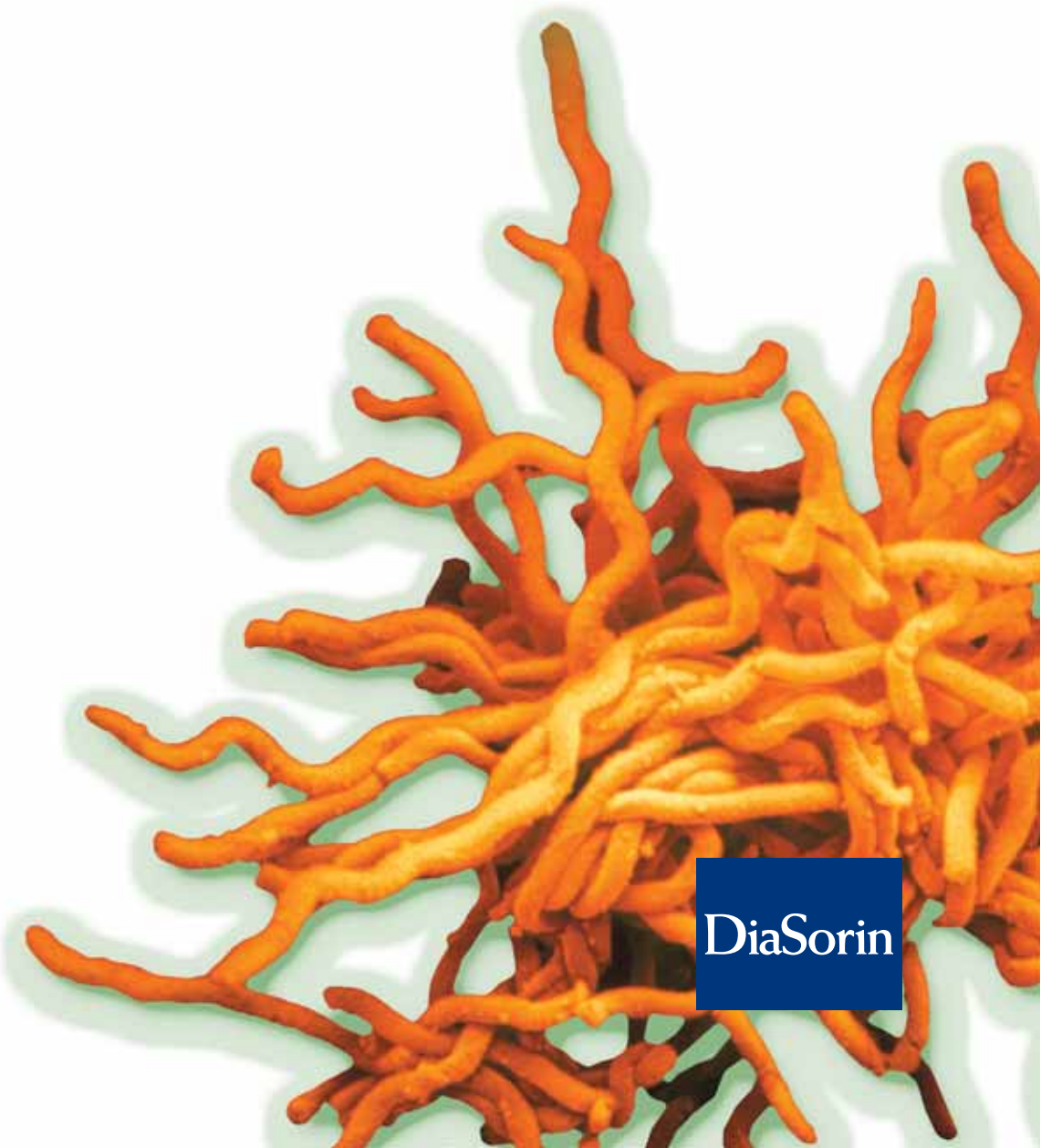
# Focus on Lyme-Borreliosis



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Prof. Elisabeth Aberer is specialized in dermatology and venereal diseases. She has a Master's Degree in Dermohistopathology. Since April 1995 she has been Senior Consultant of the Dermatology Clinic, University of Graz. Her studies have mainly focused on clinical signs and symptoms, treatment and diagnostic issues related to Lyme borreliosis. Prof. Aberer has formed a research center specialized in Lyme borreliosis at the Vienna General Hospital. She has directed two projects designed to promote scientific research: "*Die pathogenetische Bedeutung von B. burgdorferi in der Haut*" (The pathogenic meaning of B. burgdorferi on the skin, 1989-1992) and "*Harn - PCR zur Routinediagnose der Lyme-Borreliose*" (Urine PCR in routine screening for Lyme borreliosis, 1999-2002). She is the Director of the Research Center specialized in General and Self-Immune Pathology and, Director of the Quality Control Department, University Clinic of Dermatology and Venereology, Graz. Laboratory diagnostics mainly focus on immunodiagnostics in autoimmune dermatological and primary systemic diseases and on specialized diagnostics for Lyme borreliosis.

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**Volker Fingerle**

Dr. Volker Fingerle - Specialist in Microbiology and Infectious Epidemiology - has worked at the Max von Pettenkofer Institute of Hygiene and Medical Microbiology, Ludwig Maximilians Universität (LMU), Munich, since 1990. He works at the National Reference Center (NRZ) for Borreliae and oversees the consiliary lab for Ehrlichea. His studies have mainly focused on training and consulting activities targeted at colleagues and dealing with the epidemiology, diagnostics and treatment of Lyme borreliosis and of anaplasmosis (ehrlichiosis). His fields of research are epidemiology, diagnostics and pathogenesis of Lyme borreliosis and granulocytic anaplasmosis in humans.

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# Epidemiological, biological, and ecological aspects of Lyme borreliosis

Thomas Talaska

As the science of disease prevention, epidemiology plays a significant role in the planning and evaluation of public health and health politics. It is used in conjunction with laboratory research to identify risk factors for disease, elucidate the mechanisms by which they develop, and finally to formulate prevention strategies. In Germany, the instrument for epidemiological evaluation of infectious diseases is the Infectious Diseases Control Law (IfSG), which came into force on January 1, 2001; however, this law applies only to infections transmitted directly from person to person or food-borne, and does not, cover Lyme borreliosis, by far the most common vector-borne disease in central Europe. However, all of the former East German states and Berlin have laws regarding registration of both occurrence and positive laboratory results of the disease. An effective, affordable, regionally tailored prevention programme including immunisation schemes cannot be realised without knowledge of disease prevalence, incidence, and risk factors. Such epidemiological information is also essential to gauging the success of any preventive measures. Although Lyme borreliosis has an extremely low mortality rate, its frequency alone has a significant economic effect. This "economical impact" is defined by the World Health Organisation (WHO) as the total cost of visits to physicians, laboratory diagnostics, drugs, hospital stay, lost work time, additional health care and, in certain cases, invalidity [5]. Personal suffering, reduced quality of life and ability to perform should not be ignored, even though these are difficult to quantify. In occupations with high exposure rates, Lyme borreliosis has considerable significance as a work-related disease.

The WHO European regional office in Copenhagen published a report in 2004 on vector-borne diseases, including tick-borne diseases, and their significance in Europe. The incidence of such illness is significantly higher than has been

assumed by medical practitioners and public health departments – often diagnoses are not made and treatment is delayed. This requires action! In the absence of the public interest associated with dramatic outbreaks, the need for surveillance and control of not only the disease but also its vectors is easily overlooked, resulting in insufficient support for epidemiological research, even by the public health authorities [4].

## Borrelia: ticks – hosts

The epidemiology of Lyme borreliosis in humans is closely associated with the tick's life cycle, its habitat, and the interaction between tick, host – including humans – and the particular species of *Borrelia* involved. Thus it is necessary here to make a short excursion into the world of ticks in order to understand this zoonosis.

*Borrelia* are obligate parasites with no free-living stages, and their propagation relies on complex zoonotic transmission cycles involving rodents as the main reservoir hosts and ticks as vectors.

In Europe there are three species confirmed pathogenic to humans:

- *Borrelia afzelii*
- *Borrelia garinii*
- *Borrelia burgdorferi* sensu stricto

Two other species show evidence of pathogenicity:

- *Borrelia valaisana*
- *Borrelia lusitaniae*

Recently an additional *Borrelia burgdorferi* genospecies was isolated from erythema migrans lesions [7]:

- *Borrelia spielmanii (also designated A14S)*.

**Fig. 1.** A mouse with *Ixodes ricinus* larvae and nymphs

For *Borrelia*, humans are dead-end hosts.

The main vector of Lyme borreliosis in central Europe is *Ixodes ricinus* (Fig. 1). This tick species has a very broad spectrum of vertebrate hosts (reptiles, birds, especially migratory species, and mammals), compared with other native tick species, and is thus well suited for transmitting pathogens to a wide variety of species.

## How does a tick bite?

Once a tick finds a suitable attachment site on a host, it uses its mouthparts, the chelicerae, to cut through the host's skin. The tick pushes its hypostome, a chitin tube with recurved teeth, into the small wound created. In effect, the tick stabs or pricks its host. The tick hypostome is however not a completely

closed tube, as in insects, and to facilitate suction, the hypostome must be cemented into the skin by a salivary secretion at the site of penetration. While feeding, the tick is stuck fast to the host – thus the generic name *Ixodes* (from Greek *ixos,* meaning "glue" [13]).

Transmission of *Borrelia* to ticks is required for human infection and can take place in various ways. Trans-ovarial transmission to offspring in systemically infected ticks is relatively uncommon, affecting less than 5% of *Ixodes ricinus* larvae. A more common route of transmission occurs when larvae are infected by feeding on blood, usually from infected forest mice. Trans-stadial transmission is then possible to nymphs and finally adult ticks. This cross-stage transmission is highly dependent on the *Borrelia* species involved, the distribution of *Borrelia* in the tick (midgut only or systemic, including salivary glands), and on the host species. It also depends on the sensitivity of *Borrelia* species to the lytic action of the host's innate immune defences, the complement system.

If a tick infected with *Borrelia afzelii* draws a bird's blood, the complement system in this liquid "meal" is able to destroy the *Borrelia* in the tick's midgut. If the tick is infected only in the midgut, then the *Borrelia* can neither be transferred to the next tick developmental stage nor to the host (a nonpermissive system). If, however, the tick is systemically infected, deactivation in the midgut occurs, but the tick remains infected and trans-stadial transmission occurs, while the bird host is protected by its complement system (a semipermissive system). The bird has no reservoir competence for this strain. Reservoir-incompetent host species can have a zooprophylactic effect through which the dissemination of *Borrelia* can be hindered. Nuncio et al. describe an example of this effect on the Portuguese island of Madeira, where the native hosts are lizards (Madeira wall lizard, *Teira dugesii*) in either non- or semi-permissive systems (Fig. 2) [12, 15]. The circulation of *Borrelia* on Madeira can be maintained by introduced mice and rats [16].

**Fig. 2.** *Teira dugesii*, Funchal, Madeira (photo: Robert Klose)

Co-feeding is an additional transmission route between ticks. *Borrelia* can be transmitted through cofeeding when ticks are infected systemically. Ticks are

pool feeders, meaning that their biting and the activity of enzymes secreted in their saliva cause a pool of blood to accumulate at the base of the hypostome, from which the blood meal is drawn. This "washing" of the tick's feeding apparatus with saliva leads to the transfer of *Borrelia* into the wound. Ticks have preferred attachment sites where they congregate close together to feed. In the process, *Borrelia* can be transferred to neighbouring ticks without the host becoming infected or needing to be reservoir-competent (Fig. 3).

The above transmission mechanisms are responsible for the local and life stage-dependent variations in infection rates of *Ixodes ricinus*. An extremely high prevalence was found in a part of Croatia, with 45% of ticks infected with *Borrelia*, while ticks in a Prague city park showed a low prevalence of 4.9% over a four-year period. Although some risk of contracting Lyme borreliosis by tick bite exists throughout Germany, with the exception of vegetation-free areas in the mountains and Baltic and North Sea beaches, the risk varies dramatically within regions and depends strongly on local conditions. From the many studies in German states, we find mean prevalences of 7.7% in Hessen, 14.4% in Baden-Wuerttemberg, 14% in Bavaria, 15.11% in Thuringia, 16.5% in Saxony, and 22% in Brandenburg. However, the incidence of infection in a whole state cannot be used to predict the actual risk in a particular part of that state. In areas with optimal tick habitat, local prevalence can approach 40%, a fact that is not obvious if only the mean prevalence is considered.

Optimal tick habitats have two essential requirements: sufficient moisture to maintain the ticks' sensitive water balance and a mixture of sufficiently numerous hosts for tick larva, nymphs, and adults. It is interesting to note that tick behaviour is also influenced by *Borrelia*. Such phenomena are well known from other host-parasite systems (e.g. trypanosomes and tsetse flies, rat fleas and *Yersinia pestis*, *Leishmania* and *Phlebotomus* sandflies). Drs Perret and Guerin and Prof. Gern of the University of Neuchatel in Switzerland demonstrated that, in dry atmospheric conditions, infected ticks move farther and more often from their original location than uninfected ticks. This

**Fig. 3.** White-tailed deer with a heavy tick infestation (photo: USDA Northeast Area-Wide Tick Control Project, USA)

increases the likelihood of finding new hosts on which to feed – and thus of new infections with *Borrelia* [10]. Prevalence rates also vary according to tick life stage; for example in Thuringia a study of ticks found attached to patients showed infection in 0% of larvae, about 15% of nymphs, and about 19% of adult females [6].

The diversity of interactions between *Borrelia*, ticks, and hosts outlined here demonstrates the need for sophisticated biological and epidemiological analyses of Lyme borreliosis. In addition, one must also include ecological factors such as vegetation, geology, hydrology, temperature, and rainfall. At present, we are only able to give a descriptive account of the epidemiology of Lyme borreliosis in humans.

### Factors influencing the epidemiology of Lyme borreliosis in humans

- Tick species
- *Borrelia* species
- Presence/density of reservoir-competent hosts
- Presence/density of tick habitats which humans enter
- Use of forest and agricultural land
- Forest structure and kinds of biotopes
- Seasonal changes in temperature, sunshine, and rainfall
- Ground moisture and geological characteristics
- Long-term temperature developments
- Risk and recreational behaviour of humans
- Certainty of the diagnosis of Lyme borreliosis

## Epidemiology

Worldwide, epidemiological data on Lyme borreliosis as an "emerging infectious disease" are inadequate, with the exception of those from the USA (Centers of Disease Control, or CDC, and the Division of Vector-Borne Diseases). In Europe there are currently very few national notification or registration systems. The epidemiological data available for Germany are based mostly on studies aimed at only a single clinical manifestation or are limited to very small regions within a German state. Other countries have regional registration systems, such as in Austria and France. Russia has the beginnings of a regional notification system based on a highly centralised diagnosis of *Borrelia*. Slovenia probably has the oldest epidemiological databank in Europe, based on a notification system for Lyme borreliosis originating in 1998 [21].

What actually is borreliosis? *Borrelia* are not new pathogens – typical clinical cases were described as early as the end of the 1800s (acrodermatitis chronica atrophicans, erythema chronicum migrans, Bannworth's syndrome). In 1996 Ohlenbusch confirmed the presence of *Borrelia* in ticks more than 100 years old from museum collections in Berlin and Vienna (*Borrelia*

*burgdorferi* sensu stricto, *Borrelia garinii, Borrelia afzelii* [17]). The significant increase in borreliosis, considered a rarity with unknown aetiology but well-defined symptoms as recent as 20 years ago, is surprising. The establishment of clinical case definitions of Lyme borreliosis was an important requirement for collecting valid data. Those created by the CDC were very stringent and developed specifically for epidemiological purposes. However, a number of European manifestations of the disease were not included because they are practically unknown in the USA. In 1996 the European Union for Concerted Action on Risk Assessment in Lyme Borreliosis (EUCALB) established valid case definitions for Europe that allow standardisation of registered, defined clinical cases [20]. The existence of yet undefined and hence unregistered clinical manifestations must be considered.

The possibility of simultaneous transmission of multiple tick-born diseases necessitates differential diagnosis. In fact, simultaneous infection with *Borrelia* and the pathogens causing human granulocytic ehrlichiosis (HGE), *Anaplasma phagocytophilum*, or *Babesia* spp. are documented. These present a clinical picture that differs from the course typical for Lyme borreliosis. The significance of infection with *Rickettsia slovaca* and *Rickettsia helvetica* is still largely unclear.

What explains the increase in Lyme disease? Socio-ecological factors are implicated in a study of an area with endemic Lyme disease in the northeastern USA where the movement of large numbers of people from populated urban centres to forested rural areas increased the risk of exposure to tick bites [8]. At the same time, this area experienced a decrease in agricultural land use, and an increase in bushes and trees abutting directly to inhabited areas, with a concomitant increase in tick hosts. The WHO Report on Europe also describes massive ecological changes that have taken place since the end of the Second World War. In particular, intensive reforestation has brought (and continues to bring) changes in the flora and fauna. The population densities of wild boar and deer have increased, leading to greater tick density. Similar developments can also be seen in Germany, but specific studies on this problem are lacking.

It is probable that continued climatic change will influence the frequency of vector-borne diseases. The distribution and seasonality of diseases transmitted by ectothermic insects and ticks are particularly sensitive to global temperature changes. Warmer winters favour tick survival and movement into new areas where they were previously rare or absent. Global warming also leads to longer active periods for ticks in spring and autumn and probably increases human recreational activities

in these periods, resulting in higher risk of exposure to ticks. Examples include Sweden, where the distribution of tick-borne encephalitis (TBE) has expanded northward, and the Czech Republic, where (TBE) expansion has occurred over the last 30 years into higher areas of the Bohemian Forest [25]. How the situation with Lyme borreliosis will develop remains to be determined, although it is likely to follow the pattern of TBE. The first data confirming this came from the Czech Republic [5].

A temperature-related lengthening of the tick season in the presence of a large number of reservoir-competent hosts can also lead to faster tick developmental cycles and thus greater tick density; transit from egg to adult can be shortened from 54 to 18 months.

In November of 2004, the European Union project "Emerging Diseases in a Changing European Environment" was started. Prof. Sarah Randolph of Oxford University is leading the "Tick-Borne Diseases" section. Its aim is to explain the increase in tick-borne diseases on a European scale and to create predictive statistical and biological models.

## The development of borreliosis epidemiology in Brandenburg

The first studies on the epidemiology of Lyme borreliosis in Germany began in the state of Brandenburg under the auspices of the 1994 WHO Consultation on the Development and Application of Geographical Methods in the Epidemiology of Zoonoses [3]. In that year, the "Geographical Epidemiology of Borreliosis in Brandenburg" project was started, in which a voluntary notification schedule was introduced and the Lyme Disease Case Report Form adapted from the American Case Report Questionnaire in conjunction with the CDC. Starting in November 1996 the German Federal Infectious Diseases Law was extended to require that all clinical occurrences of Lyme borreliosis in Brandenburg be reported using the notification schedule and all positive laboratory results to be registered. This was the first opportunity to collect and analyse a data set covering an entire German state. In the same year, the Lyme Borreliosis Interdisciplinary Advisory Group was established by the Brandenburg State Medical Board to advise medical practitioners, patients and self-help groups. In 2001, the Brandenburg State Ministry for Work, Social Affairs, Women, and Health founded the Regional Counselling Centre for Tick-Borne Diseases. In addition to its advisory role, this service coordinates and carries out studies, evaluates tests, and makes additional analyses of registered cases of Lyme borreliosis. In addition, issues involving HGE, *Rickettsia helvetica,* and babesiosis are considered there in cooperation with, among others, the Robert Koch Institute (RKI) in Berlin, the WHO in Geneva, the CDC Division of Vector-Borne Diseases at Fort Collins, the University

of Frankfurt/Main, and Tulane University in New Orleans.

## Geographic information systems and Lyme disease

Computer-based geographic information systems (GIS) represent a modern tool for analysing risk factors and producing risk maps for the epidemiology of infectious diseases, particularly tick-borne diseases [3]. This in turn can provide the basis for locally adapted prevention strategies similar to the time-tested methods used in veterinary medicine and veterinary disease control. The GIS systems are based on area-related databases with parameters that include cases of infection, geographic coordinates, administrative structures, and population density. They contain digital coordinate reference maps showing, for example, administrative boundaries, biotopes, geology, hydrology, weather, and other relevant factors [14, 18, 19].

**Fig. 4.** Distribution of borreliosis cases in Brandenburg 2003 (database: EpiInfo 3.3; created using ArcView 3.2 [23])

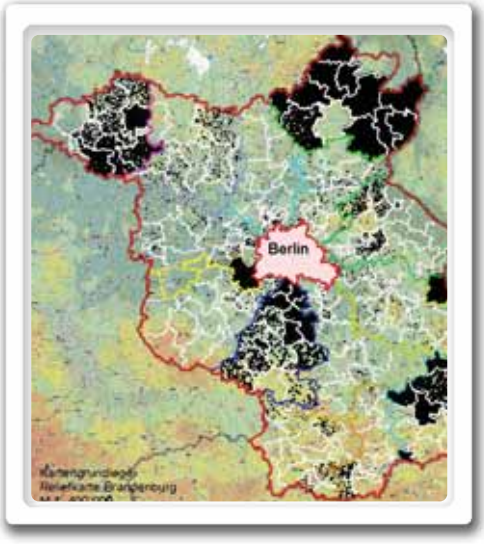
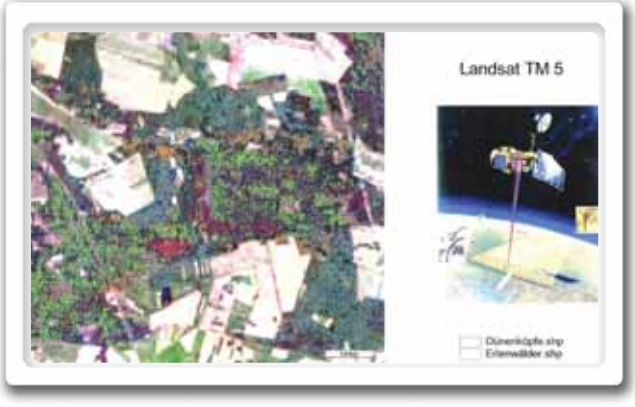



**Fig. 5.** Example of satellite-supported biotope cartography – alder sections (marked blue) in the Spree forest (photos: Landsat TM; processed with ArcView 3.2; A. Ober 2003)




The computer programme "EpiInfo", freeware for epidemiological surveillance originally developed by the CDC, includes databases, analytical tools, and EpiMap, a component program that can produce cartographic representations of epidemiological data. The databases are compatible with the GIS system ArcView, which allows coordinates from study areas or new distribution areas to be recorded locally using global positioning systems (GPS) and precisely located on coordinate reference maps. Other geographic parameters such as vegetation and watercourses can be superimposed on these maps for analysis (Fig. 4).
EpiInfo is also useful for evaluating

satellite photographs, in which for example different vegetation types produce different patterns of reflection spectra. The ability to precisely superimpose satellite photographs onto digital maps permits on-site biotope searching and analysis using GPS. Computer searches for biotypes with similar characteristics are also facilitated (Fig. 5).

Areas with a potentially higher risk of Lyme borreliosis can be located on the computer screen and related to data from regions with a higher incidence of borreliosis – a major prerequisite for the development of risk maps.

## Epidemiological data on Lyme borreliosis in Brandenburg

The first incidence data on Lyme borreliosis in Brandenburg were collected on a volunteer basis from 1994 to 1996, i.e. until introduction of the notification requirement. The incidence was ten cases per 100,000 inhabitants but with marked regional differences, which may, however, have been strongly related to notification behaviour. In 1997, 400 clinical cases were registered. Two studies in Maryland and Connecticut, USA shortly after the introduction of a notification requirement showed that only 10–15% of cases fitting the CDC case definition were actually registered. Applying this factor optimistically to Brandenburg (ignoring undiagnosed cases and those not presenting erythema migrans, as these can only be speculated) would predict 4,000 clinically relevant cases of borreliosis. Developments in the USA show that the number of registered cases increased markedly in the years following introduction of the notification system, especially when the

**Fig. 6.** Lyme borreliosis in Brandenburg – registered clinical cases per month until 31.12.2004 (graphic: T. Talaska)

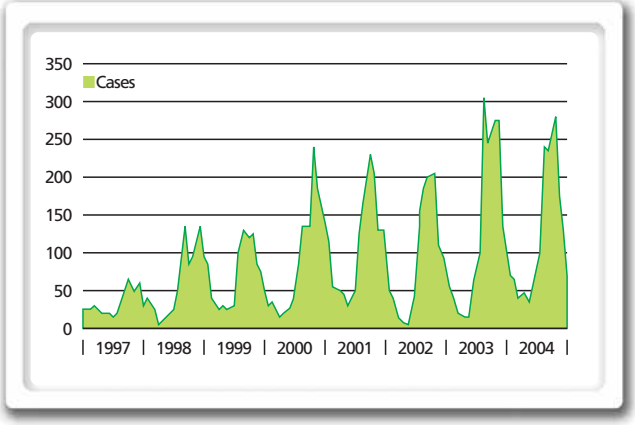



**Fig. 7.** Clinical cases of Lyme borreliosis from 1995 to 2004 (graphic: T. Talaska)

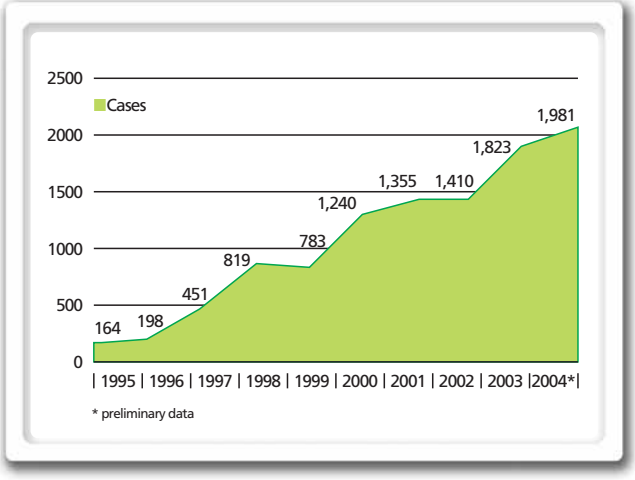


**Fig. 8.** Borreliosis in individual German states (graphic: G. Hesse, Erfurt 2004)

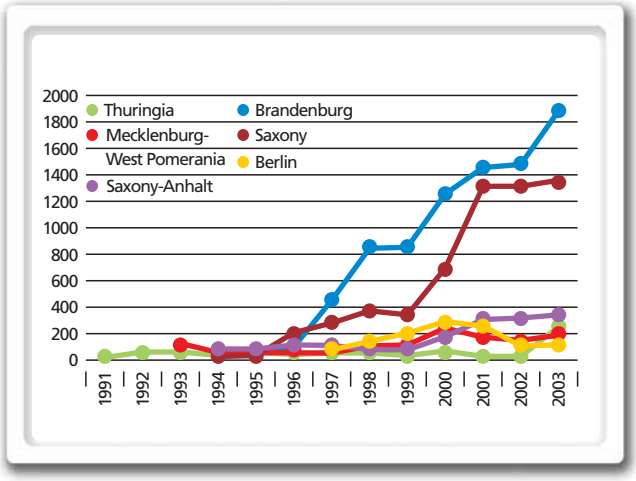
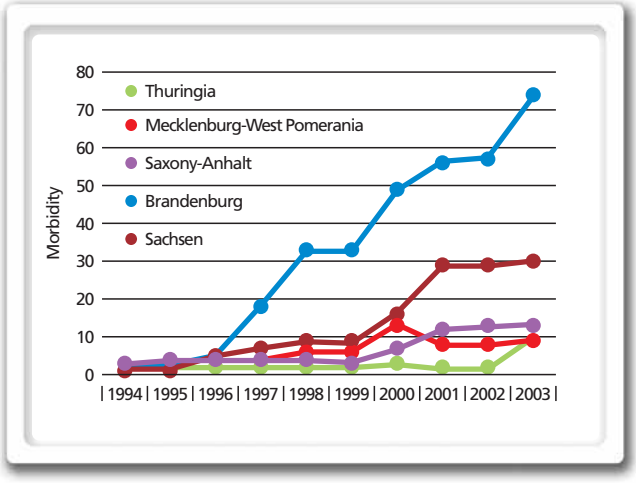



**Fig. 9.** Borreliosis morbidity from 1994 to 2003 (graphic: G. Hesse, Erfurt 2004)




notifying physician was informed on regional epidemiological data. It is assumed that with an established notification system, about a third of infections are reported (D.T. Dennis, personal communication). If this is the case, Brandenburg's 1,240 registered borreliosis cases in 2000 represent an actual value of about 4,200. Such high estimates suggest that the increased incidence of borreliosis is probably only a function of the notification changes, and that the actual estimates for the last four years were much lower.

Nevertheless, one cannot ignore that subsequent years have shown a steady increase in registered cases of 10–15% annually. This trend has also been found in other German states with notification requirements, as G. Hesse reported at the 2004 Thuringia Workshop on Tick-Borne Diseases in Erfurt (Figs. 6, 7, 8, 9, 10).

## Regional variability in the state of Brandenburg

As already indicated, there are significant differences between districts in the registered incidence of borreliosis. The highest incidence in Brandenburg was reached in 2000 in the Oder-Spree district (89.3/100,000 inhabitants), the Uckermark (89.0/100,000), and the Barnim (74.6/100,000). In general, there has been a trend toward higher incidence in eastern Brandenburg since 1996. This appears to depend not on risk behaviour but rather on the density of suitable biotopes for the ticks. It is still necessary to determine whether more and smaller habitat areas with higher contact for humans exist in the eastern part of Brandenburg than the west. A typical such case is the municipality of Scharmuetzelsee in the Oder-Spree

district, a densely forested recreational centre for people visiting the lake area. Inhabitants of the town of Bad Saarow stated they are regularly bitten by ticks in their gardens, in which mice are very common and visits by wild animals (e.g. roe deer) are not uncommon. Scharmuetzelsee had the highest incidence in Brandenburg in 2000, with 237 cases per 100,000 inhabitants. This figure does not include visitors from Berlin who were affected. This example shows considerable similarities to observations made in the USA [8]. Considering the Oder-Spree district more closely, one finds immense differences in incidence at the local level. These ranged from 10/100,000 inhabitants in Beeskow to the previously mentioned 237/100,000 in adjacent Scharmuetzelsee. Mapping cases in the Oder-Spree district on a satellite photograph, most of the registered cases were localised close to forested wetlands. Sections with very low incidence included large agricultural areas, but even there, the few registered cases were associated with small lakes or water courses.

In general Brandenburg is rich in forest and wetlands, but not all forested land is suitable tick habitat. Ticks require a moist microclimate for survival [11] and protection from the cold in winter. These are best provided by several annual layers of slowly decomposing leaf cover, especially beech and oak.

**Fig. 10.** Registered cases and incidence of borreliosis in those German states with required notification (graphic: T. Talaska)

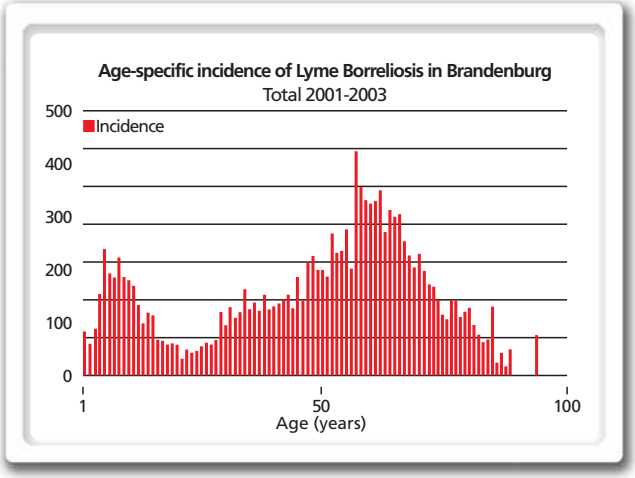
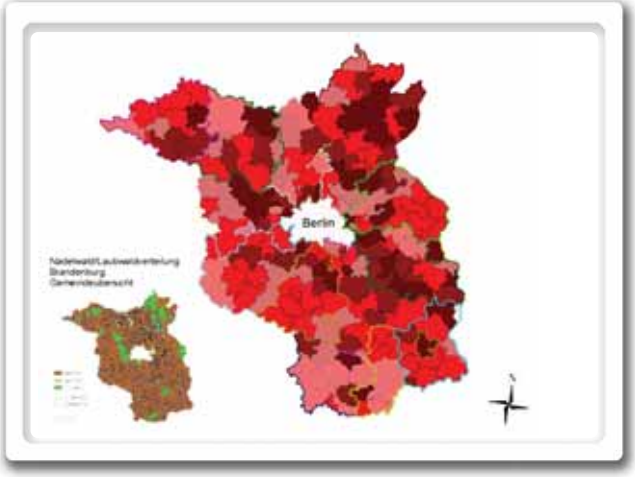



**Fig. 11.** Incidence of borreliosis in 2003 in municipalities and communities (database: EpiInfo 3.3 created using ArcView 3.2 [23])



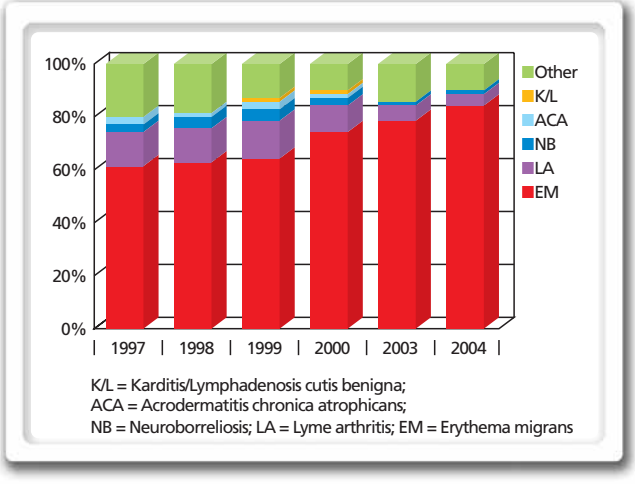

Such deciduous and mixed forest tracts together comprise less than 5% of forest cover in the state. There are large areas of commercial pine monoculture. Areas forested in this way are poor habitats for ticks because pine needle

layers retain less humidity directly over the soil surface than deciduous leaf layers in the warmer part of the year. Recent changes in forestry practice in this region have aimed at increasing the deciduous and mixed forest components to 40% over a 10-year period to return the forest to its original natural make-up. It is anticipated that the extent of suitable tick habitat will also increase, and therefore provisions have been made to monitor tick density and thus the potential risk of Lyme borreliosis in these areas (Fig. 11).

## Clinical data

Erythema migrans, an early clinical manifestation, is the most common registered sign of borreliosis in all states of Germany, and the trend from 1997 to 2004 shows that its proportion increased from 61.7% to 84.5% over that period. It is now higher than in Austria (77.7%) and the USA (76.0%) [24]. It is also evident that the growing proportion of erythema migrans cases accompanies significant reductions in the proportions of reported cases of both the more advanced disseminated borreliosis (19.2% in 1997 vs 5.6% in 2004) and cases which cannot be clearly clinically classified into the EUCALB case definitions (19.1% in 1997 to 8.7% in 2004). Given the notification requirement from 1996 and continuing education organised by the Interdisciplinary Lyme Borreliosis Advisory Group, a possible explanation for this trend is that the early stage of borreliosis is more frequently and accurately diagnosed and treated due to increasing physician and patient awareness. This would reduce the number of patients developing late manifestations such as disseminated borreliosis. However, these cannot be completely avoided, as a significant proportion of infected individuals present with the disseminated form at anamnesis. Only 21% of cases of Lyme arthritis registered in 1999 and 2000 had been previously diagnosed with erythema migrans. The decreasing proportion of "unclear" cases is an indication that Lyme borreliosis is generally being diagnosed with more certainty. However, it is still necessary to analyse these ambiguous clinical cases and if possible expand the current case definitions. When analysing cases from 2003 and 2004, it is necessary to consider a certain bias in registered

**Fig. 12.** Different clinical manifestations in percentages



manifestations toward erythema migrans due to the RKI case definition of Lyme borreliosis (Fig. 12).
Considering the conservatively estimated cost of 10,000 Euro per case of disseminated borreliosis and the relatively certain relationship between erythema migrans and development of disseminated borreliosis in the absence of intervention, the economic importance of information distribution and continuing education is evident.
According to this very simplified model, about 1 million Euros could have been saved by the prevention campaign in Brandenburg over the last few years. (Fig. 13).

## Model system in the Oder-Spree district

The Oder-Spree district was chosen as a study area to analyse risk factors for borreliosis and the prevalence of *Borrelia* in ticks due to the high incidence of Lyme borreliosis found there. The study was carried out in conjunction with the RKI from June to December 1999. In addition, the geographic distribution of cases was calculated to determine whether it was random or there were indeed high-risk areas within the district (which, as indicated previously, is very heterogeneous).
One significant risk factor, as predicted, was activity in private gardens, particularly those bordering the forest (Fig. 14).

**Fig. 13.** Estimates of borreliosis occurrence in Brandenburg with and without preventive measures (highest estimates) for 1997 and 2000

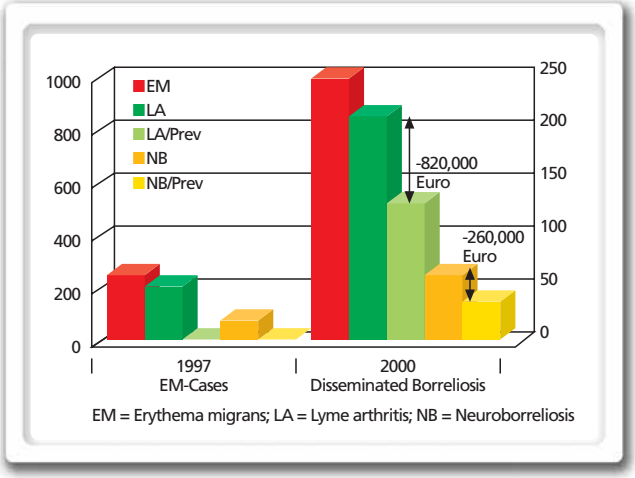
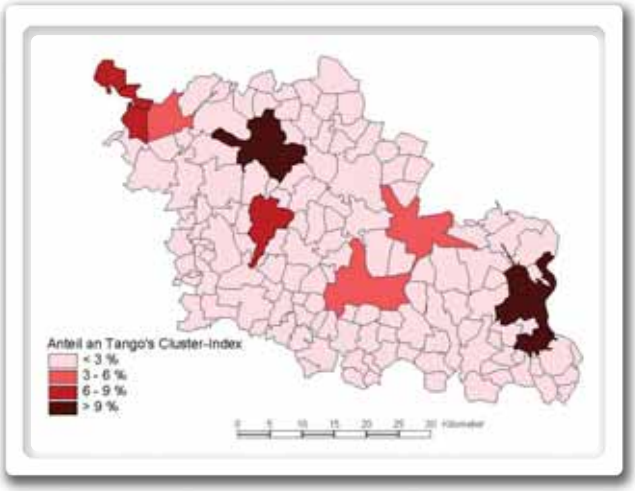



**Fig. 14.** Typical "tick biotope" in the Oder-Spree district: a garden adjacent to the forest kept close to natural condition

This is probably due to a lack of awareness of the risk, which most individuals seem to associate only with activities in the woods. Light clothing is often worn while gardening and, if nobody search for ticks follows, there

**Fig. 15.** Registered cases of Lyme borreliosis in 1998 and 1999 in the Oder-Spree district showing the proportion of municipalities on the cluster index (Borreliosis Study, Oder-Spree District; A. Ammon, RKI 1999)




is a distinct risk of infection. Unfortunately it was also determined that, in spite of familiarity with personal prevention measures, they were actually carried out by only 40% of those questioned. This requires action by the health authorities and additional prevention strategies directed not only at individual activities. It showed that personal prevention can be very effective, when actually carried out. Our experience from the Oder-Spreewald district shows that it is frequently ignored [2, 9].

In the USA a comprehensive analysis of practical prevention methods requiring

**Table 1.** Model system in the Oder-Spree district: incidence of registered Lyme borreliosis 2003 (Compare with incidence in Old Lyme municipality, USA 2001, 297, and 2002, 391)

| Municipality | Mean age | N clinical cases | Incidence |
|---|---|---|---|
| **Cases/100,000 inhabitants** | | | |
| **Beeskow** | 34 | 9 | **100** |
| **Brieskow-Finkenheerd** | 55 | 26 | **311** |
| **Eisenhüttenstadt** | 53 | 62 | **149** |
| **Erkner** | 38 | 2 | **16** |
| **Friedland** | 63 | 5 | **146** |
| **Fürstenwalde** | 51 | 25 | **73** |
| **Grünheide** | 54 | 13 | **184** |
| **Neuzelle** | 55 | 8 | **111** |
| **Odervorland** | 47 | 4 | **68** |
| **Rietz-Neuendorf** | 52 | 5 | **109** |
| **Scharmützelsee** | 49 | 25 | **298** |
| **Schlaubetal** | 54 | 5 | **58** |
| **Schöneiche bei Berlin** | 57 | 11 | **45** |
| **Spreenhagen** | 44 | 11 | **142** |
| **Steinhöfel** | 24 | 1 | **24** |
| **Storkow** | 51 | 12 | **127** |
| **Tauche** | 63 | 1 | **23** |
| **Woltersdorf** | 39 | 4 | **59** |

minimal effort was done. From the point of view of practicability, acaricide-impregnated feeding stations for wild animals, like those used in the USA, are a realistic alternative. Their application requires minimal administrative effort, and the case reduction achieved in models is very good. Unfortunately, at present this is not possible in Germany due to the difficulty in coordinating the areas of responsibility of the various authorities involved.

The geographic distribution shows a clear aggregation of cases in the Oder-Spree district in particular, and Brandenburg as a whole (Fig. 15).

Analyses of notification records show that differences in notification behaviour by physicians can be eliminated as a possible cause. Thus we can assume that the geographic factors previously discussed are responsible for this variation in risk (Table 1) [2].

## Forestry workers: a risk group

The seroprevalence of tick-borne diseases in forestry workers was examined in a study carried out in Berlin and Brandenburg [22]. Forestry workers, hunters, and forest managers are more commonly exposed to such diseases than the general population; however, they are also better informed on the risks and their prevention and hence their typical working clothing is usually well-suited to preventing tick bites.

Our study was carried out on a volunteer basis and included immunoglobulin-G (IgG) examinations for seroprevalence against *Borrelia burgdorferi* sensu lato, as well as the pathogens causing HGE (human granulocytic ehrlichiosis): *Anaplasma phagocytophilum*, *Babesia microtii* and *Rickettsia helvetica*.

The risk group as a whole showed 29% *Borrelia burgdorferi* prevalence, although borreliosis was allegedly diagnosed and treated in only 10.2% of cases. This discrepancy indicates a significant number of asymptomatic and previously undiagnosed cases. In fact, a questionnaire filled out after serodiagnosis showed that an additional 24% of seropositive subjects described symptoms compatible with Lyme borreliosis.

A significant aggregation of seropositive cases occurred in the Barnim region northeast of Berlin. This population showed 6.2% positivity for IgG antibodies to *Anaplasma phagocytophilum* (FAT, Western blot). In the subsequent case control study, no statistical relationship could be found between HGE-positive cases and potential symptoms. Thus there is evidence of contact with the pathogen, but the course of infection appears to be either asymptomatic or mild.

There are only limited data on the frequency of *Babesia microtii* antibodies in humans in Europe. Our study showed a 1.4% IgG-positive prevalence (FAT, Western blot). Only one indi-

vidual complained at anamnesis of symptoms consistent with babesiosis after a tick bite, but we found no antibodies against HGE or *Borrelia*. *Rickettsia helvetica* has been found in ticks from Switzerland, Sweden, France, and Slovenia. Clinical cases with prolonged fever, weakness, and myalgia were recorded in France. In Scandinavia, the deaths of two young athletes were attributed to perimyocarditis from training during *Rickettsia helvetica* infection. A sample from the forestry worker group was therefore tested for *Rickettsia helvetica* IgG using an in-house FAT (Raoult, Rolain, Marseilles University). A total of 28.3% of individuals tested were IgG-positive. Infection with other *Rickettsia* species were found in only two cases. These results are in agreement with Swedish studies; *Rickettsia helvetica* is apparently endemic and dominant in central and northern Europe. In a current study, we are examining the prevalence of *Rickettsia helvetica* IgG in children and the elderly. Our results to date show 0% prevalence for children less than 10 years old (without borreliosis). This increases to 25% in the elderly (>65 years, without borreliosis) (Figs. 16, 17). In a third group of patients with clinically and serologically proven borreliosis, this level increased to 27.7%, with co-infections probable. The clinical significance of these results still needs clarification.

**Fig. 16.** Rickettsia helvetica IgG seroprevalence in Brandenburg (study from 2005)

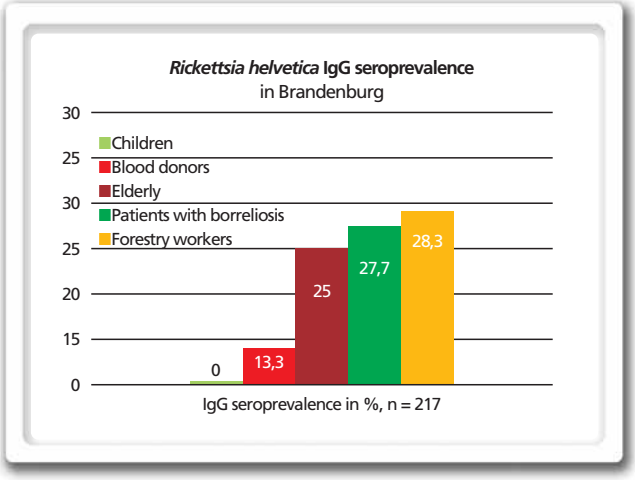



**Fig. 17.** Map showing HGE and babesiosis seropositivity in Brandenburg

## Outlook

Considering the borreliosis situation over the past 8 years, it is apparent that interest in this illness, originally considered a “fashionable disease” in

Europe, has increased markedly and that the problem of vector-associated diseases in general is being taken more seriously. Collection of the epidemiological data from Brandenburg, which according to the CDC are among the best available worldwide for Lyme borreliosis, would not have been possible without the active help of physicians, public health officials, and the Brandenburg state Department of Public Health and Ministry of Health. We hope the German federal government will also become active in the prevention of Lyme borreliosis. A positive signal can be see in the publication in 2002 of the RKI Bulletin of case definitions for this disease conforming to the new IfSG. However, the inclusion of only erythema migrans and what was previously termed neuroborreliosis remains an obstacle to effective epidemiological work. Lyme arthritis, *Borrelia*-related lymphocytoma, chronic neuroborreliosis, acrodermatitis chronica atrophicans, and Lyme carditis were not included, in contrast with both the CDC and EUCALB definitions. We know from our own epidemiological data that about 15–20% of cases fall outside of the RKI definitions. This biases the actual picture. For example, erythema migrans is found in only 21.5% of Lyme arthritis cases. Cranial nerve paralysis, such as facial nerve paralysis, by definition requires registration under neuroborreliosis only in cases where intrathecal antibodies are present, even though it is known that these are not found in all cases because central nervous system involvement is not necessary. Accurate analyses of the clinical spectrum of Lyme borreliosis and the efficiency of prevention programs are not possible using the present case definitions. Since these are still under discussion, it is hoped that more inclusive versions will be forthcoming. In 2004, the RKI wrote in a publication on zoonoses in humans: "An improved database on the frequency of Lyme borreliosis in Germany is to be strived for. A contribution to improved epidemiological data collection would be achieved by including Lyme borreliosis in the list of pathogens requiring official registration according to the IfSG" [1]. We hope this will not remain only a dream.

Another unsolved problem is the development of a safe, efficacious vaccine against Lyme borreliosis. The monovalent OspA vaccine approved for use in the USA showed good immunogenicity, but was later removed from the market. A polyvalent OspC vaccine failed to gain approval due to problems with side effects. The development of therapeutically useful polyvalent vaccines based on chimeric antigens is currently underway. However, it will be some time before they are available.

## Legal bases in those German states with notification requirements for Lyme borreliosis

- Thuringia: Thuringian Decree on the Adjustment of Notification Requirements for Infectious Diseases (Thüringer Infektionskrankheitenmeldeverordnung, or ThürIfKrMVO) of 15 February 2003
- Saxony: Decree of the Saxon State Ministry for Social Affairs on the Extension of the Notification Requirements for Transmissible Diseases and Pathogens According to the Infectious Diseases Control Law (IfSGMeldeVo) of 3 June 2002
- Saxony-Anhalt: Decree on the Extension of Notification Requirements for Transmissible Diseases in the State of Saxony-Anhalt of 24 April 1997
- Mecklenburg-West Pomerania: State Decree on the Extension of the Notification Requirements for Transmissible Diseases According to the Federal Epidemic Law of 5 February 1992 (a new version for protection against infection is currently being produced)
- Berlin: Decree on the Extension of Notification Requirements for Transmissible Diseases according to the Federal Epidemic Law of 13 January 1997
- Brandenburg: Decree on the Extension of Notification Requirements for Infectious Diseases (Infektionskrankheitenmeldeverordnung, or InfKrankMV) of 14 December 2001 (succeeding the SeuchMV of 1 November 1996)

There is no notification requirement for Lyme borreliosis or positive laboratory results in the other German states.

## References


1. Alpers K, Stark K, Hellenbrandt W, Ammon A (2004) Zoonotische Infektionen beim Menschen – Übersicht über die epidemiologische Situation in Deutschland. Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 47:611-622
2. Ammon A (2001) Risikofaktoren für Lyme-Borreliose: Ergebnisse einer Studie in einem Brandenburger Landkreis. RKI Epidemiol Bull 21:147-149
3. Anonymous (1994) WHO Consultation on development and application of geographical methods in the epidemiology of zoonoses. WHO/CDS/VPH/94.139
4. Anonymous (2004) The vector-borne human infections of Europe – their distribution and burden on public health. WHO Regional Office for Europe, Copenhagen
5. Danielova V (2005) The risk of tick-borne diseases rises in higher altitudes in central Europe (Czech Republik). Abstract. VIII International Potsdam symposium on tick-borne diseases, Jena, March 2005
6. Dorn W, Flügel C, Grübner I (2002) Data on human-biting *Ixodes ricinus* ticks in a region of Thuringia (Germany). Abstract. Int J Med Microbiol 291 [Suppl 33]:219
7. Fingerle V, Schulte-Spechtel U, Göttner G, Hizo-Teufel C, Hofmannn H, Pfister K, Leonhard S, Weber K, Wilske B (2005) Detection of a new *Borrelia burgdorferi* s.l. genospecies A14S from patient material and ticks. Abstract. VIII International Potsdam symposium on tick-borne diseases, Jena, March 2005
8. Fish D (1997) Ecoepidemiology of tick-borne pathogens (*Borrelia burgdorferi*, *Ehrlichia*) in the northeastern United States: implications for Europe? In: Süss J, Kahl O (eds) Tick-borne encephalitis and Lyme borreliosis. Pabst Science Publishers, pp 15-20
9. Fitzner J, Ammon A, Baumann I, Talaska T, Schönberg A, Stöbel K, Fingerle V, Wilske B, Petersen L (2001) Risk factors in Lyme-borreliosis: a German case-control study. Poster. VII. International Postdam symposium on tick-borne diseases
10. Gern L, Humair P-F (2002) Ecology of *Borrelia burgdorferi* sensu lato in Europe. In: Gray JS, Kahl O, Lane RS, Stanek G (eds) Lyme borreliosis – biology, epidemiology and control. CABI International 149-174
11. Kahl O (1995) Betrachtungen zur Ökologie der Lyme-Borreliose in der Region Berlin-Brandenburg. In: Talaska T (ed) Borreliosen in Brandenburg. Tagung Berufsverband Med Mikrobiol. Brandenburg, pp 6-7
12. Kahl O, Gern L, Eisen L, Lane RS (2002) Ecological research on *Borrelia burgdorferi* sensu lato: terminology and some methodological pitfalls. In: Gray JS, Kahl O, Lane RS, Stanek G (eds) Lyme borreliosis – biology, epidemiology and control. CABI International 29-46
13. Kimmig P (2000) Biologie von Zecken. In: Kimmig P, Hassler D, Braun R (eds) Zecken – Kleiner Stich mit bösen Folgen. Ehrenwert, Munich, pp 11-21
14. Kistemann T, Schweickart J, Exner M (2003) Geographische Informationssysteme. In: Krämer A, Reintjes R (eds) Infektionsepidemiologie. Springer, Berlin Heidelberg, pp 109-116
15. Kurtenbach K, Schäfer SM, Michaelis S de, Etti S, Sewell H-S (2002) *Borrelia burgdorferi* sensu lato in the vertebrate host. In: Gray JS, Kahl O, Lane RS, Stanek G (eds) Lyme borreliosis – biology, epidemiology and control. CABI International pp 117-148
16. Nuncio MS, Schouls LM, Pool I van de, Almeida V, Filipe AR (2002) Ecoepidemiology of *Borrelia* spp. on Madeira Island, Portugal. Abstract. Int J Med Microbiol 291 [suppl 33]:212
17. Ohlenbusch A (1996) Beiträge zur Diagnostik und Pathogenese der Lyme-Borreliose und zur Transmission des Erregers Borrelia burgdorferi. Cuvillier, Göttingen
18. Schöder W (2005) GIS, geostatistics, metadatabanking and tree-based models

for data analysis and mapping in environmental and epidemiology. Abstract. VIII International Potsdam symposium on tick-borne diseases, Jena, March 2005
19. Schweickart J, Kistemann T, Leisch H (1998) Der Arbeitskreis "Medizinische Geographie". Eine interdisziplinäre Antwort auf gesundheitsrelevante Fragestellungen. HHG-J 12:245-250
20. Stanek G, O'Connell S, Cimmino M, Aberer E, Kristoferitsch W, Granström M, Guy E, Gray J (1996) European Union concerted action on risk assessment in Lyme borreliosis: clinical case definitions for Lyme borreliosis. Wiener Klin Wochenschr 106:741-747
21. Strle F (1999) Lyme borreliosis in Slovenia. Zentralbl Bakteriol 289:643-652
22. Talaska T, Bätzing-Feigenbaum J (2001) Waldarbeiterstudie Berlin-Brandenburg 2000 zu zeckenübertragenen und anderen Zoonosen. RKI Epidemiol Bull 16:109-110
23. Talaska T, Ober A, Hoffmann C, Schweickart J, Pieper J, Dreissig M (2005) Epidemiological analysis of Lyme borreliosis in the Federal Land Brandenburg with GIS. Abstract. VIII International Potsdam symposium on tick-borne diseases, Jena, March 2005
24. Tylewska-Wierzbanowsk S (1995) Country Reports/WHO Workshop on Lyme borreliosis – diagnosis and surveillance. WHO/CDS/VPH/95.141-1
25. Zeman P, Benes C (2004) A tick-borne encephalitis ceiling in central Europe has moved upwards during the last 30 years: possible impact of global warming? Int J Med Microbiol 293 [suppl 37]:48-54

# Rheumatological and other internal manifestations of Lyme borreliosis

Andreas Krause

Lyme arthritis is one of the most common and clinically important manifestations of Lyme borreliosis. It was discovered and first described about 30 years ago due to a local aggregation of cases in children in the municipalities of Lyme and Old Lyme in Connecticut, USA. In addition, systemic infection with Lyme borreliosis can lead to numerous other rheumatological and generalised internal symptoms, knowledge of which is of particular importance for differential diagnosis of the various clinical pictures.

All three species of *Borrelia burgdorferi* sensu lato known to be human pathogens (*Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii*) can cause internal symptoms. Whether the other *Borrelia* species recently isolated from patients with Lyme borreliosis (e.g. *Borrelia valaisiana*, *Borrelia lusitaniae, Borrelia bissettii*) are pathogens remains unclear.

The most important cause of Lyme arthritis is *Borrelia burgdorferi* sensu stricto. The fact that this is the only species known to cause Lyme borreliosis in the USA explains why Lyme arthritis occurs more frequently there than in Europe or Asia. Although *Borrelia garinii* and *Borrelia afzelii* are considerably more common in Europe than *Borrelia burgdorferi* sensu stricto, various studies of patients with Lyme arthritis have found either comparable frequencies of all three species or predominantly *Borrelia burgdorferi* sensu stricto. These data confirm the particularly athritogenic significance of *Borrelia burgdorferi* sensu stricto while indicating that *Borrelia garinii* and *Borrelia afzelii* play an important role in causing Lyme arthritis in Europe [1, 3, 4, 9, 10, 15].

## Clinical picture

It is impractical to divide the clinical course of Lyme borreliosis into three stages, as was previously common, as this incorrectly suggests that victims must go through all these stages and

that chronic infections become more disseminated with the involvement of more and more organ systems. In truth, Lyme borreliosis follows a variable course with a wide variety of symptoms. It can spontaneously heal at any phase or be arrested through the action of antibiotics. Except for the early cutaneous manifestation, erythema migrans, Lyme borreliosis usually affects a single organ system, presuming antibiotic treatment has followed the diagnosis. Thus for example a patient suffering from acute neuroborreliosis usually does not get Lyme arthritis, and Lyme arthritis patients with antibiotic treatment need not fear developing chronic neuroborreliosis. A possible explanation for this is the organ specificity of the different *Borrelia burgdorferi* species, which attack organ systems preferentially.

It has proven clinically valuable, however, to divide Lyme borreliosis into an early or acute stage (with the pathogenic stages of local and disseminated infection), and a late or chronic stage (persistent infection) showing different clinical pictures and variable degrees of response to antibiotic treatment (Table 1).

**Table 1.** Rheumatological and other internal manifestations of Lyme borreliosis (from [3, 8])

| Organ system/phase | Symptoms | Comments |
|---|---|---|
| *General symptoms* | | |
| Early phase | Feeling of illness, headache, subfebrile temperatures, lymph node swelling | Can be pronounced, no respiratory or gastrointestinal symptoms |
| Chronic phase | As in the acute phase | Mostly less distinct |
| *Heart* | | |
| Early phase | Perimyocarditis | Uncommon, typical AV block with changing grade, usually complete recovery |
| Chronic phase | Dilated cardiomyopathy, ventricular extrasystoles | Questionable, individual cases |
| *Musculoskeletal system* | | |
| Early phase | Arthralgia and myalgia | Rarely fleeting arthritis |
| Chronic phase | Arthritis, myositis, bursitis, enthesitis | Intermittent, uncommon chronic persistent arthritis, mainly in the knee joint, not the axis skeleton, myositis is uncommon |
| *Other* | | |
| Early phase | Hepatomegaly, hepatitis, splenomegaly | Practically never clinically relevant |
| Chronic phase | Vasculitis | Uncommon, can lead to ischaemia |

Particularly in the early phase, many patients have the feeling of being ill. Swollen lymph nodes and subfebrile temperatures are also possible. These symptoms can be especially evident at the beginning of the infection. Respiratory or gastrointestinal symptoms, however, are not part of the symptomatology of Lyme disease and are therefore helpful in differentiating it from other infectious diseases. During the chronic phase, these unspecific signs of infection are less common and weaker.

Lyme carditis is rare, occurring in less that 5% of patients. As heart involvement is often subclinical or only accompanied by unspecific symptoms, it is possible for transitory rhythm disturbances or partial atrioventricular (AV) block to go unnoticed if they are not consciously sought. Dramatic and potentially lethal complete AV blocks, which can require temporary pacemaker treatment, are rare and usually are rapidly cured with antibiotic and steroid therapy. Whether Lyme borreliosis leads to dilated cardiomyopathy (DCMP) in the late phase is still controversial. On the one hand, sporadic borrelia-like structures have been detected in myocardial biopsies, while on the other, serological studies do not suggest an association between dilated cardiomyopathy and Lyme borreliosis.

The involvement of other internal organs is possible but is usually not clinical significant. Hepato- and splenomegaly, increased hepatic enzyme levels, and pathological urine results in Lyme borreliosis patients have been described, but relevant functional disturbances of the corresponding organs have not been documented.

Rheumatological symptoms can occur relatively early in the course of the disease (within weeks), with arthralgia, myalgia, or mild short-term arthritides of individual joints. The typical manifestation of Lyme arthritis occurs however in the chronic phase (several weeks to months after infection). Due to its variable latency, there is no seasonal aggregation of new cases. Arthritis usually manifests as mono- or oligoarthritis, with 85% of cases involving at least one knee joint. The ankle and elbow joints can also be involved, while involvement of finger joints, especially as polyarthritis, has only rarely been recorded. Individual exceptions are the arthropathies that appear in association with acrodermatitis chronica atrophicans and often involve toe or finger joints. Due to the frequency with which these occur together, they are commonly referred to as arthrodermatitis.
The course of Lyme arthritis is usually sporadic, with recurring inflammation interrupted by intervals in which the intensity of symptoms is reduced or they disappear completely. Over time, these intervals may shorten and the arthritis becomes chronic. Synovial

analysis shows acute arthritis with dramatically increased white cell counts of up to 50,000/µl, predominantly neutrophils. Histological findings for the synovial membrane in chronic Lyme borreliosis cannot be distinguished from those in rheumatoid arthritis.

Accompanying manifestations related to the musculoskeletal system are bursitis and tenosynovialitis. Important for the differentiation from other, clinically similar spondylarthritides is that the axial skeleton, as for example in sacroiliitis, is not involved in Lyme arthritis. Moreover, there are no typical symptoms clearly differentiating Lyme arthritis from other inflammatory joint diseases.

In addition to the myalgia frequently found in the early phase, chronic Lyme arthritis can on rare occasions develop into manifest, proximally accentuated myositis leading to muscle weakness and atrophy.

With early diagnosis and treatment, Lyme arthritis has good prognosis and usually heals without further consequences. Erosive courses with chronic arthritis have been reported but are rare. For some patients with Lyme arthritis, cure is not achieved even after multiple antibiotic treatments. In the USA this proportion is estimated at 10% of patients; the epidemiological data for Europe are not adequate for such an estimate. Evidence suggests that these antibiotic-resistant cases involve infection-triggered immunopathological mechanisms [1, 3, 5, 14].

So-called post-Lyme syndrome represents a special rheumatological problem. In spite of regression of inflammatory manifestations with antibiotic therapy, some patients show persistent unspecific symptoms such as arthralgia, myalgia, sleep disturbances, and fatigue, while others report headache as well as memory and concentration disturbances. Some patients show only transient symptoms in the previously inflamed joints. In children, both cognitive and psychiatric changes such as fear and depression have been recorded. Late start of therapy is a risk factor associated with these problems.

Many patients with post-Lyme syndrome are severely handicapped by the continuing symptoms and suffer from significantly reduced quality of life. The course is similar in many ways to that of chronic fatigue syndrome and fibromyalgia. Recent studies have shown that, contrary to the fear nourished by uncertainty and information from less authoritative sources, the risk of contracting this syndrome is very low. A comparison of previous Lyme borreliosis patients and an age-matched control group showed good health in both groups and similar frequency of common unspecific symptoms. Only those patients with, for example, neuroborreliosis who had received inadequate or late antibiotic treatment were more likely to suffer from residual neurological symptoms and pain. Patients with Lyme arthritis at anamnesis complained more of knee pain.

In two placebo-controlled studies, symptoms showed a variable course that was not influenced by antibiotic therapy. It is hypothesised that *Borrelia* infection triggers autoimmune or neurohormonal processes which continue some time after the pathogen itself has been eradicated, and which in turn cause the symptoms experienced. Thus, although an optimal pathogen-oriented treatment is still lacking, irrational use of antibiotic therapy, with its potentially severe side effects should be avoided [1, 2, 3, 6, 7, 15].

## Diagnosis and differential diagnosis

General symptoms, fever and lymph node swelling, are not specific to Lyme disease, opening up a range of differential diagnoses which must be eliminated through anamnesis and looking for more specific manifestations of the disease.

Any myocarditis can be a manifestation of Lyme borreliosis, especially when a high-grade AV block is present. As additional specific symptoms are lacking, anamnesis with tick bite, erythema migrans, and Lyme serology are of particular diagnostic importance. Other causes of myocarditis must be eliminated as well, since positive *Borrelia* serology cannot prove the presence of Lyme borreliosis (see below).

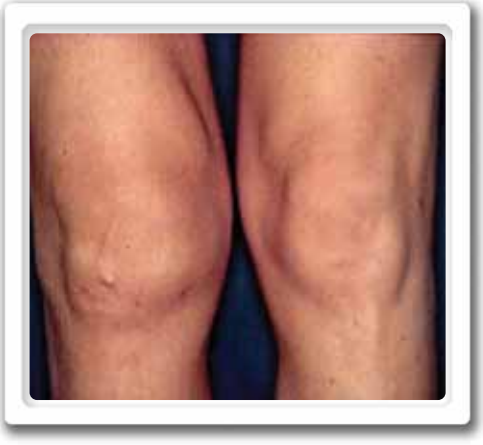
Lyme arthritis must be considered in the differential diagnosis of new cases of mono- or oligoarthritis. The diagnosis must be based on clinical findings and anamnesis, and corroborated by positive serology. Because direct detection of the pathogen is uncommon, generally Lyme arthritis can be diagnosed with certainty only after the elimination of many differential diagnoses.

**Differential diagnosis for Lyme arthritis (most important examples) includes:**

- Gout
- Pseudogout
- Septic arthritis
- Löfgren's syndrome
- Reactive arthritis
- Psoriatic arthritis
- Enteropathic arthritis
- Rheumatoid arthritis (atypical onset)

Typical for Lyme arthritis is uni- or bilateral involvement of the knee joint with synovialitis and usually voluminous effusion (Fig.1). This frequently leads to

**Fig. 1.** Lyme arthritis of the right knee



extended Baker's cysts, which often rupture. In addition, there is also a frequent discrepancy between pronounced local findings and only limited pain. Other large joints of the upper and lower extremities are less commonly involved. In contrast to infection-reactive arthritides, isolated hand and arm joint involvement is quite possible in Lyme arthritis. Otherwise, the symptoms of this disease are ambiguous and unspecific when considered alone. Polyarthritis of smaller joints suggests either a viral or autoimmune-based disease, and involvement of the axis skeleton indicates one of the spondylarthritides.

Important anamnestic clues to the presence of Lyme arthritis include increased risk of exposure to ticks (occupational or especially recreational gardening), previous tick bite, and obviously, a recent untreated or inadequately treated erythema migrans. It is highly unlikely that Lyme arthritis will develop after adequately treated erythema migrans, however if anamnesis indicates exposure to ticks and increased risk of tick bites, the possibility of a new infection should be taken into consideration. Unfortunately, less than 50% of Lyme arthritis patients remember a tick bite, and decidedly fewer, a previous erythema migrans. Previously untreated erythema migrans and neuroborreliosis are now less common due to increased knowledge of the disease and therefore of adequate antibiotic therapy. Occasionally the association of arthritis with acrodermatitis chronica atrophicans can be diagnostically suggestive. Lyme arthritis, however, is often the first and only manifestation of *Borrelia* infection and it is precisely these cases that make diagnosis so difficult [1, 4].

The diagnostic gold standard for an infectious disease is demonstration of the pathogen's presence. Due to this pathogen's characteristics and its low density, determining the presence of *Borrelia burgdorferi* using culture methods is difficult, time-consuming, and, with Lyme arthritis, of low sensitivity and hence unsuitable for clinical diagnostics. Therefore, polymerase chain reaction (PCR) is increasingly used for direct determination of the pathogen. This method can detect the presence of *Borrelia* DNA in synovia and synovial membrane from untreated patients with a sensitivity approaching 80% (Table 2) [9, 12, 13, 16]. Information on its sensitivity with muscle biopsies from myositis patients is not available. False positive results are uncommon, so this test has high specificity. It is important to note however that PCR does not demonstrate the presence of viable pathogens, only pathogen DNA. Nevertheless, a positive PCR result is now interpreted as a demonstration of the presence of the pathogen and thus as an indication for therapy. Various protocols and test systems are now available; however, their suitability to routine diagnostics needs to be validated. PCR is fast becoming an

established component of laboratory diagnostics, particularly for Lyme arthritis [9, 11, 12, 16].

The most common laboratory method for routine diagnosis is serology, i.e. determining the presence of specific antibodies against *Borrelia burgdorferi* (see the relevant chapter in this book). In the early phase of infection, *Borrelia* serology is often still negative, as measurable concentrations of antibodies develop slowly over weeks. Given continued clinical suspicion, a short-term serological examination of disease progression demonstrating seroconversion will confirm the diagnosis. In chronic phases of the illness such as Lyme arthritis, serology is less problematic, as in general there are clearly positive antibody values for a whole series of *Borrelia* antigens. Significantly elevated IgG levels can be found but, in spite of pathogen persistence, specific IgM antibodies are seldom detected. In other cases, IgM antibodies can persist for years after successful treatment and thus cannot be used as an indication for therapy or evidence of persistence of the infection.

Due to the high sensitivity of the test, a false negative serology for Lyme arthritis is very unlikely. However, the lack of IgM antibodies does not exclude a current infection or active illness. The main problem here lies in the interpretation of serological findings: positive IgG values in the chronic phase of Lyme borreliosis are indistinguishable from positive titres after an acute illness or infection (so-called serum scar). This means that the banding pattern on immunoblot does not allow determination of the time of infection or inference of a possible ongoing infection. Immune responses are individual, in part genetically determined and dependent on, for example, extruded pathogen antigens. The antibody response is therefore also variably strong and in some cases nonexistent. The value of a positive *Borrelia* serology is thus dependent on the clinical symptoms. Only in cases with sufficient clinical evidence does positive serology have high diagnostic value. In those with unspecific symptoms however, positive serology is of limited diagnostic value. Due to the high sensitivity of the test, negative

**Table 2.** Sensitivity of methods for direct proof of B. burgdorferi (from [16])

| Material | Sensitivity |
|---|---|
| Skin biopsy (erythema migrans, acrodermatitis) | 50–70% in culture or PCR |
| Liquor (acute neuroborreliosis) | 10–30% in culture or PCR |
| Synovia[a] | 50–70% in PCR |

[a]Even higher sensitivity (up to 80%) by examination of the synovial tissue

serology practically eliminates the diagnosis of Lyme arthritis.

Given the slowness of changes in immune response during the chronic phase, successive examinations over time are seldom helpful, as they produce significant findings only at intervals of several months. In such cases, chronic borreliosis can often be surmised with only a limited probability but seldom proven [3, 5, 13, 16].

An additional difficulty stems from the fact that serological tests are not standardised; tests performed in different laboratories often provide different results, which could be mistakenly interpreted as the titre course or an effect of therapy. This commonly leads to false diagnosis, unnecessary fears of continued disease progression, and unnecessary treatment. Thus Lyme arthritis can be diagnosed with sufficient certainty either by standard anamnesis and clinical symptoms together with positive serology or, in case of less typical symptoms, by demonstrating the presence of the pathogen. A careful rheumatological differential diagnosis is also always necessary.

### Criteria for the diagnosis of Lyme arthritis

- Association with pathognomonic extra-articular manifestations
- Typical pattern of joint involvement
- Exclusion of possible differential diagnoses
- IgG antibodies against *Borrelia burgdorferi*
- Positive *Borrelia* PCR for the synovia or synovial membrane

Depending on the constellation of findings, it is possible to distinguish between certain, probable, and possible Lyme arthritis. This also helps indicate the possible need for critical re-examination of the diagnosis during the course of the disease. If the first four criteria are fulfilled, e.g., when the arthritis is associated with obvious acrodermatitis chronica atrophicans in a patient with highly positive *Borrelia* serology, then the findings are unequivocal and allow definite diagnosis. The same is true for gonarthritis patients with positive serology, and positive *Borrelia* PCR result from synovial fluid once the differential diagnoses have been excluded. In practice however, the suspected diagnosis is based only on criteria 2 and 4, leaving diagnostic uncertainty, and even after eliminating other possible diagnoses, one can only speak of a probable diagnosis; or, for example, if there is also psoriasis that cannot be excluded by other cause, only a diagnosis of possible Lyme arthritis remains [2, 4, 8, 10].

## Therapy

Antibiotic therapy should be started as soon as possible after diagnosis in order to shorten the course of the disease, cure it, and prevent progression or the development of chronic disease. Treatment is stage- and symptom-oriented, with doxycycline, amoxicillin,

and ceftriaxone being the drugs of choice. The current internationally valid standard treatment recommendations are listed in Table 3.

The success rate of antibiotic therapy is high, and therapy-resistant cases are rare. In the acute phase, a single treatment cycle almost invariably leads to a cure, although general, unspecific symptoms sometimes take a long time to disappear. The success rate of the first therapy for Lyme arthritis is about 80%. If patients still have symptoms after several weeks, a second and later at most a third treatment with parenteral antibiotics should be carried out. Recent studies have shown that further antibiotic treatment is inefficacious, as are long-term therapies, high-dose pulse therapies, combination treatment, and the use of antibiotics other than those listed [6,7]. Therapy-resistant Lyme arthritis occurs in about 10% of patients in the USA but is probably less common in Europe. Useful treatment can include intra-articular steroid injections (only after antibiotic therapy!), radiosynoviorthesis, and synovectomy. Otherwise, symptom-oriented treatment is recommended together with an explanation to the patient that the disease will not spread and usually improves slowly over time, possibly many months. The general prognosis for Lyme arthritis and the other forms of Lyme borreliosis discussed is very good [1, 2, 3, 4, 17]. Early studies on Lyme arthritis have shown that 10% of cases heal spontaneously within a year, and that

**Table 3.** Therapy for Lyme borreliosis [1, 2, 3, 4, 10, 17]

| Manifestation | Drug | Dose/day (dose for children[a]) | Duration (days) |
|---|---|---|---|
| General symptoms, acute phase (corresponds to therapy for erythema migrans) | Doxycycline | 1x200 mg or 2x100 mg orally | 14-21 |
| | Amoxicillin | 3x500-750 mg or 2x1,000 mg orally (50 mg/kg) | 14-21 |
| | Azithromycin[b] | 2x500 mg orally | 1 |
| | | 500 mg | 2-5 |
| | Cefuroxime Axetil[b] | 2x500 mg orally | 14-21 |
| Carditis[c] | Ceftriaxone | 1x2 g i.v. | 14 |
| | Cefotaxim | 3x2 g i.v. | 14 |
| | Penicillin G | 4x5 million U i.v. | 14 |
| Arthritis, myositis | Doxycycline | 1x200 mg or 2x100 mg oral | 30 (-40) |
| | Amoxicillin | 3x500-750 mg or 2x1,000 mg oral | 30 (-40) |
| | Ceftriaxone | 1x2 g i.v. | 14-21 |
| | Cefotaxim | 3x2 g i.v. | 14-21 |

[a]Adult dose corresponds to maximum dose
[b]Only in case of allergies or contraindications against doxycyline and amoxicillin
[c]In patients with 1st grade AV Block, the oral therapy (14-21 days) is indicated

antibiotic therapy initiated even several years after disease onset is effective [14].

## Case studies

### First case description

A 38-year-old male patient had complained for 4 months of painful swelling in the right knee. As this problem first occurred after a skiing holiday, trauma was suspected, although the patient could not remember such incident. Magnetic resonance imaging showed minor chondropathy and degenerative changes to the outer meniscus. Symptomatic treatment with diclofenac led to only short-term improvement. Further anamnesis showed no significant previous illness. About a year previously, the patient had been bitten on the left side of the chest by a tick probably while working in the garden. The tick had been removed with a tweezers. Shortly thereafter, erythema occurred which lasted 1 day and then spontaneously disappeared. Indications for another cause of the arthritis, additional rheumatological symptoms, and extra-articular manifestations were neither reported nor found on clinical examination. The latter was unremarkable with the exception of florid, left-sided gonarthritis with substantial synovitis. Arthrosonography confirmed this finding and showed partly edematous, partly proliferative synovialitis, and joint swelling with a poor echo (estimated volume 50 ml) but no popliteal cyst.

Laboratory findings showed limited humoral inflammatory activity with increased erythrocyte sedimentation rate and elevated C-reactive protein (CRP). All other routine parameters were normal. The patient was human leukocyte antigen (HLA)-B27-negative. *Borrelia* serology was highly positive for IgG antibodies against *Borrelia burgdorferi* using enzyme-linked immunosorbent assay (ELISA). Immunoglobulin-M antibodies were not present. These results were confirmed by immunoblot using antibodies against, among others, the proteins OspC, p39, flagellin, VlsE, and p83/100. Analysis of synovial fluid from the right knee joint showed a highly inflamed knee effusion with 10,000 leukocytes/mm$^3$ (90% neutrophils). Probable Lyme arthritis was diagnosed that likely resulted from the reported tick bite. Whether the erythema following the tick bite was erythema migrans remains unclear. Its regression after only 1 day suggests that this was not the case, although erythema migrans is sometimes very pale and can go unnoticed by patients. Oral treatment with 200 mg/day of doxycycline was initiated. Acemetacin (60 mg) as required was given as an anti-inflammatory in addition to cold treatment and physiotherapy. The arthritis healed slowly under this treatment, with symptomatic treatment continuing 4 weeks after the antibiotic therapy was finished. The patient was symptom-free after 3 months.

## Second case description

A 55-year-old, obese female had complained for several months of pain in the right knee. Additionally, she had had lumbar back pain for "as long as she could remember". Anamnesis indicated psoriasis vulgaris from early adulthood and typical erythema migrans on the left upper thigh 6 years previously that had been treated for 2 weeks with an unknown antibiotic. She suffered tick bites every year. Having changed her general practitioner shortly before, the new physician conjectured that her rheumatological problems were caused by Lyme arthritis. A serological examination was positive.

From the clinical rheumatological point of view, both iliosacral joints were markedly swollen and painful when moved, and the right knee joint showed malposition of the valgus with light inflammation. Subsequent anamnesis indicated, at least for earlier years, an inflammatory character to the back pain with a distinct maximum in the early morning and reduction with movement. Radiology showed bilateral sacroiliitis and right-sided gonarthritis.

Laboratory findings showed increased values for erythrocyte sedimentation rate and CRP. Human leukocyte antigen-B27 was present. Borreliosis serology was positive, with low titres of IgG against OspC, flagellin, p58, and p60. Analysis of synovial fluid from the knee showed less than 1,000 leukocytes/$mm^3$, as seen in arthritic irritation.

A diagnosis of active right-sided valgus gonarthritis was made, with probable psoriatic spondylarthritis associated with HLA-B27, and Lyme borreliosis with erythema migrans 6 years previously. There was no evidence of florid Lyme disease.

Sacroiliitis does not manifest in Lyme arthritis. Synovial analysis clearly showed that the knee problems, which could also be related to psoriatic arthritis in differential diagnosis, did not result from Lyme arthritis but, taken together with the other clinical and radiological findings, were apparently caused by active arthritis. The serological findings also speak against Lyme arthritis, as the typical clearly positive IgG antibody response against numerous *Borrelia* proteins, e.g. VlsE and p38/100, was not present. The findings suggest a serum scar after Lyme borreliosis some years previously. The erythema migrans 6 years earlier helps to explain this serological result. An association between *Borrelia* and the current rheumatological problems could not be made due to the time span involved. In addition, the development of a disease course involving an organ cannot be expected after adequate antibiotic treatment of erythema migrans. This case shows how carefully a serological finding must be interpreted in relation to clinical symptoms and other results.

## References


1. Burmester GR, Kamradt T, Krause A (2004) Lyme disease. In: Isenberg DA, Maddison PJ, Woo P, Glass D, Breedveld FC (eds) Oxford textbook of rheumatology. pp 613-620,
2. European Union concerted action on Lyme borreliosis (EUCALB). http://www.dis.strath.ac.uk/vie/LymeEU/index.htm
3. Franz JK, Krause A: Lyme disease (Lyme borreliosis). Best Practice & Research Clinical Rheumatology 17: 241-264, 2003
4. Herzer P, Krause A. Lyme-Borreliose. In: Classen M et al. (Hrsg.) Rationelle Diagnostik und Therapie in der Inneren Medizin, Urban&Fischer, München, 2006, in press
5. Huppertz HI, Krause A. Lyme-Borreliose. Internist 44: 175-183, 2003
6. Kalish RA, et al. Evaluation of study patients with Lyme disease, 10-20-year follow-up. J Infect Dis 183: 453-460, 2001
7. Klempner MS, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 34: 85-92, 2001
8. Krause A, Herzer P. Frühdiagnostik der Lyme-Arthritis. Z f Rheumatol 2005 (in press)
9. Lünemann J, et al. Rapid typing of *Borrelia burgdorferi* sensu lato species in specimens from patients with different manifestations of Lyme borreliosis. J Clin Micrbiol 39: 1130-1133, 2001
10. Nationales Referenzzentrum für Borrelien am Max von Pettenkofer-Institut München. http://pollux.mpk.med.uni-muenchen.de/alpha1/nrz-borrelia/lb/frame-lb.html
11. Nocton JJ, Dressler F, Rutledge BJ, Rys N, Persing DH, Steere AC. Detection of *B. burgdorferi* DNA by polymerase chain reaction in synovial fluid in Lyme arthritis. N Engl J Med 330: 229-234, 1999
12. Priem S, et al. Detection of *Borrelia burgdorferi* by polymerase chain reaction in synovial membrane, but not synovial fluid from patients with persisting Lyme arthritis after antibiotic therapy. Ann Rheum Dis 57: 118-121, 1998
13. Quality standards for the microbiological diagnosis of infectious diseases“: Lyme-Borreliose (MiQ 12 2000 Lyme Borreliosis). http://pollux.mpk.med.uni-muenchen.de/alpha1/nrz-borrelia/miq-lyme/frame-miq-lyme.html
14. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. Ann Intern Med 107: 725-731, 1987
15. Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. J Clin Invest 113: 1093-1101, 2004
16. Wilske B. Diagnosis of Lyme borreliosis in Europe. Vector-Borne Zoonotic Dis 3: 215-227, 2003
17. Wormser GP, et al. Practice guidelines for the treatment of Lyme disease. Clin Infect Dis 31: 1-14, 2000

# Dermatological manifestations of Lyme borreliosis

Elisabeth Aberer

As the organ contacting the environment, the skin is exposed to many external influences. It is also a point of entry for pathogens, which spread via the skin if the barrier function or immune system is no longer intact. In contrast, the transmission of pathogens such as *Borrelia* spp. by arthropods can occur in fully healthy individuals.

Whether Lyme borreliosis (LB) manifests, leading to a specific clinical picture, depends on the genetic disposition of the patient, duration of the tick bite and virulence of the pathogen [20, 22, 26, 28].

Cutaneous symptoms of LB were already recognized many decades before discovery of the pathogen, *Borrelia burgdorferi*, by W. Burgdorfer in 1983. It has also been known since the 1950s that erythema migrans (EM), *Borrelia* lymphocytoma (BL), and acrodermatitis chronica atrophicans (ACA) can be successfully treated with penicillin [19, 21].

## What happens after a tick bite?

The transmission of Lyme borreliosis by ticks, in Europe predominantly by the *Ixodes ricinus* species, is reported in only about 60% of patients [30]. Studies have shown that insects such as mosquitoes, horse flies, sandflies, gnats, and wasps have no organ suitable for survival of the *Borrelia* pathogen as exists in the tick gut [13].

### Unspecific arthropod reactions

After a tick has attached to the skin for an extended time, a local immune reaction occurs that, however, may go unnoticed. An itchy swelling or lump may then form at the site of the bite, from which a tick granuloma can develop. An inflamed patch several centimetres in diameter can also develop and then disappears over a number of days.

## Classic cutaneous symptoms of Lyme borreliosis (Table 1)

### Erythema migrans

Erythema migrans can develop from

an unspecific reaction to a tick bite after only 1–2 days. It is characterised by a slowly expanding inflamed patch. In most cases however there is a latent phase of 4–20 days before the formation of this patch. If not treated, it can increase to 50 cm or more in diameter. The erythema can vary in form from round to oval to irregular. Subjectively it can lead to slight itching. Local lymph node swelling is possible [11, 28].

### Clinical forms of erythema migrans

- The color can be bluish in the center while the edge is bright red. There are often streaky or patchy radial branches at the periphery (Fig. 1).
- A bright red, moving ring can form, with healing in the center (Fig. 2).
- A small vesiculous variant can appear (Fig. 3).
- A homogenous erythema without a marked border can form (Fig. 4).

Children often show a unilateral reddening of a cheek or a stripe that is only visible on some days. Temporary disappearance followed by reappearance of the erythema has been reported. Rubbing a suspect location with a wooden spatula can lead to re-appearance of the erythema.

Fig. 2.

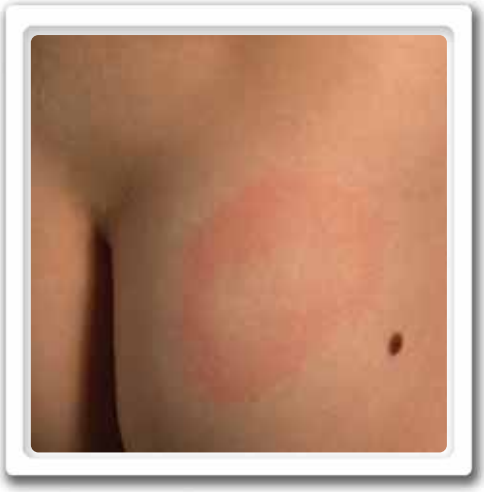


Fig. 1.

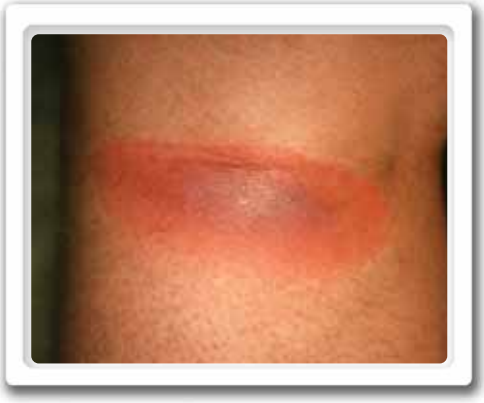


Fig. 3.

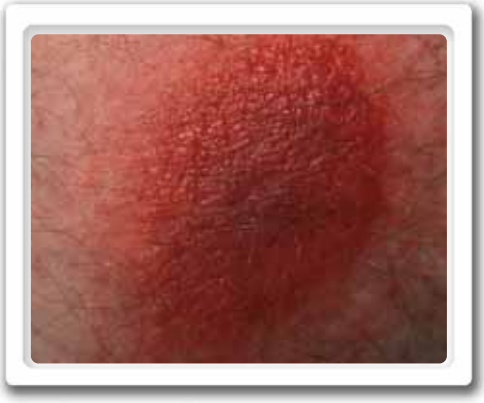
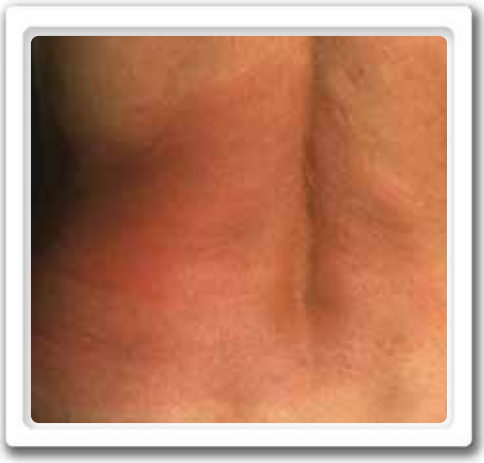


Fig. 4.



## Disseminated early infection

While only about two thirds of patients have only cutaneous infection (erythema migrans minor), the remaining third have simultaneous, generalised symptoms as a sign of haematogenic dissemination [34]. Patients with this EM major form report flu-like symptoms, light fever, headache, joint and muscle pain, swollen lymph nodes, and fatigue. Occasionally, rapid heartbeat and cardiac rhythm disturbances are observed [23]. Multiple EM lesions can also result from haematogenic dissemination as well as from

**Table 1.** Differential diagnosis of Lyme borreliosis

| Cutaneous manifestation | Differential diagnosis |
|---|---|
| *Erythema migrans* | |
| Bluish center | Fixed drug eruption<br>Morphea |
| Homogenous reddening | Fixed drug eruption<br>Erysipeloid<br>Subacute cutaneous lupus erythematosus |
| Vesicular form | Contact dermatitis<br>*Herpes simplex* |
| Multiple erythemata | Urticaria |
| *Borrelia lymphocytoma* | |
| On the ear | Othaematoma<br>Erysipelas<br>Relapsing polychondritis |
| On the mamilla | Breast cancer |
| In other places | Malignant lymphoma |
| *Acrodermatitis chronica atrophicans* | |
| Light red discoloration of the distal extremities | Stasis dermatitis |
| Diffuse livid discoloration | Chronic venous insufficiency<br>Venous thrombosis of the leg |
| Acral form: fingers/toes | Acrocyanosis |
| Livid reticular discoloration on the extremities | Phospholipid antibody syndrome<br>Livedo reticularis/racemosa |

simultaneous infection from multiple tick bites.

### Erythema chronicum migrans

If not treated, EM can last several weeks or months while growing continuously. Spontaneous healing is possible, but its frequency is difficult to determine.

### Borrelia lymphocytoma

Borrelia Lymphocytoma (BL) was first described as lymphadenosis cutis benigna (Bäfverstedt's disease), a pseudolymphoma with mixed polyclonal B and T cell proliferation [6]. After a latent period of several weeks, BL most commonly manifests in children as a reddish to bluish node in the area of the earlobe (Fig. 5), livid reddish discoloration or swelling in the area of the helix, and a flat node one or more centimetres in size in the scrotal area. BL may also occur on one mammilla or areola mammae as an enlargement or nodular lesion (Fig. 6). These nodes can start in an expanding EM that the patient may or may not remember. As EM often occurs on the heads of children, the redness frequently goes unnoticed and a lymphocytoma on the ear is the first recognised symptom.

### Acrodermatitis chronica atrophicans

Acrodermatitis chronica atrophicans begins as an inconspicuous, poorly defined reddening of the skin (Fig. 7), subjectively without symptoms, which becomes slowly darker. It occurs on the distal extremities, elbows, extensor sides

Fig. 5.

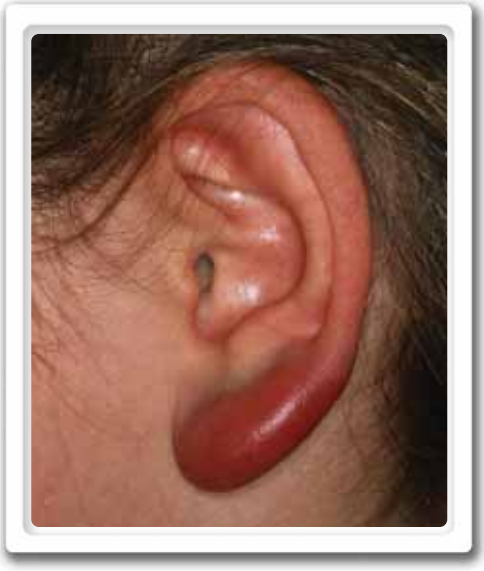


Fig. 6.

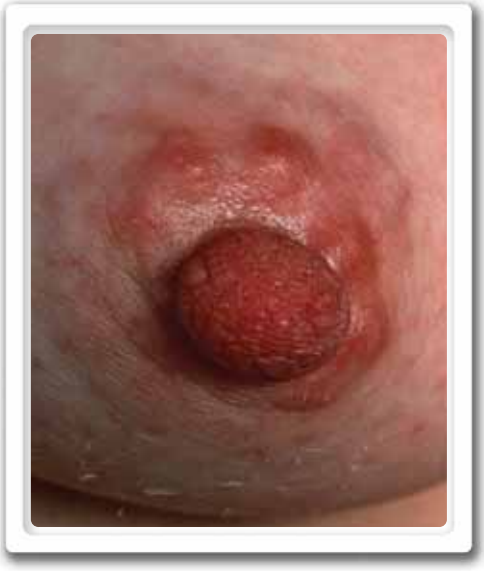
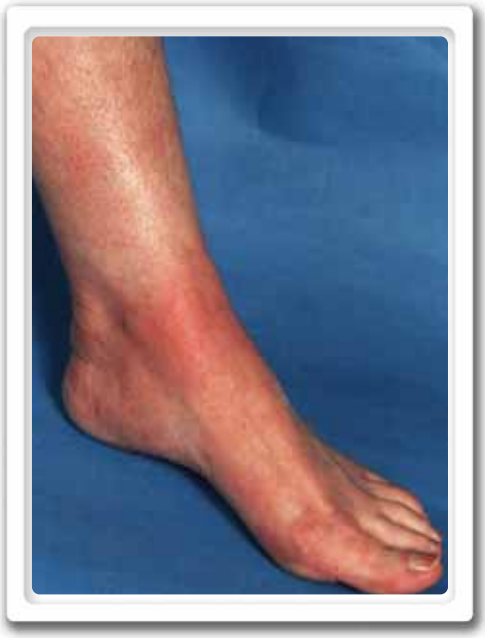


Fig. 7.



of the upper arms, back of the hand, and upper and lower legs.

## Case reports

The development of ACA with neuropathy. A 57-year-old female observed an EM on her lower leg after a tick bite. Correct diagnosis was not made, and the erythema spread to the upper thigh and buttocks over the next 2 years. After 3 years an enlarged lymph node was removed from the right inguinal area which showed unspecific lymphadenitis. The patient reported intermittent redness on the buttocks. Two years later, left-sided bartholinitis appeared and later disappeared. Twelve years after the tick bite, the patient noted a burning sensation and weakness in both thighs as well as a bright red discoloration with undefined borders and light swelling in the left ankle region. This led her to visit a dermatologist, where finally ACA was diagnosed clinically and verified with a biopsy and serology (Fig. 7). The dermatological changes healed under antibiotic treatment with 2 g of ceftriaxone daily for 15 days. The accompanying neuropathy improved slowly.

### Comments

The clinical course of ACA can rarely be studied so clearly. This patient distinctly shows the clinical persistence of the disease as described in the literature [14, 29]. Reactivation of *Borrelia burgdorferi* and the development of ACA were also found in two other patients, one injured on a rose thorn while gardening and the other on a metal spike at home. This indicates that latent infections occur in the extremities where temperature is lower, the optimum *Borrelia* growth temperature being 33–34°C.

Bluish, cushion-like swellings also develop in ACA. Sometimes individual fingers are swollen and discolored blue (Fig. 8). Swelling of the feet can lead to the patient's requiring a larger shoe size. More reticulate discoloration can occur on the extensor sides of the upper arms and thighs. The skin gradually becomes thin and wrinkled, and the bluish

Fig. 8.

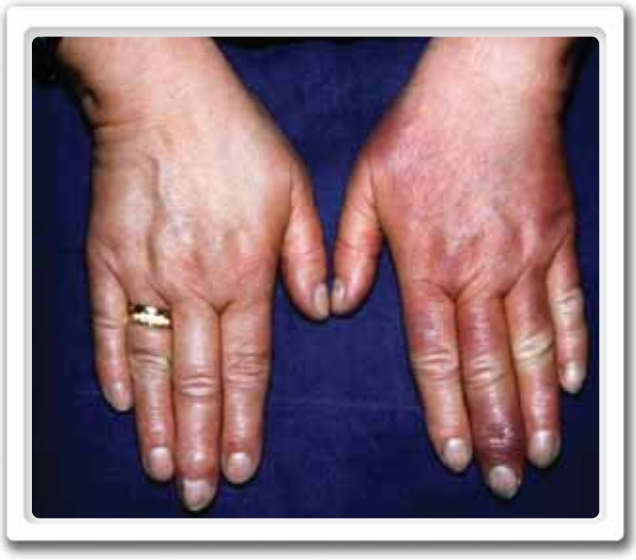
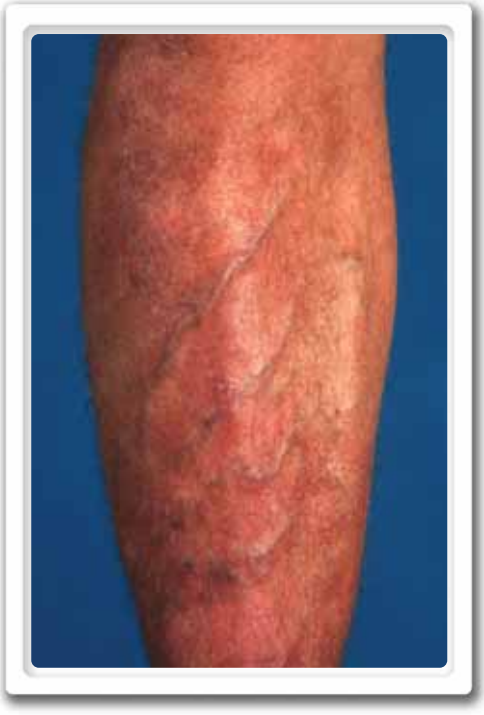


Fig. 9.



discoloration decreases. A thin, almost transparent skin remains, with blood vessels visible underneath (Fig. 9).

The neuropathy often reported by patients does not respond to antibiotics as well as the dermatological changes do [12]. Solid, extra-articular (fibrous) nodes form over the elbows, knees, and finger joints. These can easily be lifted in a fold of the skin and, unlike rheumatic nodes, are not painful.

Skin sclerosis is possible in shin and underarm extensor regions (tibial and ulnar bands) where ACA is present. This can be differentiated from linear scleroderma by its undefined edges. In addition to classic ACA, anetodermic lesions can occur also.

## Genospecies-specific courses of Lyme borreliosis

The species *Borrelia burgdorferi* sensu lato is subdivided into three genospecies: *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii,* and *Borrelia garinii;* all three of these can cause EM. Various genospecies-specific courses of EM have been reported [7]. Of these, only *Borrelia burgdorferi* sensu stricto is found in the USA. This species causes the least number of *Borrelia* infections in Europe. In our study in the Vienna area, *Borrelia burgdorferi* sensu lato was isolated from the skin of 28 patients. Of these, 23 had *Borrelia afzelii* (17 with EM, six with ACA), three had *Borrelia garinii* (all with EM), and two had *Borrelia burgdorferi* sensu stricto

infection (one with EM, one positive blood culture in disseminated LB). In and around the city of Graz, Austria 11 strains were isolated, of which five were *Borrelia garinii* from EM and six *Borrelia afzelii* (four EM, one ACA, one morphea). A similar pattern has been found in other studies [25]. For the other cutaneous symptoms, BL is most commonly caused by *Borrelia afzelii* (eight of nine cases) [18] and ACA almost exclusively by *Borrelia afzelii* [24], with mixed infections also possible. This explains why no secondary cutaneous clinical courses (e.g. BL or ACA) of LB have been found in the USA, with the exception of patients who were infected in Europe. Differences between EM caused by *Borrelia burgdorferi* sensu stricto and EM caused by *Borrelia afzelii* are: shorter incubation time (4 days vs 14), more frequent localisation in the trunk area (50% vs 27%), central lightening of colour in only a third of patients compared with two thirds of *Borrelia afzelii* infections, and more frequent systemic signs and seropositivity (89% vs 30%) [30]. In addition, EM caused by *Borrelia afzelii* differs from that caused by *Borrelia garinii*–infections with the former occurring more often in June and with *Borrelia garinii* in September, while *Borrelia afzelii* EM appears more often on the extremities and less often on the trunk. Growth of the reddened area by *Borrelia afzelii* reaches 3 cm/day and by *Borrelia garinii* 12.5 cm/day. Generalised and local symptoms occur in three quarters of *Borrelia garinii* patients but only a third of *Borrelia afzelii* patients [5, 17].

## Symptoms after erythema migrans

A case of EM is clinically cured with 2–3 weeks of antibiotic treatment, sometimes within a few days and sometimes towards the end of the therapy period. Occasionally there is still a livid erythema after 3 weeks that fades several weeks later. If severe symptoms exist at the start of therapy, they may continue after the end of medication and completely disappear several weeks later. Severe symptoms can also arise during therapy (Herxheimer-like reaction) and should not be interpreted as treatment failure. The prescribed antibiotic must not be stopped. Duration of therapy should be increased to 3 weeks if originally set at 2 weeks based on apparently localised EM. About 7% of patients suffer relapse in the following weeks (for up to 6 months) with repeated wandering joint and muscle pain and headaches that can last for days. These symptoms also disappear after several weeks. In severe cases, an additional course of antibiotics is usually prescribed. The occurrence of arthritis or neuroborreliosis has not been observed in LB patients with correctly treated EM over an observation period of 20 years. In two patients, erythema nodosum (EN) accompanied the EM.

### Case report of erythema migrans with erythema nodosum (EN)

A 34-year-old male had a 7 x 5 cm annular erythema on the right leg for 2 months and painful, bright red nodes on both shins for a week. He could not remember being bitten by a tick. He also complained of joint pain that had lasted for a year. X-ray of the thorax showed bilateral lymphadenopathy (Löfgren's syndrome). Pathological findings included C-reactive protein of 89, erythrocyte sedimentation rate of 44, and angiotensin-converting enzyme of 57.4 U (normal range 18.0–55.0 Units). Immunoblot for *Borrelia* antibodies was negative before and after therapy. Polymerase chain reaction (PCR) for *Borrelia* in the urine was positive on five occasions from the 5th to 150th day of treatment but negative on days 210 and 360 [2]. Anti IgM and IgG antibodies specific for *Ehrlichia* were positive (1:64) over 5 months without a change in titre, but PCR for *Ehrlichia* in the blood was negative. A pulmonary specialist diagnosed pulmonary sarcoidosis I. Bronchoscopy and bronchoalveolar lavage showed no evidence of pulmonary involvement. A double therapy cycle of 200 mg of doxycyline for 3 weeks and glucocorticosteroid treatment led to cure of the EM, EN, and lymph node swelling.

**Comments**

Due to the steady titre values and negative PCR, ehrlichiosis could be eliminated as a cause of the EN and bilateral lymphadenopathy. There were also no fever or unusual laboratory values such as leukopenia. The conclusion is that the EN is associated with the EM. Arthralgia, myalgia, memory disturbances, fatigue syndrome, conjunctivitis, febrile periods, headache, tinnitus, and acute loss of hearing are referred in the literature as symptoms of post-Lyme syndrome. Seltzer et al. [27] examined the symptom profiles of 212 patients 1–11 years after LB and compared them to a group with equivalent age and place of residence. No differences were found between the groups. It is still unclear how to classify this clinical picture. Perhaps the symptoms are overrated and were influenced by fear of further organ involvement from the LB.

## Culture and polymerase chain reaction confirmed atypical forms

Clinical observations have shown that the potency of *Borrelia burgdorferi* infection lies in immune stimulation of B cells and the induction of cutaneous sclerosis.

### Cutaneous lymphoma

The role of *Borrelia burgdorferi* in atypical cutaneous proliferation, pseudolymphoma, and malignant lymphoma has been confirmed in many publications. As a cause of malignant lymphoma, it acts similarly to *Helicobacter pylori* or Epstein-Barr

virus. A targeted antibiotic (virostatic) therapy can lead to cure in some cases. Comparative studies with lymphoma patients in the USA or Asia showed no association with *Borrelia burgdorferi* [16, 32], while those in Austria, France, Scotland, Denmark, and Germany clearly did [9, 15].

## Circumscribed scleroderma (morphea) and lichen sclerosis

Starting in 1985, an association between *Borrelia* infection and the development of morphea was described in Switzerland, Germany, and Austria by various authors from Basel, Munich, Berlin, and Vienna. According to PCR results and positive *Borrelia* cultures from skin biopsies and their typing, aggregation of *Borrelia*-induced morphea cases was geographic in nature [4, 10]. Only *Borrelia afzelii* could be isolated from both morphea and lichen sclerosis.

## Diagnostic and therapeutic management of these patients

In principle, circumscribed scleroderma has a variety of causes. It can occur after insect bites, immunisations, and trauma. Independently of this, a childhood and early adolescent form exists which is localised to the lines of Blaschko (e.g. linear forms, morphea en coup de sabre) and therefore could be induced during embryonal development. Congenital atrophic morphea has been cured by treatment with ceftriaxone [1]. The significance of *Borrelia* to the emergence of this disease is not yet clear.

*Borrelia* serology using immunoblot and *Borrelia* PCR of a skin biopsy are recommended for diagnosis. The IgG ELISA titres are often negative, but specific bands appear on immunoblot. In *Borrelia* associated skin sclerosis it is likely that cellular immune reactions predominate, as shown by lymphocyte proliferation tests. Stimulation of lymphocytes by *Borrelia burgdorferi* was also observed in seronegative patients with morphea and ACA patients with fibrous nodules [3].

Patients with morphea react better to systemic therapy with ceftriaxone (for 20 days) than to oral treatment; the same has been observed in seronegative patients. This is the treatment of choice for all patients with progressive morphea and distinct lilac rings, particularly in children.

## Dermatoses in which *Borrelia burgdorferi* infection must be considered

All patients with unexplained sclerosis [31], myositis, and especially dermatomyositis must be examined for the presence of *Borrelia burgdorferi* infection. In the literature this infection has also been associated with cases of livid erythema with myopathy, relapsing febrile panniculitis, annular erythema, trigger-finger, Schönlein-Henoch and thrombocytopenic purpura, granulomatous thrombophlebitis, temporal arteritis, sarcoidosis, pityriasis rosea, pityriasis lichenoides, anetoderma, Buschke's scleroderma, eosinophilic

fasciitis (Shulman's syndrome), facial hemiatrophy, granuloma annulare, and interstitial granulomatous dermatitis.

## Diagnosis of Lyme borreliosis

Consult the European Union for Concerted Action on Risk Assessment in Lyme Borreliosis guidelines at http://www.oeghmp.at/eucalb/.

### Erythema migrans

Erythema migrans is usually diagnosed on purely clinical criteria! Anamnesis of a growing red patch is the most important feature; patients frequently do not remember a bite. This patch should be >5 cm. The patch may have to be observed over the course of a week, with a control examination sometimes required. Histological examination is unspecific, showing perivascular lymphohistiocytic infiltrate with sparse eosinophils. Biopsy is needed only when the clinical picture is unclear.

Serological examination can show positive IgM antibodies, which should however be confirmed by immunoblot. Depending on the duration of the erythema, IgG antibodies may also be present. These could also result from an earlier infection of known or unknown origin (seroprevalence in healthy individuals 10–30%). Since only 50% of patients have antibodies, *serological examination is not required for diagnosis*.

### *Borrelia* lymphocytoma

The clinical diagnosis is also decisive here. Biopsy shows polyclonal B and T cell proliferation. In children, biopsy can be avoided if there is a classic manifestation. In cases of lymphocytoma of the mammilla or other localised areas, a skin biopsy should be carried out. In about 70% of patients, antibodies can be demonstrated. Healing after antibiotic therapy can be slow, taking several weeks.

### Acrodermatitis chronica atrophicans

Positive IgG titre, typical clinical picture, and histological examination are crucial to diagnosing this manifestation of LB. Histology shows atrophy of the epidermis and dermis, telangiectasia, and occasionally band-like lympho-histiocytic infiltrates with plasma cells. The cure by therapy is very slow, requiring months. Follow-up after a year is recommended, possibly also a repeat biopsy to determine whether inflammatory infiltrates are present.

### Serology

*Borrelia* serology will be dealt with in detail in the chapter on diagnosis. Here only a possible serological picture for EM is presented (Table 2).

## Therapy

Treatments of LB with oral penicillin, tetracycline, cefuroxime axetil, and azithromycin are equally effective (Table 3). With antibiotic treatment, EM with or without accompanying symptoms heals without complications

**Table 2.** Serological picture in erythema migrans (Borrelia antibodies)

| ELISA IgM | ELISA IgG | IB IgM | IB IgG | Interpretation |
|---|---|---|---|---|
| - | - | - | - | EM/no borreliosis? |
| + | - | - | - | EM/unspecific |
| + | - | + | - | EM Confirmation/antibody persistence from older infections |
| + | + | + | +/- | EM Confirmation/reinfection |
| - | + | - | + | Older infections |
| +/- | - | + | + | EM Confirmation/older infections |

in >90% of cases. If treatment fails, headaches, joint and muscle pain, and fatigue can occur for a period of 6 months but usually disappear within a few weeks. Secondary manifestations are extremely rare after appropriate treatment. The most recent publications on duration of therapy show that post-Lyme syndrome occurs in 5-10% of patients independently of whether treatment lasts 2 or 3 weeks [33]. Three-week treatment is recommended if the erythema lasts >2 months and in case of severe symptoms. The choice of antibiotic depends on patient compatibility and possible allergies. Tetracyclines should be avoided if exposure to sun is likely, and are also contraindicated during pregnancy, while breast-feeding, and for children <8 years old. In cases accompanied by fever, severe general symptoms, or when co-infection with ehrlichiosis is suspected, tetracycline is the treatment of choice (Table 3).

**Table 3.** Antibiotic therapy for Lyme borreliosis

| | | | |
|---|---|---|---|
| *Erythema migrans* | | | |
| Penicillin V | 3x1 | 1,5 Mio | 14-20 days |
| Amoxicillin | 3x1 | 500-750 mg | 14-20 days |
| Doxycycline | 1x1 | 200 mg | 14-20 days |
| Azithromycin | 2x1<br>1x1 | 500 mg<br>500 mg | 1st day<br>7 days |
| *Borrelia lymphocytoma* | | | |
| Oral therapy | | | 20 days |
| *Acrodermatitis chronica atrophicans* | | | |
| Oral therapy | | | 30 days |
| Or ceftriaxone | 1x1 | 2g | 20 days |
| **A second treatment with an alternative antibiotic is recommended for the following findings:** | | | |
| Positive serology and unspecific symptoms | Severe suffering | | |
| EM | Continuing arthralgia, headaches for >3 months | | |
| BL | No cure within 3 months | | |
| ACA | Histologically verified infiltrate after 1 year | | |

## Prognosis

Pathogen persistence was found in three of 48 patients with late symptoms [29]. Re-infection is possible. It is noteworthy that chronic *Borrelia* infections stimulate lymphocytes, with the possibility of monoclonal lymphocytic proliferation. Persistent, severe disease courses could be due to infection with exceptionally pathogenic *Borrelia burgdorferi* clones (RST1 isolate [8]). In addition to interspecies variation, additional genetic factors can lead to organ-specific manifestations. In general, there is no danger of developing LB when EM is properly treated. Therapy can fail however, when less efficacious antibiotics such as first-generation cephalosporins, roxithromycin, or erythromycin are used in an irregular manner; for example if therapy is interrupted on weekends, as sometimes happens with ceftriaxone therapy. Uncertainty concerning serology remains, as the variable prevalence in the population and persistence of antibodies after therapy complicate the assessment of serology results, making diagnosis difficult.

## References


1. Aberer E, Weissenbacher G (1997) Congenital anetoderma induced by intrauterine infection? Arch Dermatol 133(4):526-527
2. Bergmann AR, Schmidt BL, Derler AM, Aberer EJ (2002) Importance of sample preparation for molecular diagnosis of lyme borreliosis from urine. J Clin Microbiol 40(12):4581–4584
3. Breier P, Klade H, Stanek G, Poitschek C, Kirnbauer R, Dorda W, Aberer E (1996) Lymphoproliferative responses to Borrelia burgdorferi in circumscribed scleroderma. Br J Dermatol 134(2):285–291
4. Breier FH, Aberer E, Stanek G, Khanakaha G, Schlick A, Tappeiner G (1999) Isolation of Borrelia afzelii from circumscribed scleroderma. Br J Dermatol 140(5):925–930
5. Carlsson SA, Granlund H, Jansson C, Nyman D, Wahlberg P (2003) Characteristics of erythema migrans in Borrelia afzelii and Borrelia garinii infections. Scand J Infect Dis 35(1):31–33
6. Colli C, Leinweber B, Müllegger R, Chott A, Kerl H, Cerroni L (2004) Borrelia burgdorferi-associated lymphocytoma cutis: clinicopathologic, immunophenotypic, and molecular study of 106 cases. J Cutan Pathol 31(3):232–240
7. Dam AP van, Kuiper H, Vos K, Widjojokusumo A, Jongh BM de, Spanjaard L, Ramselaar AC, Kramer MD, Dankert J (1993) Different genospecies of Borrelia burgdorferi are associated with distinct clinical manifestations of Lyme borreliosis. Clin Infect Dis 17(4):708–717
8. Dolan MC, Piesman J, Schneider BS, Schriefer M, Brandt K, Zeidner NS (2004) Comparison of disseminated and nondisseminated strains of Borrelia burgdorferi sensu stricto in mice naturally infected by tick bite. Infect Immun 72(9):5262–5266
9. Fouchardiere A de la, Vandenesch F, Berger F (2003) Borrelia-associated primary cutaneous MALT lymphoma in a nonendemic region. Am J Surg Pathol 27(5):702–703
10. Fujiwara H, Fujiwara K, Hashimoto K, Mehregan AH, Schaumburg-Lever G, Lange R, Schempp C, Gollnick H (1997) Detection of Borrelia burgdorferi DNA (B garinii or B afzelii) in morphea and lichen sclerosus et atrophicus tissues of German and Japanese but not of US patients. Arch Dermatol 133(1):41–44
11. Hengge UR, Tannapfel A, Tyring SK, Erbel R, Arendt G, Ruzicka T (2003) Lyme borreliosis. Lancet Infect Dis 3(8):489-500; Erratum in: Lancet Infect Dis 3(12):815
12. Kindstrand E, Nilsson BY, Hovmark A, Pirskanen R, Asbrink E. Peripheral neuropathy in acrodermatitis chronica atrophicans - effect of treatment. Acta Neurol Scand. 2002 Nov;106(5):253
13. Kosik-Bogacka D, Kuzna-Grygiel W, Bukowska K (2004) The prevalence of spirochete Borrelia burgdorferi sensu lato in ticks Ixodes ricinus and mosquitoes Aedes spp. within a selected recreational area in the city of Szczecin. Ann Agric Environ Med 11(1):105–108
14. Kuiper H, Dam AP van, Spanjaard L, Jongh BM de, Widjojokusumo A, Ramselaar TC, Cairo I, Vos K, Dankert J (1994) Isolation of Borrelia burgdorferi from biopsy specimens taken from healthy-looking skin of patients with Lyme borreliosis. J Clin Microbiol 32(3):715–720
15. Leinweber B, Colli C, Chott A, Kerl H, Cerroni (2004) Differential diagnosis of cutaneous infiltrates of B lymphocytes with follicular growth pattern. Am J Dermatopathol 26(1):4–13
16. Li C, Inagaki H, Kuo TT, Hu S, Okabe M, Eimoto T (2003) Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathologic study of 24 asian cases. Am J Surg Pathol 27(8):1061–1069
17. Logar M, Ruzic-Sabljic E, Maraspin V, Lotric-Furlan S, Cimperman J, Jurca T, Strle F (2004) Comparison of erythema migrans caused by Borrelia afzelii and Borrelia garinii. Infection 32(1):15–19
18. Maraspin V, Cimperman J, Lotric-Furlan S, Ruzic-Sabljic E, Jurca T, Picken RN, Strle F

(2002) Solitary borrelial lymphocytoma in adult patients. Wien Klin Wochenschr 31;114(13-14):515–523
19. Miescher G, Storck H (1951) Successful penicillin therapy of acrodermatitis atrophicans. Dermatologica 102(4-6):370–371
20. Nahimana I, Gern L, Blanc DS, Praz G, Francioli P, Peter O (2004) Risk of Borrelia burgdorferi infection in western Switzerland following a tick bite. Eur J Clin Microbiol Infect Dis 23(8):603–608
21. Paschoud JM (1958) Lymphadenosis benigna cutis as a communicable disease. IV. Discussion of study results. Part B: penicillin therapy; lymphadenosis benigna cutis & erythema chronicum migrans, connections & etiology; summary. Der Hautarzt 9(7):311–315
22. Piesman J (1993) Dynamics of Borrelia burgdorferi transmission by nymphal Ixodes dammini ticks. J Infect Dis 167(5):1082–1085
23. Pikelj-Pecnik A, Lotric-Furlan S, Maraspin V, Cimperman J, Logar M, Jurca T, Strle F (2002) Electrocardiographic findings in patients with erythema migrans.Wien Klin Wochenschr 31;114(13-14):510–514
24. Rijpkema SG, Tazelaar DJ, Molkenboer MJ, Noordhoek GT, Plantinga G, Schouls LM, Schellekens JF (1997) Detection of Borrelia afzelii, Borrelia burgdorferi sensu stricto, Borrelia garinii and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. Clin Microbiol Infect 3(1):109–116
25. Schaarschmidt D, Oehme R, Kimmig P, Hesch RD, Englisch S (2001) Detection and molecular typing of Borrelia burgdorferi sensu lato in Ixodes ricinus ticks and in different patient samples from southwest Germany. Eur J Epidemiol 17(12):1067–1074
26. Seinost G, Dykhuizen DE, Dattwyler RJ, Golde WT, Dunn JJ, Wang IN, Wormser GP, Schriefer ME, Luft BJ (1999) Four clones of Borrelia burgdorferi sensu stricto cause invasive infection in humans. Infect Immun 67(7):3518–3524
27. Seltzer EG, Gerber MA, Cartter ML, Freudigman K, Shapiro ED (2000) Long-term outcomes of persons with Lyme disease. JAMA 2;283(5):609–616
28. Stanek G, Strle F (2003) Lyme borreliosis. Lancet 15;362(9396):1639–1647
29. Strle F, Cheng Y, Cimperman J, Maraspin V, Lotric-Furlan S, Nelson JA, Picken MM, Ruzic-Sabljic E, Picken RN (1995) Persistence of Borrelia burgdorferi sensu lato in resolved erythema migrans lesions. Clin Infect Dis 21(2):380–389
30. Strle F, Nadelman RB, Cimperman J, Nowakowski J, Picken RN, Schwartz I, Maraspin V, Aguero-Rosenfeld ME, Varde S, Lotric-Furlan S, Wormser GP (1999) Comparison of culture-confirmed erythema migrans caused by Borrelia burgdorferi sensu stricto in New York State and by Borrelia afzelii in Slovenia. Ann Intern Med 5;130(1):32–36
31. Wackernagel A, Bergmann AR, Aberer E (2005) Acute exacerbation of systemic scleroderma in Borrelia burgdorferi infection. J Eur Acad Dermatol Venereol 19(1):93–96
32. Wood GS, Kamath NV, Guitart J, Heald P, Kohler S, Smoller BR, Cerroni L (2001) Absence of Borrelia burgdorferi DNA in cutaneous B-cell lymphomas from the United States. J Cutan Pathol 28(10):502–507
33. Wormser GP, Ramanathan R, Nowakowski J, McKenna D, Holmgren D, Visintainer P, Dornbush R, Singh B, Nadelman RB (2003) Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 6;138(9):697–704
34. Wormser GP, McKenna D, Carlin J, Nadelman RB, Cavaliere LF, Holmgren D, Byrne DW, Nowakowski J (2005) Brief communication: hematogenous dissemination in early Lyme disease. Ann Intern Med 3;142(9):751–755

# Neuroborreliosis

Reinhard Kaiser

For laymen the terms *Borrelia* infection, borreliosis, and neuroborreliosis are commonly used as synonyms. The reason for this confusion is the incorrect assumption that unspecific, generalised symptoms are usually caused by a disease of the nervous system, and that a borrelial infection discovered by chance during examination is causing these symptoms. Here the uncritical request for *Borrelia* serology analysis in response to unspecific symptoms has a high *a priori* chance of returning positive results due to the high seroprevalence in the population. Even using an optimal test method, such as ELISA using recombinant proteins from three *Borrelia* spp., only a negative result provides useful information. The following is a critical discussion of the typical neurological symptoms of neuroborreliosis, the problem of unspecific symptoms, and post-Lyme syndrome.

## Pathogenesis

Laboratory investigations have shown that *Borrelia burgdorferi* sensu lato has a natural affinity for cells of the central and peripheral nervous systems, where it concentrates in the region of the meninges and nerve roots. This because these cells have surface proteoglycans and galactocerebrosides, which provide binding sites for the outer surface protein Osp A of *Borrelia burgdorferi* sensu lato [2]. The OspA lipoprotein is also of particular importance in the pathogenesis of neuroborreliosis. Initially it induces proliferation of astrocytes and lymphocytes via the synthesis of pro-inflammatory cytokines (e.g. IL-1, IL-6, and IL-8). Later however, it causes their apoptosis from increased production of tumour necrosis factor alpha [30]. The general feeling of illness and, in cases of acute neuroborreliosis, frequently pain can be explained by the increased synthesis of immunotransmitters and their attachment to neuronal receptors, but the mechanisms leading to the disturbance of neurological function in chronic forms are not yet known. Antibodies and autoreactive T lymphocytes specific for neuronal and glial proteins are commonly

found, but their significance is also unclear. A correlation between these laboratory findings and the clinical course and prognosis of the disease has not been established [14, 18].

## Clinical picture

*Borrelia* infection of the nervous system is usually acute, and seldom chronic. Symptoms lasting for ≥6 months are criteria for the chronic form.

### Diseases affecting the peripheral nervous system

#### Meningopolyradiculoneuritis (Bannwarth's syndrome)

After erythema migrans, meningopolyradiculoneuritis (Bannwarth's syndrome) is the second most common manifestation of acute *Borrelia* infection in Europe [19, 34]. Diagnosis is characterised by the effects of *Borrelia*-induced inflammation in the meninges (meningitis), spinal cord root (radiculitis), and cranial nerves, which may occur concurrently or consecutively but also alone.

#### Meningitis

Meningitis is relatively uncommon in adult Europeans but can be a major problem in children [4, 7]. Headaches often are only moderately pronounced, however their intensity can fluctuate substantially within a few days or weeks. Meningitis, fever, nausea, vomiting, and vertigo are uncommon.

Suspected diagnosis is confirmed by demonstrating pleocytosis in the CSF and an elevated *Borrelia*-specific antibody index.

#### Radiculitis

The symptoms of radiculitis develop on average within 4–6 weeks (range 1–12 weeks) after the tick bite. The first symptoms are predominantly night time radicular pain in the extremities and circling pain in the trunk that do not respond to simple analgesics. Maximum pain is often reached within a few hours or days. It reaches an intensity that patients report not having previously experienced, is constantly present, and often prevents sleep. As there is usually no demonstrable neurological deficit during this phase, the possibility of borreliosis is often not considered. Most patients however show sensory neurological irritation and neurological deficits within a few weeks: symptoms involving sensitivity to stimulus are commonly reported although corresponding deficits are rare. Occasionally the segment with the most intense pain later becomes numb. Paresis is more common than sensitivity disturbances and is typically distributed asymmetrically, more commonly affecting the limb bitten by the tick or where the erythema migrans occurred. In cases of radiculitis, the pain usually decreases within a few days after beginning antibiotic treatment. Differential diagnoses for such extreme pain and pleocytosis are *Varicella zoster* virus reactivation and carcinomatous meningitis. Pathological findings on

magnetic resonance imaging are uncommon for radiculitis.

### Cranial nerve paresis

About 60% of patients with Bannwarth's syndrome have cranial nerve paresis. With the exception of the olfactory nerve, reports indicate that any brain region can be affected. In most cases, the paresis occurs within a few weeks after the erythema migrans. The facial nerve paresis is by far the most common, affecting about 80% of cases, and is bilateral in 40% of these (acute > chronic course). Facial paresis in neuroborreliosis is occasionally isolated, in which case it cannot be differentiated from idiopathic cases. Clarification is best made by analysis of the CSF. If pleocytosis is absent–even with the presence of *Borrelia*-specific IgG antibodies in the serum–an idiopathic case is most likely. If *Borrelia*-specific IgM antibodies are found in the serum, it is not possible to exclude borreliosis as a cause of the paresis, even in the absence of pleocytosis and antibodies in the CSF. In such cases (particularly if there are also *Borrelia*-specific IgG antibodies in the serum), a 14-day oral course of antibiotics (e.g. 1x200 mg doxycycline or 3x750 mg amoxicillin per day) is recommended. As with idiopathic facial paresis, about 5–10% of patients suffer from subsequent disturbances [5, 17].

Second in frequency after facial paresis is abducens paresis, which is bilateral in about 10% of cases. All the other cranial nerves are affected much less often. The significance of *Borrelia* infection in vestibulocochlear nerve or vestibular organ diseases is still unconfirmed. A number of large epidemiological studies were unable to show a definite causal relationship between acute vertigo and serologically determined borreliosis infection [27]. Only in a single study of patients with acute hearing loss did analysis of CSF by lumbar puncture reveal inflammatory changes. It is noteworthy that patients with such inflammatory CSF signs and positive *Borrelia* serology responded better to treatment and/or had better prognoses than those with acute idiopathic hearing loss [13]. These findings suggest a recommendation not only for *Borrelia* serology but also for lumbar puncture in cases of acute hearing loss. Chronic neuroborreliosis can occasionally also be associated with a significant loss in hearing.

### Polyneuritis/polyneuropathy

The term polyneuritis is used to describe cases in which there is evidence of an inflammatory cause of a polyneuropathy (PNP). In PNP, disturbances in neurological function or dysaesthesia are usually distal, affecting the extremities. In European patients distal polyneuritis resulting from *Borrelia* infection is almost always found in association with acrodermatitis chronica atrophicans (ACA) [16]. All other cases of *Borrelia*-induced polyneuritis should be considered with scepticism, as most

publications on the subject lack sufficient proof and an adequate differential diagnosis is not defined. The major hindrances to collecting evidence are the large number of possible causes of PNP combined with the very high seroprevalence of *Borrelia* antibodies in the general population (between 10% and 30% depending on region). Additionally, only a small proportion of *Borrelia* infections (<5%) manifest clinically. Thus the association of PNP and serum *Borrelia* antibodies is more coincidental than causal in most patients. Only with additional evidence of pathological CSF changes can causality be accepted. This however, would suggest a diagnosis of radiculitis, because CSF involvement is not generally associated with the predominantly distal changes seen in a typical PNP. Using such diagnostic and therapeutic methods, we have been unable to find a proven *Borrelia*-induced PNP in the last 15 years.

## Diseases of the central nervous system

### Encephalitis

Clinical symptoms of borreliosis involving the central nervous system have seldom been observed. When present, they occur more frequently in the chronic than the acute course. Cases of encephalitis involving *Borrelia* show no characteristics pointing to such aetiology. Reported disturbances have included quantitatively and qualitatively reduced states of consciousness, focal and generalised seizures, mono- and hemiparesis, hemianopsia, aphasia, dysarthria and coordination disturbances. Individual cases have presented with choreiform and dystonic motor disturbances, transitory Parkinson's disease-like symptoms, pseudotumour cerebri in association with autoimmune thyroiditis, obstructive hydrocephalus, temporary organic brain syndrome with memory and concentration disturbances, opsoclonus, and cerebral "pseudo-lymphoma" (lymphocytic infiltrate as a consequence of local collection of *Borrelia* in the cerebrum).

### Myelitis

The clinical symptoms of the rare, acute *Borrelia* myelitis include transverse sensory and motor symptoms as well as bladder disturbances, usually accompanied by fever. These cannot be differentiated from myelitis of viral aetiology. Chronic myelitis, which was diagnosed more frequently in the past, is now relatively rare and usually develops slowly over months to years. At first, the patients notice increased fatigue while walking, more frequent stumbling, progressive reduction in walking distance and finally the development of a spastic-ataxic gait disorder and urinary dysfunction. Two thirds of such patients with para- or tetraparesis described in the literature showed severe clinical symptoms [1, 9]. In about 60% of patients with myelitis, there were also

signs of encephalitis, and of these, 40% had additional cranial nerve paresis. Magnetic resonance imaging seldom showed signal changes in the spinal cord. *Borrelia*-induced inflammation of the spinal cord and nerve roots (myeloradiculitis) can sometimes be painless or lack changes in sensitivity such as those in a motor neuron illness. The causal relationship between *Borrelia* infection and the development of amyotrophic lateral sclerosis suggested by some authors is based only on seroprevalence, and no case has been confirmed using appropriate diagnostic tests.

### Cerebral vasculitis

In rare cases, cerebral symptoms are caused by *Borrelia*-induced cerebral vasculitis. More than a third of the reported cases occur in patients younger than 30 [24, 32]. Its course is usually acute, involving an infarct in the posterior basin (thalamus, brainstem) (Fig. 1), and can be fatal. Autopsies have revealed obliterative vasculitis with substantial intima swelling and only discrete fibrosis of the media but marked thickening of the adventitia with numerous lymphocytic infiltrates. Additionally, chronic lymphocyte infiltration of the meninges was present. Diagnosis is based on positive serology, pathological CSF findings, MRI, and magnetic resonance angiography. Due to its rare aetiology, regular checks for antibodies in cases of cerebral insult are not necessary.

### Myositis

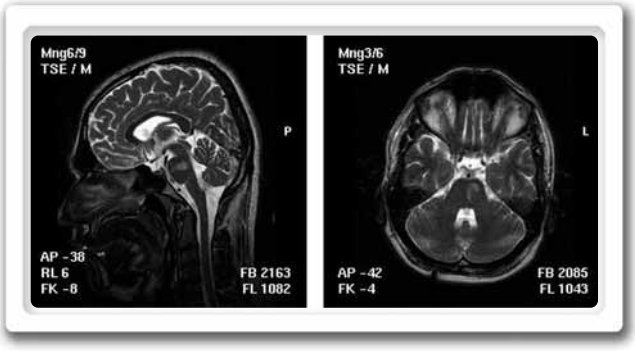
Myositis is considered a very rare manifestation of borreliosis affecting the musculoskeletal system. The pain and paresis are usually focal, and muscle enzyme levels are seldom elevated. Histopathology shows nodular or interstitial myositis with little degeneration of the muscle fibres and few macrophage but many CD4-positive T-lymphocytes [31]. This finding differentiates *Borrelia* myositis from other inflammatory myopathies in which B-lymphocytes usually predominate. Diagnosis is supported by electromyography, clinical symptoms conforming to the serology, and immunological and molecular demonstration of *Borrelia burgdorferi* sensu lato in the biopsy.

## Problem cases in clinical practice

### Borrelia encephalopathy

This diagnosis comes predominantly from the American literature and shows various unspecific symptoms such as reduced performance, increased fatigue,

**Fig. 1.** Brainstem infarct as a result of cerebral vasculitis due to Borrelia



irritability, emotional instability, as well as disturbances in sleep, concentration, and memory [22]. A causal relationship between these symptoms and serologically determined *Borrelia* infection is hardly conceivable without evidence of inflammatory signs in the CSF and the persistence of symptoms after antibiotic therapy. The positive *Borrelia* serology in patients lacking inflammatory changes in the CSF, should be interpreted instead as resulting from a clinically unapparent past infection (seroprevalence), and possible causes for these symptoms other that *Borrelia* must be sought in the differential diagnostic. When, a positive *Borrelia* serology is associated with inflammatory changes in the CSF and other neurological deficits, all of these indications, singly or in combination, can be interpreted as symptoms of neuroborreliosis.

### Post-Lyme syndrome

Post-Lyme syndrome (PLS) was defined in 1996 as a complex of symptoms that persist more than 6 months after appropriately treated Lyme disease [6]. Two groups of 23 patients of comparable age and gender who had received antibiotic treatment were compared, one group without persistent symptoms and the other without. Fibromyalgia was diagnosed in seven patients with PLS, three had chronic fatigue syndrome, and ten had "other" mild symptoms. Patients with PLS showed conspicuous deficits in concentration and memory tests and complained more frequently of sleep disturbances and mood changes than the control population. It was concluded from these results that Lyme borreliosis could lead to persistent complaints in a proportion of patients despite antibiotic treatment. It was not possible to show what factors, other than the *Borrelia* infection, were responsible for development of these symptoms.

Elkins et al. examined 30 patients with PLS using psychological and neuropsychological tests [12]. Unlike other authors, they could not find relevant deficits compared with a normal group. The only feature observed was a reduced proportion of positive emotions. However, such conspicuous emotional features are also found in chronic fatigue syndrome patients with no history of Lyme borreliosis, calling into question the credibility of PLS as a cause.

Klempner et al. examined 78 *Borrelia* antibody-positive and 51 antibody-negative patients with a history of borreliosis and PLS in a double-blind, placebo-controlled study to test the value of long-term antibiotic therapy (2 g ceftriaxone for 30 days plus 200 mg doxycycline for 60 days or placebo). The study had to be stopped prematurely, as an interim analysis showed that symptoms such as muscle pain, fatigue, and dysaesthesia could not be eliminated by such treatment [25]. Similar results were found in two other large-scale antibiotic studies, of which neither showed a positive effect on the unspecific symptoms [23, 26].

A critical review of this literature shows no plausible evidence for a chronic Lyme disease or PLS without proof of presence of the pathogen. In particular, immunological and microbiological findings that could explain the persistence of these unspecific symptoms are lacking.

### Fibromyalgia syndrome

The diagnostic criteria of fibromyalgia are:

- Generalised pain by anamnesis (i.e. left- and right-sided, upper and lower body, axis skeleton)
- Pain on at least 11 of 18 tender points with finger pressure (nine on each body side)
- Insertion point of the cisternal muscles
- Transverse processes of the spine C5–7
- Central upper edge of the M. trapezius and M. supraspinatus
- Cartilage/bone boundary of the second rib
- Radial epicondyle
- Regio glutaea lateralis
- Trochanter major
- Medial fat pad of the knee proximal to the line of the joint

In 1992 Dinerman and Steere described the appearance of fibromyalgic symptoms during acute Lyme arthritis or up to 5 months after acute Lyme disease [10]. Additional antibiotic therapy led to temporary improvement that lasted only a few months. The authors attributed this temporary success to a suggestive or placebo effect of the antibiotic, since sleep deficit, lack of training, and changed muscle metabolism can act as trigger functions, as can infections with various pathogens, head trauma, and emotional factors. Steere discussed the clinical differentiation between Lyme arthritis and fibromyalgia in his 1995 review article. Local joint inflammation predominates in Lyme arthritis; whereas patients with fibromyalgia show generalised symptoms such as increased fatigue, head, joint, and muscle pain, diffuse paraesthesia, and concentration disturbances [35]. The appearance of fibromyalgia weeks or months after Lyme disease will be discussed in a subsequent article by a panel of experts who are however quite critical of any causal relationship to previous *Borrelia* infection.

A retrospective study conducted by Shadick et al. compared the long-term results of 186 patients with clinically probable Lyme disease with those of 167 control individuals with no evidence of earlier *Borrelia* infection [33]. Six years after the acute illness, the frequency of neurological and cognitive deficits in the Lyme disease patients was not higher than that of the controls. During the initial illness, patients in the borreliosis group complained mostly of fever, headache, photophobia, and neck stiffness (symptoms of meningitis). The authors deduce from their results that the long-term prognosis for borreliosis

is usually good and that fibromyalgia is not a typical residual effect.

As neither the pathogenesis nor aetiology of fibromyalgia is known, the cause of the syndrome by some earlier event, e.g. a *Borrelia* infection, cannot be determined. This needs to be taken into consideration when expert opinion is given. Most authors agree that additional antibiotic treatment does not lead to a cure of the symptoms. A proportion of patients do however benefit from anti-depressive therapy with serotonin re-uptake inhibitors.

### Chronic fatigue syndrome

Diagnostic criteria for chronic fatigue syndrome (CFS).

The main criterion of CFS is persistent fatigue or rapid tiring for at least 6 months which:

- Cannot be explained by another illness and is recent
- Does not result from a chronic stress situation and can not be significantly improved by bed rest
- Is so pronounced that average performance is distinctly reduced

At least four secondary criteria of CFS must be present at or after the onset of fatigue and for diagnosis must persist for at least 6 months:

- Neck pain
- Painful cervical or axillary lymph nodes
- Muscle pain
- Wandering, noninflammatory arthralgia
- Newly developed headaches
- Concentration problems and disturbances in short-term memory
- No recovery after sleep
- Increased, generalised fatigue persisting >24 h after a previously tolerated activity

Coyle et al. (1994) questioned the importance of *Borrelia* infection to the development of CFS. They examined 13 patients who had had borreliosis and 12 controls without previous clinical evidence of such disease [8]. The only remarkable result was notable variations in the cerebrospinal fluid of one third of subjects in both groups; however, a specific influence of *Borrelia* infection on the cerebrospinal fluid could not be found. A similar result was found by Pollark et al., who examined 138 CFS patients for antibodies to *Borrelia burgdorferi* sensu lato in a risk area for this disease [29]. Only eight serum samples were borderline positive, but using immunoblot they were negative. In various case control studies a large number of pathogens, including *Borrelia burgdorferi* sensu lato, and various other factors (gender, number of childbirths, silicon implants, tendency to depression) have been investigated as possible causes of CFS, however no aetiology has been identified.

To summarise, the aetiology and pathogenesis of CFS as well as fibromyalgia are still unknown, and a causal relationship with previous *Borrelia* infection has not been demonstrated.

## Pain syndrome

Pain of unknown origin. Such pain, with no cause found by differential diagnosis, could indicate the beginning of *Borrelia* radiculitis if *Borrelia* antibodies are present in the serum. The next step is lumbar puncture in order to test this possible diagnosis. If the CSF findings are normal, it is highly unlikely that the pain is related to a *Borrelia* infection, and the search for other causes must be intensified.

*Borrelia* infections can cause, in rare cases, a complex regional pain syndrome (previously called Sudeck's dystrophy). Treatment involves eliminating the infection (e.g. by 1x2 g ceftriaxone/day for 14 to 21 days) and symptomatic therapy of the sympathetic nervous system-supported pain syndrome.

### Health disturbances

A whole spectrum of health problems are attributed to chronic borreliosis by patients with positive *Borrelia* serology. This self-diagnosis is supported by appropriate questionnaires patterned after that of Burrascano. Even when there is no doubt about the presence of chronic *Borrelia* infection, as with ACA or chronic neuroborreliosis (with appropriate inflammatory indications in the CSF), persistent health problems cannot be assumed to stem from chronic borreliosis in the absence of organ-specific pathology consistent with this diagnosis, despite positive *Borrelia* serology. Cost-benefit analyses speak against speculative treatment of unspecific symptoms in cases of positive *Borrelia* serology.

## Differential diagnosis

In cases of neuroborreliosis it is necessary, in principle, to consider other pathogens (bacterial and viral), autoimmunity (multiple sclerosis, lupus erythematosus), and granulomatous diseases of the nervous system (neurosarcoidosis) in the differential diagnosis. In the USA it is necessary, in rare cases of polyneuropathy after a tick bite, to consider a toxic or allergic neuropathy caused by tick saliva.

## Diagnostics

The diagnosis of neuroborreliosis is initially determined clinically, then confirmed by serology and CSF analysis. The probability of correct diagnosis can be divided into difference grades depending on clinical and laboratory findings [19].

*Possible neuroborreliosis:*

- Typical clinical picture (cranial nerve deficits, meningitis/meningoradiculitis, focal neurological deficits)
- *Borrelia*-specific IgG and/or IgM antibodies in serum
- CSF findings not available

*Probable neuroborreliosis* is as "possible borreliosis" but also with:

- Positive CSF findings with lymphocytic pleocytosis, blood/CSF barrier

disturbances, and/or intrathecal immunoglobin synthesis and
- Elimination of other possible causes of the symptoms present

*Certain neuroborreliosis* is as "probable borreliosis" but also includes:
- Intrathecal synthesis of *Borrelia*-specific antibodies (IgG and/or IgM) in the CSF or
- Positive PCR for the CSF

Typical CSF findings for neuroborreliosis show lymphocytic pleocytosis with numerous plasma cells and a definite barrier disruption (increased albumin and total protein levels). In addition, the acute form also displays pronounced intrathecal IgM that progresses to include synthesis of both IgG and IgA in the chronic form. The diagnosis can be confirmed by demonstration of *Borrelia*-specific intrathecal antibody synthesis. This can be done most easily by comparing specific antibody concentrations in the CSF and serum with respect to the total immunoglobulin concentrations in both compartments [21]. An antibody concentration at least 1.5 times higher per unit of immunoglobin in the CSF indicates intrathecal synthesis. Pathogen-specific intrathecal antibody synthesis can also be demonstrated by different banding patterns using immunoblot when the immunoglobulin concentrations in the CSF and serum are equivalent. Intrathecal *Borrelia burgdorferi*-specific antibody production can persist for many years or decades.

## Therapy

Ceftriaxone and cefotaxim, given for sufficient duration, are equally effective for treating neuroborreliosis [28]. The former shows very good *in vitro* results for minimum inhibitory concentration and availability in brain tissue. Both antibiotics reach CSF concentrations up to ten times higher than required to inhibit bacterial growth, even under the physiological conditions of the blood/CSF barrier. The clinical value of ceftriaxone for the treatment of erythema migrans, Lyme arthritis, and neuroborreliosis has been described in numerous studies and case reports. As a general rule, a dosage of 1x2 g ceftriaxone/day given over 30 min is adequate. If the infusion is too rapid and/or the dose higher, then precipitation of a calcium salt of ceftriaxone in the gallbladder may occur (accumulation of biliary sludge). Such precipitates usually do not cause symptoms and disappear at the conclusion of therapy. If the therapy lasts more than 2 weeks, sonography of the gallbladder should be done to check for biliary sludge. The effect of treatment duration on outcome has been clearly shown in several studies. In one case, *Borrelia burgdorferi* sensu lato could be isolated from the CSF of a patient 7 months after a 10-day ceftriaxone treatment regimen [28, 36]; while another study showed a substantial number of

patients complaining of unspecific problems weeks after short duration treatment. Our own study with 101 patients diagnosed with certain neuroborreliosis showed no such findings or problems after 14-day–21-day for chronic neuroborreliosis–treatment with ceftriaxone [20]. There are no criteria supporting a longer duration. Thus a minimum 14-day treatment should be given.

Although ceftriaxone showed clinical efficacy equivalent to that of penicillin G in comparative studies, some authors find significant advantage in the once-a-day drug application and the higher bactericidal CSF concentrations attained by the former. Pharmacological data on ceftriaxone can be found at www.rxlist.com/cgi/generic3/ceftriax_cp.htm.

The advantage of cefotaxim over ceftriaxone lies in its lower biliary excretion (no accumulation of biliary sludge); its disadvantage is a shorter half-life with the resulting need of more frequent application. Study results demonstrate cefotaxime's efficiency in both acute and chronic forms of borreliosis.

Penicillin has a similar efficiency to doxycycline for certain forms of acute borreliosis. In a Danish prospective study, 170 neuroborreliosis patients were successfully treated with 2x10 mega penicillin G over 10 days [15].

Doxycycline is probably the most commonly used antibiotic for borreliosis. Studies testing a new antibiotic normally use doxycycline for comparison. In most of these studies, it was as effective as the alternative test substances. It has been tested using various doses and durations of treatment, and it is generally agreed that the minimum dose is 200 mg/day, with a minimum duration of 14 days. In cases of uncomplicated meningopolyneuritis, 300 mg/day of doxycycline over 21 days is a possible alternative to 2 g/day ceftriaxone over 14 days [11]. Controlled tests determining the optimal dose of doxycycline are not available.

## Ophthalmological diseases

### Clinical picture

In certain cases, *Borrelia* infection can also lead to ophthalmological manifestations: follicular conjunctivitis, interstitial keratitis, vitritis, iridocyclitis, episcleritis, anterior uveitis, choroiditis, and retinal vasculitis [3]. Visual deterioration in cases of optic neuritis usually develops sub-acutely over several weeks and only with early treatment can a good prognosis be given.

### Diagnostics

Anamnesis, the clinical picture, and evidence of specific IgM and IgG antibodies support the diagnosis. However, they seldom demonstrate causality between *Borrelia* infection and the ophthalmological illness. Only in the case of papillitis can CSF findings confirm the diagnosis. Culture of the pathogen and PCR do not play a role in confirming

diagnosis.

### Therapy

Prospective studies are lacking due to the rarity of these diseases. For treatment, primarily third-generation cephalosporins are recommended (e.g. 2 g/day of ceftriaxone or 3x2 g/day of cefotaxime) due to their favourable penetration.

## Case report

### Case 1: acute neuroborreliosis

A 45-year old male programmer first experienced mild back pain about 3 weeks after a long hike in the Black Forest. Within a few days the pain had disseminated to include the right leg, and its intensity increased to such an extent that he visited his local physician. The patient showed a positive nerve stretch reflex, but neurological findings were otherwise normal. The physician suspected a slipped disk and ordered MRI of the lumbar spine. A small lateral protrusion was found at L4/5 but there was no nerve compression. Primary therapy therefore included analgesics, physiotherapy, and application of warmth. Despite these measures, the pain increased and the patient was unable to sleep at night. Ten days after the pain began, he developed facial paralysis. The physician prescribed cortisone (100 mg prednisolone), which did not affect the paralysis, but the other symptoms improved. Four days after beginning this treatment, the pain increased again, this time to the extent that the patient was admitted to hospital. Neurological examination showed peripheral, bilateral facial paralysis and mild impairment of foot lifting. *Borrelia* serology was positive for IgM and IgG antibodies, and CSF analysis showed lymphocytic pleocytosis with 200 cells/µl, an increased albumin quotient of $10x10^{-3}$, and intrathecal synthesis of IgM. At 1.3, the *Borrelia*-specific antibody index was still within normal limits. In spite of this, acute neuroborreliosis was suspected based on anamnesis and symptoms. Treatment with 2 g/day of ceftriaxone i.v. was started. After 3 days the patient reported marked reduction in pain and at the end of the 14-day treatment it had disappeared completely. In addition, the leg paralysis was no longer apparent on discharge from the hospital. The bilateral facial paralysis disappeared completely about 6 weeks after the end of treatment. Follow-up CSF examination at 3 months showed normal cell count and an intact blood/CSF barrier, decreasing IgM synthesis, and low IgG synthesis with evidence of oligoclonal bands. A year later, the *Borrelia*-specific antibody index was significantly higher at 2.5, indicating previous *Borrelia* infection of the nervous system, but CSF findings remain normal. The patient was symptom-free 6 weeks after the end of treatment.

### Case 2: chronic neuroborreliosis

A 40-year-old female resumed jogging

in March 2000 after a 3-month winter pause and noticed a considerable reduction in her normal running distance. Despite training three times a week, she remained unable to reach her previous year's distance, 5 km at a stretch. She also noted an increased tendency to stumble not only in the forest but also on any uneven or rough surface. In June 2000 she noticed light urinary incontinence and increased micturition, which could not be explained through urinalysis. The following August she stumbled over a curb in the darkness, fell to the ground, and broke her lower arm after an evening of dancing, when she had also noticed a certain "stiffness". On admittance to hospital, she mentioned instability while walking, so a neurologist was consulted. Further examination showed pronounced ataxia while standing and walking as well as light spastic paraparesis in the legs. Magnetic resonance imaging of the brain and spinal cord for suspected multiple sclerosis was pathologically unremarkable. Subsequent lumbar puncture showed pleocytosis at 100 cells/µl, total protein of 2500 mg/l, and intrathecal IgG synthesis of 70%. Test results for sarcoidosis, vasculitis, neurolysis, and HIV infection were negative. However, the high *Borrelia*-specific antibody index of 5.8 was occasion for initiating a 3-week treatment regimen with 2 g of ceftriaxone per day. Control examination of the patient's CSF 1 year later was unremarkable except for the clear presence of oligoclonal bands, and both general and *Borrelia*-specific IgG synthesis.

## References


1. Ackermann R, Gollmer E, Rehse-Kupper B (1985) [Progressive Borrelia encephalomyelitis. Chronic manifestation of erythema chronicum migrans disease of the nervous system] Progressive Borrelien-Enzephalomyelitis. Chronische Manifestation der Erythema-chronicum-migrans-Krankheit am Nervensystem. Dtsch Med Wochenschr 110:1039–1042
2. Bay I, Narayan K, Dail D, Sondey M, Hodzic E, Barthold SW, Pachner AR, Cadavid D (2004) Spinal cord involvement in the nonhuman primate model of Lyme disease. Lab Invest 84:160–172
3. Balcer LJ, Winterkorn JM, Galetta SL (1997) Neuro-ophthalmic manifestations of Lyme disease. J Neuroophthalmol 17: 108–121
4. Belman AL, Iyer M, Coyle PK, Dattwyler R (1993) Neurologic manifestations in children with North American Lyme disease. Neurology 43:2609–2614
5. Berglund J, Stjernber L, Ornstein K, Tykesson-Joelsson K, Walter H (2002) 5-y Follow-up study of patients with neuroborreliosis. Scand J Infect Dis 34:421–425
6. Bujak DI, Weinstein A, Dornbush, RL (1996) Clinical and neurocognitive features of the post Lyme syndrome. J Rheumatol 23:1392–1397
7. Christen HJ (1996) Lyme neuroborreliosis in children. Ann Med 28:235–240
8. Coyle PK, Krupp LB, Doscher C, Amin K (1994) Borrelia burgdorferi reactivity in patients with severe persistent fatigue who are from a region in which Lyme disease is endemic. Clin Infect Dis 18 [Suppl 1]:S24–S27
9. Depre A, Sindic CJ, Bukasa K, Bigaignon G, Laterre C (1988) [Borrelia burgdorferi encephalomyelitis] Formes encephalo-myelitiques de l'infection a Borrelia burgdorferi. Rev Neurol (Paris ) 144:416–420
10. Dinerman H, Steere AC (1992) Lyme disease associated with fibromyalgia. Ann Intern Med 117:281–285
11. Dotevall L, Hagberg L (1999) Successful oral doxycycline treatment of Lyme disease-associated facial palsy and meningitis. Clin Infect Dis 28:569–574
12. Elkins LE, Pollina DA, Scheffer SR, Krupp LB (1999) Psychological states and neuropsychological performances in chronic Lyme disease. Appl Neuropsychol 6:19–26
13. Finizia C, Jonsson R, Hanner P (2001) Serum and cerebrospinal fluid pathology in patients with sudden sensorineural hearing loss. Acta Otolaryngol 121:823–830
14. Garcia-Monco JC, Benach JL (1997) Mechanisms of injury in Lyme neuro-borreliosis. Semin Neurol 17:57–62
15. Hansen K, Lebech AM (1992) The clinical and epidemiological profile of Lyme neuroborreliosis in Denmark 1985–1990. A prospective study of 187 patients with Borrelia burgdorferi specific intrathecal antibody production. Brain 115 (Pt 2):399–423
16. Hopf H (1975) Peripheral neuropathy in acrodermatitis chronica atrophicans (Herxheimer). J Neurol Neurosurg 38:458
17. James DG (1997) Differential diagnosis of facial nerve palsy. Sarcoidosis Vasc Diffuse Lung Dis 14:115–120
18. Kaiser R (1995) Intrathecal immune response in patients with neuroborreliosis: specificity of antibodies for neuronal proteins. J Neurol 242:319–325
19. Kaiser R (1998) Neuroborreliosis. J Neurol 245:247–255
20. Kaiser R (2004) [Clinical courses of acute and chronic neuroborreliosis following treatment with ceftriaxone]. Nervenarzt 75:553–557
21. Kaiser R, Lucking CH (1993) Intrathecal synthesis of specific antibodies in neuroborreliosis. Comparison of different ELISA techniques and calculation methods. J Neurol Sci 118:64–72
22. Kaplan RF, Jones-Woodward L (1997) Lyme encephalopathy: a neuropsychological perspective. Semin Neurol 17:31–37
23. Kaplan RF, Trevino RP, Johnson GM, Levy

L, Dornbush R, Hu LT, Evans J, Weinstein A, Schmid CH, Klempner MS (2003) Cognitive function in post-treatment Lyme disease: do additional antibiotics help? Neurology 60:1916–1922 these are out of alphabetical order
24. Keil R, Baron R, Kaiser R, Deuschl G (1997) [Vasculitis course of neuroborreliosis with thalamic infarct] Vaskulitische Verlaufsform einer Neuroborreliose mit Thalamusinfarkt. Nervenarzt 68:339–341
25. Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, Trevino RP, Norton D, Levy L, Wall D, McCall J, Kosinski M, Weinstein A (2001) Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 345:85–92
26. Krupp LB, Hyman LG, Grimson R, Coyle PK, Melville P, Ahnn S, Dattwyler R, Chandler B (2003) Study and treatment of post Lyme disease (STOP-LD): a randomized double masked clinical trial. Neurology 60:1923–1930
27. Peltomaa M, Pyykko I, Seppala I, Viljanen M (1998) Lyme borreliosis – an unusual cause of vertigo. Auris Nasus Larynx 25:233–242
28. Pfister HW, Preac-Mursic V, Wilske B, Schielke E, Sorgel F, Einhaupl KM (1991) Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis. J Infect Dis 163:311–318
29. Pollark RJ, Komaroff AL, Telford SR, Gleit, Fagioli L, Brunet LR, Spielman A (1995) Borrelia burgdorferi infection is rarely found in patients with chronic fatigue syndrome. Clin Infect Dis 20:467–468
30. Ramesh G, Alvarez AL, Roberts ED, Dennis VA, Lasater BL, Alvarez X, Philipp MT (2003) Pathogenesis of Lyme neuroborreliosis: Borrelia burgdorferi lipoproteins induce both proliferation and apoptosis in rhesus monkey astrocytes. Eur J Immunol 33:2539–2550
31. Reimers CD, Koning J de, Neubert U, Preac-Mursic V, Koster JG, Muller-Felber W, Pongratz DE, Duray PH (1993) Borrelia burgdorferi myositis: report of eight patients. J Neurol 240:278–283
32. Schmiedel J, Gahn G, Kummer R von, Reichmann H (2004) Cerebral vasculitis with multiple infarcts caused by lyme disease. Cerebrovasc Dis 17:79–81
33. Shadick NA, Phillips CB, Sangha O, Logigian EL, Kaplan RF, Wright EA, Fossel AH, Fossel K, Berardi V, Lew RA, Liang MH (1999) Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. Ann Intern Med 131:919–926
34. Stanek G, Flamm H, Groh V, Hirschl A, Kristoferitsch W, Neumann R, Schmutzhard E, Wewalka G (1987) Epidemiology of borrelia infections in Austria. Zentralbl Bakteriol Mikrobiol Hyg [A] 263:442–449
35. Steere AC (1995) Musculoskeletal manifestations of Lyme disease. Am J Med 98:44S–48S
36. Treib J, Fernandez A, Haass A, Grauer MT, Holzer G, Woessner R (1998) Clinical and serologic follow-up in patients with neuroborreliosis. Neurology 51:1489–1491

# Lyme borreliosis diagnostics

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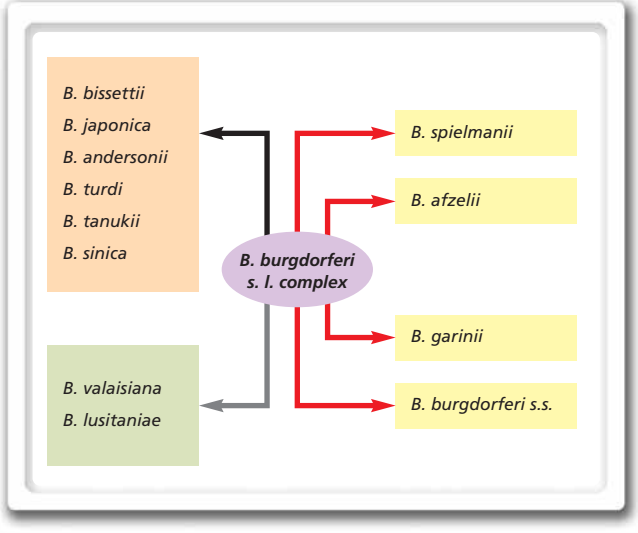
## Introduction

Lyme borreliosis is the most common tick-borne disease in the northern hemisphere. It does not occur in the southern hemisphere. As a multisystem disease it affects many organs, in particular the skin, nervous system, joints, and heart [58, 69, 70]. Due to the wide variety of symptoms, testing for antibodies against *Borrelia* spp. is the most commonly requested serological test. In Europe, microbiological diagnostics must take into account the heterogeneity of the pathogen.

## Heterogeneity of *Borrelia burgdorferi* sensu lato and its significance for the diagnostics of Lyme borreliosis

At least four *Borrelia* species cause Lyme disease in Europe, *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, and the till now rarely found *Borrelia spielmanii* (Fig. 1). Up to now, we have found *Borrelia spielmanii* four times (all from patients with erythema migrans) after analysing 242 isolates from European patients (Fingerle and Wilske, unpublished data). In contrast, *Borrelia burgdorferi* sensu stricto is the only *Borrelia spp.* pathogenic to humans in the USA [76]. The three most important human pathogenic species in Europe comprise at least seven outer surface protein A

**Fig. 1.** Heterogeneity of *Borrelia burgdorferi* sensu lato. *Yellow rectangles*, known human pathogens; *green rectangle*, human pathogenicity unclear; *orange rectangle* nonpathogenic for humans



(OspA) serotypes [83]. Skin isolates are predominantly *Borrelia afzelii* (OspA type 2), particularly those from patients with acrodermatitis chronica atrophicans, which incidentally is also not found in the USA [10, 53, 83]. Isolates from CSF and ticks are variable but show a predominance of *Borrelia garinii* (OspA types 3-7) [18, 72, 79, 83]. Sequence analysis of OspA amplicons using polymerase chain reaction (PCR) on arthrocentesis from patients with Lyme arthritis are distinctly heterogeneous [17, 73]. Other PCR studies showed mostly *Borrelia burgdorferi* sensu stricto (5S/23S rRNA intergenic spacer PCR [48] or flagellin PCR [35, 36]).

*Borrelia afzelii* and *Borrelia garinii* are the most common European species. The third most common, *Borrelia burgdorferi* sensu stricto, is particularly rare in eastern Europe (see review by Hubalek and Halouzka 1997 [32]). A great deal of heterogeneity can occur even in very small areas [18, 21, 49, 61, 64] however, distinct regional accumulations of certain species or subspecies have also been observed [45, 49, 55]. Multispecies infections in ticks have also been reported [32], although such infections are less commonly found in humans [14, 73, 79]. The heterogeneity of the pathogen (Fig. 1) makes the diagnostics of Lyme borreliosis in Europe substantially more difficult than in the USA and must be taken into consideration in the development of diagnostic reagents such as PCR primers and antigens. Thus when considering the frequently used OspA PCR, it is important that not only representatives of all human pathogenic species be detected but also the various OspA types of the heterogeneous *Borrelia garinii* group [18]. In addition, PCR should also distinguish *Borrelia valaisiana* and *Borrelia lusitaniae* because both are potential human pathogens, as indicated by a number of PCR and culture results [63, 75, 78]. We recently developed a powerful OspA PCR assay to determine and differentiate the various European species and OspA subtypes [49]. In serodiagnostic procedures, the strains' hetergeneity must also be taken into account, as certain important diagnostic proteins are decidedly heterogeneous and thus differ in their reactivity with patient sera. For example, among representative strains of *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii* (B31, PKo, and PBi), amino acid sequence identities for DbpA (Osp17) are 40–44%, and those for OspC are 54–68%. DbpA in particular has much greater amino acid sequence heterogeneity than DNA heterogeneity, which indicates immunoselection. Interestingly, highly heterogeneous proteins can still show highly conserved immunogenic epitopes (e.g. the C6 peptide from VlsE) [46, 47].

## Microbiological diagnostics

### Quality standards for the microbiological diagnosis of Lyme borreliosis

The German Society for Hygiene and Microbiology (DGHM) has published quality standards for the diagnosis of Lyme borreliosis formulated by a committee of experts (MiQ 12 Lyme-Borreliose) [86]. The free English version is available at http://www.dghm.org/red/index. html?cname=MIQ.

Except for the pathognomic clinical manifestation of erythema migrans, the diagnosis of Lyme borreliosis normally requires microbiological confirmation. For this purpose, determination of the presence of antibodies is usually used, while demonstration of the pathogen using culture or PCR is limited to special cases, for example with ambiguous serological test results or difficult clinical cases. Demonstration of the pathogen's presence should be made only in specialised laboratories.

## Detection of the pathogen

### Detection using culture

Culture using modified Kelly medium [59, 85], which expends considerable time and material, can help in special cases when the clinical picture strongly suggests Lyme borreliosis despite negative serology (seronegative Lyme borreliosis) [86]. Such cases may involve atypical erythema migrans, especially acute neuroborreliosis with short disease duration and no proof of antibodies in the CSF, or patients with immunodeficiency. The sensitivity of pathogen detection in culture is generally poor (Table 1).

### Detection using PCR

Currently no standard procedures are available for either sample preparation or the PCR itself. *Borrelia* PCR should enable diagnosis of the *Borrelia* species, i.e. the laboratory report should supply information on which human pathogenic species is present. The diagnostic sensitivity of PCR is approximately the same as for culture, whereas it is distinctly easier to detect *Borrelia* in tissue than in body fluids [2, 6, 35, 39, 44, 71, 87]. Only in the case of synovial fluid is PCR appreciably better than culture (Table 1; [52]).

**Table 1.** Sensitivity of culture and PCR methods for detecting *Borrelia burgdorferi*

| Sample | Sensitivity |
|---|---|
| Skin (erythema migrans, acrodermatitis) | 50–70% with culture or PCR |
| Cerebrospinal fluid (acute neuroborreliosis) | 10–30% with culture or PCR |
| Synovial fluid* (Lyme arthritis) | 50–70% with PCR<br>(culture is extremely seldom positive) |

*higher sensitivity with synovial biopsy compared to synovial fluid

## Detection of antibodies to *Borrelia burgdorferi*

It is generally accepted that serological examination should follow a two-step approach [12, 37, 85, 86]: (1) serological screening which, only if reactive, should be followed by (2) a confirmatory examination. A sensitive screening procedure is recommended, e.g. enzyme-linked immunosorbent assay (ELISA) or a similar procedure such as chemiluminescence immunoassay (CLIA), which can be confirmed by immunoblot if reactivity is present.

**Screening procedure.** At least an improved second-generation test (using for example recombinant material as

**Table 2.** Reactivity of the various homologues of VlsE and DbpA using line immunoblot for early manifestations. Data from Goettner et al. 2005

**A. IgG reactivity**

| | | DbpA from | | | | DbpA | VlsE from | | | VlsE |
|---|---|---|---|---|---|---|---|---|---|---|
| | Quantity (n) | PBi[e] n (%) | PBr n (%) | PKo n (%) | B31[e] n (%) | reactive n (%) | PBi[e] n (%) | PKo[e] n (%) | PKa2 n (%) | reactive n (%) |
| EM[a] | 15 | 0 (0,0) | 2 (13,3) | 4 (26,7) | 1 (6,7) | 5 (33,3) | 12 (80,0) | 9 (60,0) | 6 (40,0) | 12 (80) |
| NB[b] | 50 | 19 (38,0) | 20 (40,0) | 17 (34,0) | 6 (12,0) | 39 (78,0) | 44 (88,0) | 41 (82,0) | 41 (82,0) | 46 (92,0) |
| Controls[c] | 110 | 2 (1,8) | 2 (1,8) | 0 (0,0) | 0 (0,0) | 4 (3,6) | 1 (0,9) | 3 (2,7) | 0 (0,0) | 4 (3,6) |

**B. IgM reactivity**

| | | DbpA from | | | | DbpA | VlsE from | | | VlsE |
|---|---|---|---|---|---|---|---|---|---|---|
| | Quantity (n) | PBi[e] n (%) | PBr[e] n (%) | PKo[e] n (%) | B31[e] n (%) | reactive[d] n (%) | PBi[e] n (%) | PKo[e] n (%) | PKa2[e] n (%) | reactive[d] n (%) |
| EM[a] | 15 | 0 (0,0) | 0 (0,0) | 1 (6,7) | 0 (0,0) | 1 (6,7) | 8 (53,3) | 4 (26,7) | 3 (20,0) | 8 (53,3) |
| NB[b] | 50 | 9 (18,0) | 13 (26,0) | 2 (4,0) | 0 (0,0) | 22 (44,0) | 26 (52,0) | 7 (14,0) | 6 (12,0) | 28 (56,0) |
| Controls[c] | 110 | 0 (0,0) | 1 (0,9) | 0 (0,0) | 0 (0,0) | 1 (0,9) | 6 (5,4) | 0 (0,0) | 0 (0,0) | 6 (5,4) |

[a]Erythema migrans
[b]Neuroborreliosis
[c]Sera from blood donors were used as controls *n*=60, patients with syphilis *n*=10, patients with fever of unknown origin *n*=30, rheumatic factor-positive sera *n*=10
[d]At least one homologous protein reactive
[e]The *Borrelia* strains belong to the following species: PBi to *Borrelia garinii* OspA type 4, PBR to *B. garinii* OspA type 3, PKo to *Borrelia afzelii*, and B31 and PKa2 to *Borrelia burgdorferi*

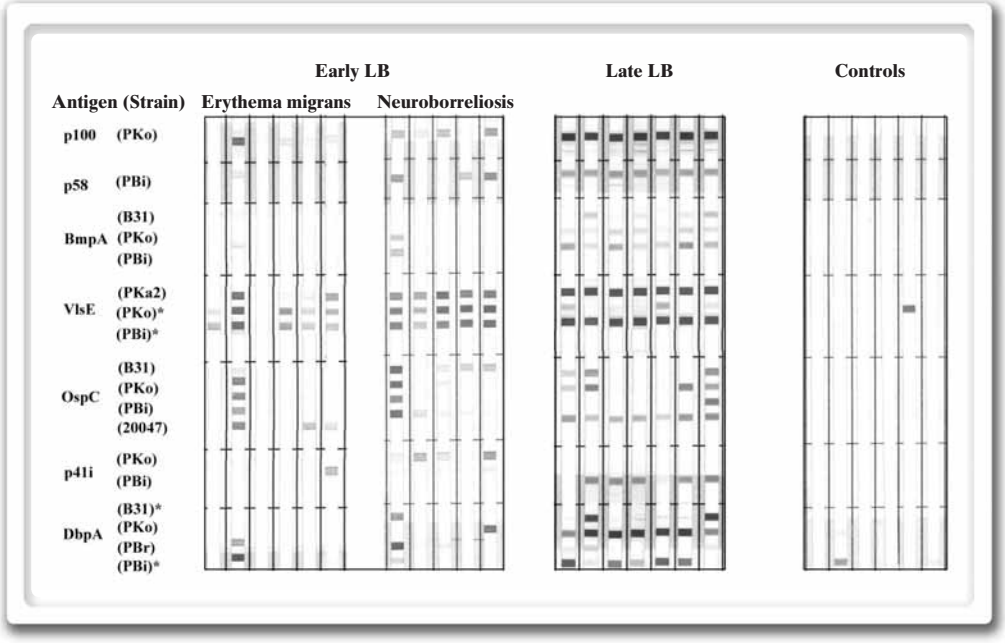
antigen) should be used for the screening procedure [86] due to possible cross-reactions with other bacteria. The strains used as a source of antigens should express OspC, the dominant antigen of the IgM immune response, and DbpA, an immunodominant antigen of the IgG response [86]. Recently, specific recombinant antigens (e.g VlsE) or synthetic peptides (e.g. the C6 peptide derived from VlsE) were used successfully on samples from American [4, 43, 47] and European patients [20, 22, 46, 54]. Moreover, DbpA which, like VlsE, is often hard to extract from *Borrelia* in culture, has also proved to be a sensitive antigen [22, 54] (Table 2).

**Confirmatory test (immunoblot).** As a confirmatory test, immunoblot requires a high specificity of at least 95%. Using recombinant antigens, the identification of diagnostic bands is substantially easier than with lysate blots.

The American immunoblot criteria recommended by the centers for disease control cannot be applied in Europe [28, 29, 65]. Dressler et al. [16] have shown that the immune response of European patients is confined to a markedly narrower spectrum of *Borrelia* proteins than that of American patients. In a study using sera from Germany and another using sera from various European countries, Hauser et al. have shown that strain-specific criteria for interpretation must be defined [28, 29]. Different rules for interpretation must therefore be established in order to achieve equivalent sensitivity and specificity using different *Borrelia* strains for immunoblot antigens. The criteria for interpreting immunoblots recommended by the DGHM were published in "MiQ 12 Lyme-Borreliose" [86].

Patients with early manifestations have an immune response based on only a few proteins, while those with late manifestations (acrodermatitis or arthritis) have IgG antibodies against a broad spectrum of antigens (Fig. 2). Using recombinant antigens has a number of advantages over the use of whole cell lysate antigen: (a) specific antigens can be selected (e.g. p83/100, BmpA), (b) homologous antigens from various strains can be combined (e.g. DbpA [Osp17], OspC, BmpA), (c) truncated antigens with a higher specificity can be produced (internal flagellin fragment), and (d) antigens expressed primarily *in vivo* but not in culture are available (e.g. DbpA and in particular VlsE [30, 67, 81]). Commercial recombinant blots can be better standardised than whole cell lysate blots. The sensitivity of immunoblots based on recombinant antigens has been improved considerably in the last few years by additional antigens and the establishment of line immunoblots [22, 67, 81]. For the first time, this has allowed significantly higher sensitivity with recombinant immunoblots than with whole cell lysate blots.

**Fig. 2.** Line immunoblot: IgG immune response in patients with various manifestations of Lyme borreliosis. Late Lyme borreliosis: sera from patients with acrodermatitis chronica atrophicans or Lyme arthritis. The *Borrelia* strains belong to the species: PBi to *Borrelia garinii* OspA type 4, PBR to *Borrelia garinii* OspA type 3, 20047 to *Borrelia garinii* unknown OspA type, PKo to *Borrelia afzelii*, and B31 and PKa2 to *Borrelia burgdorferi* sensu stricto




Recombinant immunoblot technology is an essential step towards standardising and increasing sensitivity, as homologous antigens from various strains (especially those with low sequence identity such as DbpA) and antigens that are only expressed *in vivo* (e.g. VlsE) can be used.

## Serological results at various stages of the disease

Serological test results must always be interpreted in relation to the clinical findings. For this, case definitions are helpful [60, 68, 86]. In stage I (erythema migrans), only 20–50% of patients are seropositive for IgM and/or IgG antibodies [3, 26, 77] (Table 3), and IgM antibodies are prevalent. A possible exception is the immune response to VlsE. In American patients with erythema migrans, VlsE IgG antibodies were detectable before the *Borrelia*-specific IgM response (44% vs 19% of patients in acute erythema migrans and 59% vs 43% in convalescent erythema migrans) [4]. In European patients with erythema migrans, earlier IgG response against VlsE was observed in 20 of 23 cases confirmed by culture (87%). The IgM

**Table 3.** Sensitivity for the presence of antibodies in cases of Lyme borreliosis

| Stage | Sensitivity | Antibody class |
|---|---|---|
| I | 20-50% | Predominantly IgM |
| II | 70-90% | With short-term illness predominantly IgM, with longer-term illness predominantly IgG |
| III | Near 100% | Usually only IgG[a] |

[a]The presence of IgM antibodies without IgG is not diagnostic for late manifestation

response was not studied [46]. In stage II (acute neuroborreliosis), the rate of seropositivity increases (IgM and/or IgG antibodies) to 70–90% [24, 25, 80, 82]. In principle, patients with early manifestations can be seronegative, particularly those with short-term illness. A check of the serological course should be done in such cases. At 6 weeks or more after onset of the disease, 100% of stage II neuroborreliosis patients are seropositive [24]. In cases with late manifestations (stage III, acrodermatis and arthritis), IgG antibodies could be demonstrated in all patients examined [26, 37, 80, 82]. A negative IgG test therefore speaks against diagnosis of late-stage Lyme borreliosis. A positive IgM test with negative IgG is likewise not diagnostically relevant for late-stage Lyme borreliosis [86].

For the diagnosis of neuroborreliosis, evidence of intrathecal antibody production (i.e. in the CSF/serum index) is particularly important [84]. Combined with additional changes in the CSF such as lymphocytic pleocytosis and barrier disturbance, this provides decisive diagnostic evidence. For diagnosing chronic borreliosis of the central nervous system, a positive IgG CSF/serum index is essential (see EUCALB case definitions [68, 86]), whereas chronic peripheral neuropathy usually displays no intrathecal antibody production [42].

As serological results can vary substantially and antibodies can persist for a long time even in successfully treated patients, serological course controls are not suitable for determining therapy failure. Until recently, a fall in VlsE specific antibodies was considered an indicator for the success of therapy [57]. This could not however be confirmed in a European study [56].

The presence of specific antibodies does not indicate the presence of a clinical manifestation. Antibody tests can also be positive due to previous clinical or subclinical infection. The less specific the patients' symptoms are, the less is the positive predictive value. In normal, healthy individuals, seropositivity varies with age and outdoor activity (e.g. in a study from Bavaria between 5% and 20% [62]).

## Methods not recommended for the diagnosis of Lyme borreliosis

### Evidence of borreliosis from a tick removed from a patient

Examination of ticks for the presence of *Borrelia burgdorferi* is valuable only if (1) with a negative test result there is sufficient certainty that no infection with *Borrelia burgdorferi* has occurred or (2) with a positive result there is sufficient likelihood of a clinical manifestation of the infection.

The following points must be considered for evaluating this method of examination:

1. Not every tick bite is discovered. *Ixodes ricinus* larvae and nymphs can hardly be recognised by normal vision and are often unconsciously removed with a fingernail. Thus, negative results can lead to a false sense of security, and infection can be contracted through the unrecognised bite.
2. Infected ticks usually do not transmit the pathogen through biting, and, should an infection result, only some cases become clinically relevant (e.g. Nahimana et al. [51] in which only three of 17 individuals who sero-converted became clinically ill).
3. The diagnostic sensitivity and specificity of the various methods for analysing ticks is largely unclear. Influencing factors include transport time, DNA extraction, presence of inhibitory substances (e.g. haemoglobin or scutum of the tick), laboratory contamination and the genetic heterogeneity of *Borrelia burgdorferi* sensu lato.
4. The great majority of the resulting illnesses are simple to diagnose and efficiently to treat [34].
5. No European study has been able to demonstrate that antibiotic prophylaxis or therapy following a bite by an infected tick has more advantages than disadvantages.

To summarise, examining ticks removed from humans for *Borrelia burgdorferi* sensu lato cannot be recommended. The reasons are the low infection and manifestation rates, insufficient negative and positive predictive value of the method, good disease prognosis, and no evidence of a reasonable prophylactic treatment. Action useful after tick bites has been proposed by a group of experts [38].

## Significance of the lymphocyte transformation test

In principle, the lymphocyte transformation test (LTT) measures proliferation of lymphocytes in patient blood after stimulation with an antigen (e.g. sonicated *Borrelia,* recombinant *Borrelia* antigens). The test is therefore an attempt to determine cellular immunity against specific antigens. In 1988, Dattwyler et al. showed that LTT could be positive even in cases of seronegative chronic borreliosis that developed after prompt treatment for

an early manifestation [13]. However, a series of studies published since then have shown no advantage of LTT over serology in routine Lyme borreliosis diagnostics [5, 9, 15, 19, 31, 33, 40, 66, 74]. Krause et al. used LTT to examine 24 patients with various manifestations of Lyme disease and 30 patients with arthritis of unrelated aetiology as a control group, including also 20 healthy individuals [40]. For proof of antibodies, the sensitivity was 83% and specificity 92%. For LTT with a specificity of 92%, the sensitivity was 71%, while for a sensitivity of 83% the specificity was 72%. Krause recently questioned whether LTT could contribute to routine clinical diagnostics. In addition, due to the test's low specificity, he re-emphasised the high likelihood of false positive results and concluded that LTT should not be used for the diagnosis of Lyme borreliosis [41]. Dressler et al. examined 42 patients with chronic Lyme borreliosis and 77 control individuals [15]. A sonicated *Borrelia burgdorferi* sensu stricto strain was used as antigen. The sensitivity of the test was given at 45% (serology 93%) with specificity of 95%. (No data were provided for serology.) Four of nine laboratory personnel examined also were positive using LTT.

In 1996 Huppertz et al. examined 55 children with Lyme arthritis and 48 control patients [33]. Two unsonicated *Borrelia burgdorferi* sensu stricto strains were used as the antigen source. The sensitivity of the test was 77% with a specificity of 78%. For eight of the children with Lyme arthritis, the stimulation index was negative before therapy but positive after therapy. In the authors' opinion, their study demonstrates that the test is valuable for neither prognosis nor control of the disease course. They concluded: "Lymphocyte proliferation assay will rarely aid in finding the correct diagnosis when clinical presentation and anti-borrelial serology do not match."

The studies of LTT to date show that it is not well standardised and, compared with serology, is less sensitive and particularly less specific. Therefore, LTT is not suited for routine diagnosis of Lyme borreliosis.

Meanwhile, the ELISPOT test (a commercially available LTT modification) has been recommended for human granulocytic ehrlichiosis, including an indication that co-infection has considerable significance for the prognosis and course of infection with *Borrelia*. Here we would point out that to date no case of autochthonous human granulocytic ehrlichiosis in Germany has been published and therefore use of this test is already questionable on epidemiological grounds.

## The significance of cystic forms of *Borrelia burgdorferi*

So-called cystic forms, also known as spheroblasts, forms without cell walls, or L forms, can be induced *in vitro* by various stress factors such as culture in

CFS, extreme changes in pH value, and increased temperature [1, 7, 8, 50]. Purely L forms proved infectious to mice [23]. Whether these forms are significant for pathogenesis of the disease or to diagnostics is however still unclear. In the meantime, assays allegedly specific for the *Borrelia burgdorferi* sensu lato form without cell walls are available. However, they have not been adequately evaluated and thus cannot be recommended for Lyme borreliosis diagnostics [11].

## The visual contrast sensitivity test

The visual contrast sensitivity test (VCS) has been recommended by Hartmann and Müller-Marienburg for chronically ill patients with unspecific symptoms such as myalgia, muscle twitching, joint pain, low energy, fatigue, diarrhea, constipation, and similar symptoms [27]. This test is based on the hypothesis that *Borrelia burgdorferi* produces a lipophilic neurotoxin which, among other activities, binds to the optic nerves, making the disease detectable using a VCS test due to reduced function, i.e. a deficit in recognising grey tones [27]. We do not know of any scientific publication that supports the suppositions on which this test is based. As the neurotoxin is subject to the enterohepatic circulation, it is also recommended that cholestyramine be used to break the toxic cycle.

From our point of view, we urgently advise against therapy with cholestyramine and the use of VCS tests for the diagnosis of Lyme borreliosis.

## References


1. Alban, PS, Johnson, PW, Nelson, DR. Serum-starvation-induced changes in protein synthesis and morphology of *Borrelia burgdorferi*. Microbiol 146(2000)119-127
2. Arnez M, Ruzic-Sabljic E, Ahcan J, Radsel-Medvescek A, Pleterski-Rigler D, Strle F. Isolation of *Borrelia burgdorferi* sensu lato from blood of children with solitary erythema migrans. Pediatr Infect Dis J 2001; 3:251-255
3. Åsbrink, E.; Hovmark, A.; Hederstedt, B. Serologic studies of erythema chronicum migrans Afzelius and acrodermatitis chronica atrophicans with indirect immunofluorescence and enzyme-linked immunosorbent assays. Acta Derm Venereol 1985; 65:509-514
4. Bacon, RM, Biggerstaff, BJ, Schriefer, M, Gilmore, RD, Philipp, MT, Steere, A, Wormser, GP, Marques, AR, and Johnson, BJB. Improved serodiagnosis of Lyme disease by kinetic ELISAs using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with two-tiered testing. J Infect Dis; 2003; 187:1187-1199
5. Bauer, Y.; Hofmann, H.; Jahraus, O.; Mytilineos, J.; Simon, M. M.; Wallich, R. Prominent T cell response to a selectively in vivo expressed *Borrelia burgdorferi* outer surface protein (pG) in patients with Lyme disease. Eur. J. Immunol.: 31(2001)767-776
6. Brettschneider, S.; Bruckbauer, H.; Klugbauer, N.; Hofmann, H. Diagnostic value of PCR for detection of *Borrelia burgdorferi* in skin biopsy and urine samples from patients with skin borreliosis. J. Clin. Microbiol.: 36(1998)2658-2665
7. Brorson, O.; Brorson, S. H. In vitro conversion of *Borrelia burgdorferi* to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H medium. Infection: 26(1998)144-150
8. Brorson, O.; Brorson, S. H. A rapid method for generating cystic forms of *Borrelia burgdorferi* and their reversal to mobile spirochetes. APMIS 106 (1998)1131-1141
9. Buechner, S. A.; Lautenschlager, S.; Itin, P.; Bircher, A.; Erb, P. Lymphoproliferative responses to *Borrelia burgdorferi* in patients with erythema migrans, acrodermatitis chronica atrophicans, lymphadenosis benigna cutis, and morphea. Arch. Dermatol.: 131(1995)673-677
10. Canica, MM, Nato, F, Du Merle, L, Mazie, JC, Baranton, G, Postic, D. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand J Infect Dis 1993; 25:441-448.
11. Centers for Disease Control and Prevention. Caution regarding testing for Lyme disease. Morbid Mortal Wkly Rprt 2005; 54:125
12. Centers for Disease Control and Prevention. Recommendations for Test Performance and Interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Morbid Mortal Wkly Rprt 1995;44:590
13. Dattwyler, R. J.; Volkman, D. J.; Luft, B. J.; Halperin, J. J.; Thomas, J.; Golightly, M. G. Seronegative Lyme disease: Dissociation of specific T- and B-lymphocyte responses to *Borrelia burgdorferi*. N. Engl. J. Med.: 319(1988)1441-1446
14. Demaerschalck, I, Messaoud, AB, De Kesel, M, Hoyois, B, Lobet, Y, Hoet, P, Bigaignon, G, Bollen, A, Godfroid, E. Simultaneous presence of different *Borrelia burgdorferi* genospecies in biological fluids of Lyme disease patients. J Clin Microbiol: 1995; 33:602-608
15. Dressler, F, Natalino, H, Yoshinari, Steere, A C. The T-cell proliverative assay in the diagnosis of lyme borreliosis. Ann Int Med 115(1991)533-539
16. Dressler, F, Ackermann, R, Steere, AC. Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European Lyme borreliosis. J Infect Dis 1994; 169:313-318
17. Eiffert, H, Karsten, A, Thomssen, R, Christen H-J. Characterization of *Borrelia burgdorferi* strains in Lyme arthritis. Scand J Infect Dis 1998; 30:265-268

18. Eiffert, H, Ohlenbusch, A, Christen, H-J, Thomssen, R, Spielman, A, Matuschka, F-R. Nondifferentiation between Lyme disease spirochetes from vector ticks and human cerebrospinal fluids. J Infect Dis 1995; 171:476-479
19. Ekerfelt, C.; Forsberg, P.; Svenvik, M.; Roberg, M.; Bergström, S.; Ernerudh, J. Asymptomatic Borrelia-seropositive individuals display the same incidence of Borrelia-specific interferon-gamma (IFN-y)-secreting cells in blood as patients with clinical Borrelia infection. Clin. Exp. Immunol.: 115(1999)498-502
20. Fingerle, V, Schulte-Spechtel, U, Wilske, B. Evaluation of an ELISA based on the C6-peptide of VlsE for diagnosis of early neuroborreliosis. Int J Med Microbiol 2002; 292 (Suppl. 34): 217
21. Gern, L, Hu CM, Kocianova E, Vyrestekova V, Rehacek J. Genetic diversity of *Borrelia burgdorferi* sensu lato isolates obtained from *Ixodes ricinus* ticks collected in Slovakia. Europ J Epidemiol (1999)15, 665-669
22. Goettner, G., U. Schulte-Spechtel, R. Hillermann, G. Liegl, B. Wilske, and V. Fingerle. 2005. Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant IgG and IgM line immunoblot and addition of VlsE- and DbpA homologues. J.Clin.Microbiol. in press
23. Gruntar, I, Malovrh, T, Murgia, R, Cinco, M. Conversion of Borrelia garinii cystic forms to motile spirochetes in vivo. APMIS 105(2001)383-388
24. Hansen, K, Hindersson, P, Pedersen, NS. Measurement of antibodies to the *Borrelia burgdorferi* flagellum improves serodiagnosis in Lyme disease. J Clin Microbiol 1988; 26:338-346
25. Hansen, K, Lebech, A-M. Lyme neuroborreliosis: a new sensitive diagnostic assay for intrathecal sythesis of *Borrelia burgdorferi* - specific immunoglobulin G, A, and M. Ann. Neurol.: 30(1991)197-205
26. Hansen, K.; Åsbrink, E. Serodiagnosis of erythema migrans and acrodermatitis chronica atrophicans by the *Borrelia burgdorferi* flagellum enzyme-linked immunosorbent assay. J Clin Microbiol 1989; 27:545-551
27. Hartmann und Müller-Marienburg. Indirekter Neurotoxinnachweis durch den „Visual Contrast Sensitivity“ Test bei Patienten mit chronischer Borreliose. Neurologie 2003. 248-251
28. Hauser, U, Lehnert, G, Lobentanzer, R, Wilske, B. Interpretation criteria for standardized western blots for three european species of *Borrelia burgdorferi* sensu lato. J Clin Microbiol 1997. 35:1433-1444
29. Hauser, U, Lehnert, G, Wilske, B. Diagnostic value of proteins of three *Borrelia* species (*Borrelia burgdorferi* sensu lato) and implications for development and use of recombinant antigens for serodiagnosis of Lyme borreliosis in Europe. Clin Diagn Lab Immunol 1998; 5:456-462
30. Heikkilä, T, Seppälä, I, Saxen, H, Panelius, J, Yrjänäinen, H, Lahdenne, P. Speciesspecific serodiagnosis of Lyme arthritis and neuroborreliosis due to *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* by using decorin binding protein A. J Clin Microbiol 2002; 40:453-460
31. Horowitz, HW, Pavia, CS, Bittker, S, Forseter, G, Cooper, D, Nadelman, RB, Byrne, D, Johnson, RC, Wormser, GP. Sustained cellular immune response to *Borrelia burgdorferi*: Lack of correlation with clinical presentation and serology. Clin Diagn Lab Immunol 1(1994)4:373-378
32. Hubalek, Z. Halouzka, J. Distribution of *Borrelia burgdorferi* sensu lato genomic groups in Europe, a review. Eur J Epidemiol 1997; 13: 951-957
33. Huppertz, H.-I.; Mösbauer, S.; Busch, D. H.; Karch, H. Lymphoproliferative responses to *Borrelia burgdorferi* in the diagnosis of Lyme arthritis in children and adolescents. Eur. J. Pediatr.: 155(1996)297-302
34. Huppertz, H. I.; Böhme, M.; Standaert, S. M.; Karch, H.; Plotkin, S. A. Incidence of Lyme borreliosis in the Würzburg region

of Germany. Eur. J. Clin. Microbiol. Infect. Dis.: 18(1999)697-703

35. Jaulhac, B, Chary-Valckenaere, I, Sibilia, J, Javier, R-M, Piemont, Y, Kuntz, J-L, Monteil, H, Pourel, J Detection of *Borrelia burgdorferi* by DNA amplification in synovial tissue samples from patients with Lyme arthritis. Arthritis Rheum 1996; 39:736-745
36. Jaulhac, B.; Heller, R.; Limbach, F. X.; Hansmann, Y.; Lipsker, D.; Monteil, H.; Sibilia, J.; Piemont, Y. Direct molecular typing of *Borrelia burgdorferi* sensu lato species in synovial samples from patients with Lyme arthritis. J. Clin. Microbiol.: 38(2000)1895-1900
37. Johnson, BJB, Robbins, KE, Balley, RE, Cao, B-L, Sviat, SL. Craven R B, Mayer L W, Dennis D T: Serodiagnosis of Lyme disease: accuracy of a two-step approach using a flagella-based ELISA and immunoblotting. J Infect Dis 1996; 174:346-353
38. Kaiser, R. Teilnehmer der Expertenkonferenz: Frühsommermeningoenzephalitis und Lyme-Borreliose-Prävention vor und nach Zeckenstich. Dtsch med Wochenschr 1998; 123:847-853
39. Karlsson, M, Hovind-Hougen, K, Svenungsson, B, Stiernstedt, G. Cultivation and characterization of spirochetes from cerebrospinal fluid of patients with Lyme borreliosis J Clin Microbiol 1990; 28:473-479
40. Krause, A, Brade, V, Schoerner, C, Solbach, W, Kalden, J R, Burmester, G. T cell proliferation induced by *Borrelia burgdorferi* in patients with lyme borreliosis. Arthr Rheum 34(1991)393-402
41. Krause, A. Verraten Lymphos die Borreliose? Medical Tribune 38(2003)14:26
42. Kristoferitsch, W. Chronical peripheral neuropathy. in: Weber, K.; Burgdorfer, W. (eds.) Aspects of Lyme borreliosis. Berlin, Heidelberg, New York, Springer (1993) 219-227
43. Lawrenz, MB, Hardham, JM, Owens, RT, Nowakowski, J, Steere, AC, Wormser, GP, Norris, SJ. Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi*. J Clin Microbiol 1999; 37:3997-4004
44. Lebech, A. M., K. Hansen, F. Brandrup, O. Clemmensen, and L. Halkier-Sorensen. 2000. Diagnostic value of PCR for detection of *Borrelia burgdorferi* DNA in clinical specimens from patients with erythema migrans and Lyme neuroborreliosis. Mol.Diagn. 5:139-150
45. Lencakova D, Hizo-Teufel C, Petko B, Schulte-Spechtel U, Stanko M, Wilske B, Fingerle V. Prevalence of Borrelia burgdorferi s.l. OspA types in Ixodes ricinus ticks from selected localities in Slovakia and Poland.. Int J Med Microbiol. 2006; Suppl 1:108-18.
46. Liang, FT, Aberer, E, Cinco, M, Gern, L, Hu, CM, Lobet, YN, Ruscio, M, Voet, PE, Weynants, VE, Philipp, MT. Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among european pathogenic genospecies of *Borrelia burgdorferi* sl. J Infect Dis 2000; 182:1455-1462.
47. Liang, FT, Alvarez, AL, Gu, Y, Nowling, JM, Ramamoorthy, R, Philipp, MT. An immunodominant conserved region within the variable domain of VlsE, the variable surface antigen of *Borrelia burgdorferi*. J Immunol 1999; 163:5566-5573.
48. Lünemann, JD, Zarmas, S, Priem, S, Franz, J, Zschenderlein, R, Aberer, E, Klein, R, Schouls, L, Burmester, GR, Krause, A. Rapid typing of *Borrelia burgdorferi* sensu lato species in specimens from patients with different manifestations of Lyme borreliosis. J Clin Microbiol 2001; 39:1130-1133
49. Michel, H.; Wilske, B.; Hettche, G.; Goettner, G.; Heimerl, C.; Reischl, U.; Schulte-Spechtel, U.; Fingerle, V. An *ospA*-polymerase chain reaction/restriction fragment length polymorphism-based method for sensitive detection and reliable differentiation of all European *Borrelia burgdorferi* sensu lato species and OspA-types. Med. Microbiol. Immunol.: 193(2004)219-226
50. Murgia, R.; Cinco, M. Induction of cystic forms

by different stress conditions in *Borrelia burgdorferi*. APMIS: 112(2004)57-62
51. Nahimana, I.; Gern, L.; Blanc, D. S.; Praz, G.; Francioli, P.; Peter, O. Risk of *Borrelia burgdorferi* infection in western Switzerland following a tick bite. Eur. J. Clin. Microbiol. Infect. Dis.: 23(2004)603-608
52. Nocton, J J, Dressler, F, Rutledge, B J, Rys P N, Persing, D H, Steere, A C. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med 1994; 330:229-234
53. Ohlenbusch, A, Matuschka, F-R, Richter, D,Christen, H-J, Thomssen, R, Spielman, A, Eiffert, H. Etiology of the acrodermatitis chronica atrophicans lesion in Lyme disease. J Infect Dis 1996; 174:421-423
54. Panelius, J., P. Lahdenne, H. Saxen, S. A. Carlsson, T. Heikkila, M. Peltomaa, A. Lauhio, and I. Seppala. 2003. Diagnosis of Lyme neuroborreliosis with antibodies to recombinant proteins DbpA, BBK32, and OspC, and VlsE IR6 peptide. J. Neurol. 250:1318-1327
55. Péter, O, Bretz, A.-G., Bee, D. Occurence of different genospecies of *Borrelia burgdorferi* sensu lato in ixodid ticks of Valais, Switzerland. Eur J Epidemiol 1995; 11:463-467
56. Peltomaa,M., McHugh,G., and Steere,A.C. (2003). Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of *Borrelia burgdorferi* after successful antibiotic treatment of Lyme disease. J. Infect. Dis. *187*, 1178-1186
57. Philipp,M.T., Marques,A.R., Fawcett,P.T., Dally,L.G., and Martin,D.S. (2003). C6 test as an indicator of therapy outcome for patients with localized or disseminated lyme borreliosis. J. Clin. Microbiol. *41*, 4955-4960
58. Pfister, H-W, Wilske, B, Weber, K. Lyme borreliosis: basic science and clinical aspects. Lancet 1994; 343:1013-1016
59. Preac-Mursic, V, Wilske, B, Reinhardt, S. Culture of *Borrelia burgdorferi* on six solid media. Eur J Microbiol Infect Dis. 1991; 10:1076-1079
60. Rauer, S., R. Kaiser, H. W. Kölmel, H. W. Pfister, and B. Wilske. Leitlinien für Diagnostik und Therapie in der Neurologie. Herausgegeben von der Kommission "Leitlinien" der Deutschen Gesellschaft für Neurologie, Stuttgart:Georg Thieme Verlag, 2005, 1-21
61. Rauter, C, Oehme, R, Diterich, I, Engele, M, Hartung, T. Distribution of clinically relevant Borrelia genospecies in ticks assessed by a novel, single-run, real-time PCR. J Clin Microbiol 2002;40:36-43
62. Reimer, B.; Marschang, A.; Fingerle, V.; Wilske, B.; v. Sonnenburg, F.; v. Hoecke, C. Epedimiology of Lyme borreliosis in South-Eastern Bavaria (Germany). Zent. bl. Bakteriol. 1999; 289:653-654
63. Rijpkema S G T, Tazelaar D J, Molkenboer M J C H, Noordhoek G T, Plantinga G, Schouls L M, Schellekens J F P. Detection of Borrelia afzelii, *Borrelia burgdorferi* sensu stricto, Borrelia garinii and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. Clin Microbiol Infect. 1997;3:109-116
64. Rijpkema, S, Golubic, D, Molkenboer, M, Verbeck-De Kruif, N, Schellekens, J. Idenfication of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. Exp Appl. Acarol 1996; 20: 23-30
65. Robertson, J, Guy, E, Andrews, N, Wilske, B, Anda, P, Granström, M, Hauser, U, Moosmann, Y, Sambri, V, Schellekens, J, Stanek, G, Gray, J. A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. J Clin Microbiol (2000)38:2097-2102
66. Schempp, C.; Owsianowski, M.; Lange, R.; Gollnick, H. Comparison of *Borrelia burgdorferi* ultrasonicate and whole *B. burgdorferi* cells as a stimulus for t-cell proliferation and GM-CSF secretion in vitro. Zbl. Bakt.: 279(1993)417-425
67. Schulte-Spechtel, U. Lehnert, G.; Liegl, G, Fingerle, V, Heimerl, C, Johnsson, BJB,

Wilske,B. Significant improvement of the recombinant Borrelia IgG immunoblot by addition of VlsE and a DbpA homologue derived from *B. garinii* for the diagnosis of early Neuroborreliosis. J Clin Microbiol. 2003; 41:1299-1303

68. Stanek, G, O'Connell, S, Cimmino, M, Aberer, E, Kristoferitsch, W, Granström, M, Guy, E, Gray, J. European Union concerted action on risk assessment in Lyme borreliosis: clinical case definitions for Lyme borreliosis. Wien Klin Wochenschr 1996; 108/23:741-747
69. Stanek, G, Strle, F. Lyme borreliosis. Lancet 2003; 362:1639–1647
70. Steere, AC. Medical progress - Lyme disease. N Engl J Med 1989; 321:586-596.
71. Strle, F.; Nadelman, R. B.; Cimperman, J.; Nowakowski, J.; Picken, R. N.; Schwartz, I.; Maraspin, V.; Aguero-Rosenfeld, M. E.; Varde, S.; Lotric-Furlan, S.; Wormser, G. P. Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by Borrelia afzelii in Slovenia. Ann. Intern. Med.: 130(1999)32-36
72. Van Dam, AP, Kuiper, H, Vos, K, Widjojokusumo, A, De Jongh, BM, Spanjaard, L, Ramselaar, ACP, Kramer, MD, Dankert, J. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin Infect Dis 1993 ; 17:708-717
73. Vasiliu, V, Herzer, P, Rössler, D, Lehnert, G, Wilske, B. Heterogeneity of *Borrelia burgdorferi* sensu lato demonstrated by an *ospA*-type-specific PCR in synovial fluid from patients with Lyme arthritis. Med Microbiol Immunol 1998; 187:97-102
74. Vaz, A.; Glickstein, L.; Field, J. A.; McHugh, G.; Sikand, V. K.; Damle, N.; Steere, A. C. Cellular and humoral immune responses to *Borrelia burgdorferi* antigens in patients with culture-positive early Lyme disease. Infect. Immun.: 69(2001)7437-7444
75. Wang, G, van Dam, AP, Dankert, J. Phenotypic and genetic characterization of a novel *Borrelia burgdorferi* sensu lato isolate from a patient with Lyme borreliosis. J Clin Microbiol 1999a; 37:3025-3028
76. Wang, G, van Dam, AP, Schwartz, I, Dankert, J. Molecular typing of *Borrelia burgdorferi* sensu lato taxonomic, epidemiological, and clinical implications. Clin Microbiol Rev 1999b; 12:633-653
77. Weber, K, Preac-Mursic, V, Wilske, B, Thurmayr, R, Neubert, U, Scherwitz, C. A randomized trial of ceftriaxone versus oral penicillin for treatment of early Lyme borreliosis. Infection 1990; 18:91-96
78. Wilske, B. Microbiological diagnosis in Lyme borreliosis. Int. J. Med. Microbiol. 2002, 291,(Suppl.)33:114-119
79. Wilske, B, Busch, U, Eiffert, H, Fingerle, V, Pfister, H-W, Rössler, D, Preac-Mursic, V. Diversity of OspA and OspC among cerebrospinal fluid isolates of *Borrelia burgdorferi* sensu lato from patients with neuroborreliosis in Germany. Med Microbiol Immunol 1996; 184:195-201
80. Wilske, B, Fingerle, V, Herzer, P, Hofmann, A, Lehnert, G, Peters, H, Pfister, H-W, Preac-Mursic, V, Soutschek, E, Weber, K. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis, comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med Microbiol Immunol 1993a; 182:255-270
81. Wilske, B, Habermann, C, Fingerle, V, Hillenbrand, B, Jauris-Heipke, S, Lehnert, G, Pradel, I, Rössler, D, Schulte-Spechtel, U. An improved recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis. Med Microbiol Immunol 1999; 188:139-144
82. Wilske, B.; Preac-Mursic, V. Microbiological diagnosis of Lyme borreliosis. in: Weber, K.; Burgdorfer, W. (eds.) Aspects of Lyme borreliosis. Berlin, Heidelberg, New York, Springer(1993b)267-300
83. Wilske, B, Preac-Mursic, V, Göbel, U B, Graf, B, Jauris-Heipke, S, Soutschek, E, Schwab, E, Zumstein, G. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal

antibodies and OspA sequence analysis. J Clin Microbiol 1993c; 31:340-350

84. Wilske, B, Schierz, G, Preac-Mursic, V, von Busch, K, Kühbeck, R, Pfister, HW, Einhäupl, K. Intrathecal production of specific antibodies against *Borrelia burgdorferi* in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). J Infect Dis 1986; 153:304-314
85. Wilske, B, Schriefer, M. Borrelia. In Manual of Clinical Microbiology (8th edition). Edited by Murray, P. R.; Baron, E. J.; Jorgensen, J. H.; Pfaller, M. A.; Yolken, R. H. (eds.) Manual of Clinical Microbiology (8th edition) Volume 1. Washington, D. C., ASM Press(2003)937-954
86. Wilske, B, Zöller, L, Brade, V, Eiffert, M, Göbel, UB, Stanek, G unter Mitarbeit von HW Pfister. MIQ 12 Lyme-Borreliose. In Qualitätsstandards in der mikrobiologisch infektiologischen Diagnostik. Edited by Mauch H and Lütticken R; im Auftrag der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM). Urban & Fischer Verlag, München Jena; 2000 (english version: http://www.dghm.org/red/index.html?cname=MIQ)
87. Zore, A.; Ruzic-Sabljic, E.; Maraspin, V.; Cimperman, J.; Lotric-Furlan, S.; Pikelj, A.; Jurca, T.; Logar, M.; Strle, F. Sensitivity of culture and polymerase chain reaction for the etiologic diagnosis of erythema migrans. Wien. Klin. Wochenschr.: 114(2002)606-609

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