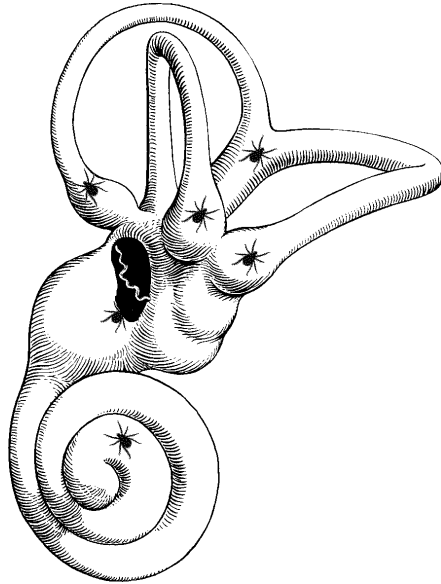


LYME BORRELIOSIS IN NEUROOTOLOGICAL
PATIENTS AND THE PREVALENCE OF
BORRELIA BURGDORFERI S.L. IN
URBAN *IXODES RICINUS* TICKS



by

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Helsinki 1999

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Academic dissertation

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To Kaisa, Aino, Anni, Juhana and Joona

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ABBREVIATIONS

ACA	acrodermatitis chronica atrophicans
<i>B. burgdorferi</i>	<i>Borrelia burgdorferi</i> sensu lato (including <i>B. burgdorferi</i> sensu stricto, <i>B. afzelii</i> and <i>B. garinii</i>)
BRA	brainstem response audiometry
BSK-II	Barbour-Stoenner-Kelly-II medium for <i>B. burgdorferi</i> cultivation
CDC	Centers for Disease Control and Prevention
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
DNA	deoxyribonucleic acid
DFM	dark field microscopy
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EM	erythema migrans
FP	facial paralysis
HL	hearing level
IFA	immunofluorescence assay
IgG	immunoglobulin G
IgM	immunoglobulin M
kHz	kiloherz
LA	Lyme arthritis
LB	Lyme borreliosis
MRI	magnetic resonance imaging
PCR	polymerase chain reaction
PTA	pure tone average
SD	standard deviation
SDS	sodium dodecylsulphate
SHL	sudden sensorineural hearing loss

LIST OF ORIGINAL PUBLICATIONS

This study is based on the following original publications, which are

- I Peltomaa M, Viljanen M, Seppälä I and Pyykkö I. Lyme borreliosis and facial paralysis - a prospective analysis of risk factors and outcome. *Am J Otolaryngol*, submitted.

- II Peltomaa M, Saxen H, Seppälä I, Viljanen M and Pyykkö I. Pediatric facial paralysis caused by Lyme borreliosis: a prospective and retrospective analysis. *Scand J Inf Dis* 1998;30:269-275.

- III Peltomaa M, Pyykkö I, Seppälä I and Viljanen M. Lyme borreliosis, an etiologic factor of sensorineural hearing loss? *Eur Arch Oto-Rhino-Laryngol* 1999; in press.

- IV Peltomaa M, Pyykkö I, Seppälä I and Viljanen M. Lyme borreliosis – an unusual cause of vertigo. *Auris Nasus Larynx* 1998;25:233-242.

- V Junttila J, Peltomaa M, Soini H, Marjamäki M, Aaltonen P and Viljanen MK. Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks in urban recreational areas of Helsinki. *J Clin Microbiol* 1999;37:1361-1365.

1. INTRODUCTION

Symptoms of the spirochetal infection currently known as Lyme borreliosis (LB) have been familiar to physicians in Europe for more than 100 years. The discovery of the causative organism, *Borrelia burgdorferi* (*B. burgdorferi*), by William Burgdorfer (Figure 1.) and his associates in the United States in 1981 elicited active scientific work all around the northern hemisphere. LB is by far the most common tick-borne infection in the United States and Europe.



Figure 1. Dr. William Burgdorfer discovered the causative agent of Lyme borreliosis in 1981.

Of the subspecies thus far recognized as belonging to the *B. burgdorferi* sensu lato complex, only three are unambiguously pathogenic for humans. *B. burgdorferi* sensu stricto appear in North America and Europe, whereas, in Europe, the subspecies *B. afzelii* and *B. garinii* also cause LB. The vector of the LB is usually an infected tick, and in Europe it is primarily the sheep tick *Ixodes ricinus* (*I. ricinus*). The enzootic cycle is maintained by a broad spectrum of reservoir animals, the major ones for *B. burgdorferi* being small and medium sized mammals.

The clinical spectrum of LB is also broad. The initial symptom, and most common clinical finding and hallmark of the disease, is an expanding rash, erythema migrans (EM). In this early stage the disease is usually easily treatable with antimicrobial therapy. Without treatment some patients develop disseminated infection with many kinds of symptoms.

Even in the early clinical studies neurootological symptoms were reported for patients with LB. Cranial nerve neuropathies are common manifestations of neuroborreliosis in both Europe and North America. The facial nerve is the most commonly

affected cranial nerve, but the disease can also affect other cranial nerves, including the vestibulocochlear nerve.

The cornerstone in the diagnostics of LB is the clinical picture presented by the patient. Laboratory tests can provide valuable information especially for the diagnosis of cases with ambiguous symptoms and signs. If pathognomonic EM is present, the diagnosis can be made without laboratory tests. Laboratory tests include the detection of *B. burgdorferi* in either body fluids or tissues and the assessment of antibodies to the spirochete in serum or cerebrospinal fluid (CSF).

The significance of *B. burgdorferi* cultivation is limited as a diagnostic tool, because it is rarely successful from clinical specimens. The determination of DNA from *B. burgdorferi* by polymerase chain reaction (PCR)

is gaining in use in the diagnosis of LB. Detection of specific antibodies against *B. burgdorferi* by enzyme-linked immunosorbent assay (ELISA) is the most commonly used method to screen patients for LB. ELISA can, however, provide false positive results because of cross-reactions, and a positive result in ELISA should be confirmed by Western blotting. Unfortunately, Western blotting lacks universally, or even locally, accepted interpretation criteria.

In this study, the prevalence of LB was evaluated for patients with facial nerve paralysis (FP), sudden sensorineural hearing loss (SHL) and vertigo. The infestation rate of ticks with *B. burgdorferi* in the patient enrollment area was assessed by dark field microscopy (DFM), PCR and cultivation. The epidemiology of LB, patients' signs and symptoms of LB and the recovery of neurootological patients from LB, were also studied.

2. REVIEW OF THE LITERATURE

2.1. History of Lyme borreliosis

In 1883, the German Alfred Buchwald described a case of long-lasting “diffuse idiopathic skin atrophy” (Buchwald 1883). More than 20 case reports of similar observations were published during the next 20 years, until Herxheimer and Hartman, in 1902, introduced the term *acrodermatitis chronica atrophicans* (ACA) for this late manifestation of LB (Herxheimer et al. 1902). The expanding rash around the site of a tick bite was first described in Sweden in 1909 (Afzelius 1910). The symptom was then called *erythema chronicum migrans*, nowadays known as *erythema migrans* (EM). The association between EM after a tick bite and *radiculoneuritis* with meningitis was first reported in France in 1922 (Garin et al. 1922). Subsequent publications reported a patient with meningoencephalitis after EM (Hellerström 1930) and 15 patients with “a chronic lymphocytic meningitis with cerebral symptoms” (Bannwarth 1941).

Already at that time Afzelius speculated that EM could be infective and produced by either a virus transmitted by ticks or a toxic agent con-

taminating the ticks. In their conclusion Garin and Bujadoux stated that “a virus (probably a spirochete)” may be the etiologic factor of the neurologic symptoms appearing after a tick bite (Garin et al. 1922). In 1923 Lipschütz, a dermatologist from Vienna, stated (Lipschütz 1923) that “...attention should be directed towards microscopic/bacteriologic investigations of intestinal tract and salivary gland secretions of the tick.” - Nobody followed his proposal.

The suspicion of an infectious agent behind the symptoms often led to successful attempts to treat patients with antimicrobial medication already several decades before the bacterial etiology was discovered (Bianchi 1950; Thyresson 1950; Hollström 1951; Hellerström 1951; Weber 1974). The infectious nature of EM was demonstrated with volunteers used to transmit the disease from one person to the other (Binder et al. 1955; Sonck 1965).

The modern era in the history of LB began in the community of Lyme, Connecticut in the United States at the request of two concerned mothers, Mrs. Polly Murray and Mrs. Judith Mensch (Burgdorfer 1993). One of the mothers was suffering

from multisystem disease, which had affected the children of both mothers and also some members of neighboring families. The systematic study of these patients (Steere et al. 1977; Steere 1989) led to the discovery of the causative agent (Burgdorfer et al. 1982) of this multisystem spirochetal disease, then called Lyme disease but later preferentially referred to as Lyme borreliosis.

2.2. Characteristics of *Borrelia* spirochetes

Order *Spirochaetales*

The causative agent of LB, *B. burgdorferi*, belongs to the order *Spirochaetales*. Another well-known human pathogen of this order is *Treponema pallidum*, the causative agent of syphilis. Other members of the *Borrelia* genus, causing tick-borne and louse-borne relapsing fever, also have a zoonotic cycle.

B. burgdorferi sensu lato

The Swiss-American scientist William Burgdorfer, along with his associates isolated *B. burgdorferi* from *Ixodes dammini* ticks collected from Shelter Island in New York (Burgdorfer et al. 1982). Genetic studies on the

DNA of *B. burgdorferi* sensu lato have shown considerable heterogeneity between different strains, and thus far nine different species of the *B. burgdorferi* sensu lato complex, including *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. lusitaniae*, *B. valaisiana*, *B. japonica*, Gr. DN127, *B. andersonii*, *B. tanukii* and *B. turdi*, have been named and even more have been recognized (Baranton et al. 1998). However, only three of them, *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* are unambiguously pathogenic to humans. All these three pathogenic species are found in Europe and also in Finland. In this study *B. burgdorferi* sensu lato is collectively called *B. burgdorferi*.

Morphology of *B. burgdorferi*

B. burgdorferi is a motile gram-negative helical bacterium with approximately 7-11 flagellae in its periplasmic space (Figure 2.). The cells are 8 to 22 microns long, 0.25-0.30 microns wide and composed of 3-10 loose coils. The cytoplasmic membrane is trilaminar and closely approximated to the cell wall. (Barbour et al. 1986) The major outer surface proteins are lipoproteins. Similar to other spirochetes, under unfavourable conditions *B. burgdorferi* forms spheroids or bleb-like struc-

tures, the function of which has not been determined. The spheroids may play a role in the transfer of genetic material (Dorward et al. 1991).



Figure 2. *Borrelia burgdorferi* is a heli-
coid gramnegative bacterium

B. burgdorferi can be visualized in CSF, blood, solid tissues and cultures by several staining methods, including Giemsa, carbol-fuchsin and silver impregnation. Gram-staining is less suitable, because *Borreliae* change their form considerably during fixation and staining procedures (Wilske et al. 1993). As motile, living organisms *B. burgdorferi* can be detected also by means of dark-field or phase contrast microscopy. However, these microscopic techniques cannot differentiate the *Borrelia* species from each other (Preac-Mursic et al. 1993).

Growth conditions

Microaerophilic *B. burgdorferi* spirochetes grow slowly in vitro, dividing every 8 to 12 hours (Barbour 1984).

One essential step in the success of the initial isolation of *B. burgdorferi* by Burgdorfer *et al.* was the earlier work of Richard Kelly, who described a successful primary cultivation of the tick-borne relapsing fever spirochete *Borrelia hermsii* in a complex artificial medium (Kelly 1971). Modifications of this medium, Barbour-Stoenner-Kelly (BSK-II) (Barbour et al. 1983) and modified Kelly medium Preac-Mursic (Preac Mursic et al. 1986) are now used in the cultivation of *B. burgdorferi* from ticks, other vectors, reservoir animals and patients.

Molecular biology of *B. burgdorferi*

The whole genome of *B. burgdorferi* has been cloned and sequenced. The function of most of the genes, however, is still unknown (Fraser et al. 1997). The genome of *B. burgdorferi* is composed of one linear chromosome of 950 kb (Baril et al. 1989), linear plasmids, and supercoiled circular plasmids (Barbour et al. 1987; Hyde et al. 1988). Both the linear and circular plasmids can be lost during in vitro cultivation and result in decreased infectivity of the bacteria.

Molecular analysis of *B. burgdorferi* has concentrated on the characterization of proteins. The impetus for

this direction has been the need to find molecular determinants to identify different *B. burgdorferi* isolates and to distinguish them from other species. The need to identify antigens for more specific and more sensitive serologic tests and for the development of vaccines has been another important motivation for studying *B. burgdorferi* proteins. The lipid and carbohydrate components of borrelia are by far less well known than its proteins.

About 40 immunoreactive bands are obtained from sodium dodecylsulphate (SDS) lysates of whole cells of *B. burgdorferi* in SDS polyacrylamide gel electrophoresis (Hauser et al. 1997). The 60 kDa polypeptide p60, also designated as "common antigen", and the 41 kDa polypeptide p41, which represents the flagellin, belong to the most prominent borrelial antigens. However, proteins cross-reactive with them occur in several bacterial antigens even outside the genus *Borreliae* (Hansen et al. 1988; Bruckbauer et al. 1992).

The immunodominant proteins of *B. burgdorferi* are lipoproteins in the outer membrane within molecular weights from 20 to 35 kDa and designated as outer surface proteins

(Osp) A-F. The expression of Osps varies between isolates and the Osps are immunologically and genetically variable (Barbour et al. 1986; Wilske et al. 1993; Wilske et al. 1996). Osps A, B and C are highly immunogenic. Osp C and flagellar protein p41 are the major immunodominant proteins in early LB, whereas Osp A appears in the later course of the disease (Howe et al. 1985; Wilske et al. 1993). Other specific antigens frequently recognized in patients with LB include proteins p83/100, p58, p43, p39, p30, p21, p17 and p14 (Hauser et al. 1997).

2.3. Ecology and epidemiology of Lyme borreliosis

I. ricinus - principal vector of *B. burgdorferi*

I. ricinus, also known as the sheep tick or Castor bean tick, is the most common tick in Europe and it is responsible for the transmission of LB to humans (Figure 3).

Other members of the subgenus *Ixodes* are also capable of transmitting spirochetes to their hosts. These species include *I. dammini*, *I. scapularis*, *I. pacificus*, *I. dentatus* and *I. neotomae* in North America and *I. persulcatus*

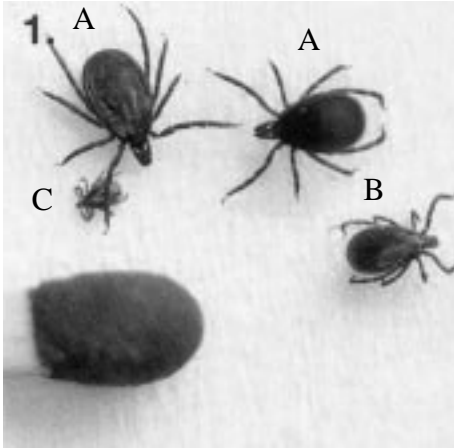


Figure 3. Adult female (A), adult male (B) and a nymph (C) *Ixodes ricinus* tick from a Helsinki city park. (Photo: M. Peltomaa)

in Europe and northern Asia. (Sonenshine 1993). The distribution of the Ixodid ticks is presented in Figure 4.

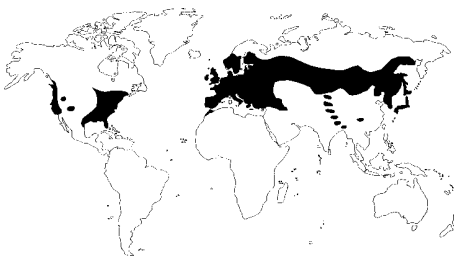


Figure 4. Geographical distribution of Ixodid ticks

Other *I. ricinus*-associated human pathogens include, among others, tick-borne encephalitis (TBE) virus, louping ill virus, *Ehrlichia* species, Uukuniemi-virus, *Babesia divergens*, *Babesia microti* and *Francisella tularensis*. In Europe and North America 18 and 19 different tick species, respec-

tively, are able to transmit *B. burgdorferi*, (Lise Gern, personal communication).

In addition to ticks other vectors can also transmit *B. burgdorferi*, including the biting fly (Luger 1990; Oksi et al. 1994), mosquitoes (Sinton et al. 1939; Magnarelli et al. 1986; Halouzka et al. 1998) and fleas (Doby et al. 1991).

Life cycle of *I. ricinus*

The length of the life cycle of the *I. ricinus* tick varies greatly in the geographic range of the tick, but it generally takes 2-6 years. In northern parts of its geographic range, as in Scandinavia, the four-staged life cycle (egg, larva, nymph and adult) usually takes 2-4 years (Gray 1991; Sonenshine 1993). The three latter motile stages of *I. ricinus* have a bimodal seasonal host seeking pattern with peaks in late spring or early summer and in late summer or early fall (Milne 1943; Milne 1946; Milne 1950; Lees et al. 1951).

Reservoirs of *B. burgdorferi*

The major reservoirs of *B. burgdorferi* are small and medium-sized mammals. In Europe 9 small mammals,

7 medium-sized mammals and 16 bird species are known to be capable of acting as sources of spirochetes to ticks (Gern et al. 1998). The most numerous of the reservoir animals in the zoonotic cycle of *B. burgdorferi* are small rodents of the genera *Peromyscus*, *Apodemus* and *Clethrionomys*. Various, especially ground-foraging birds including thrushes, blackbirds, robins, wrens and pheasants have been shown to be reservoirs of *B. burgdorferi* (Olsen et al. 1995; Gern et al. 1998). The prevalence of different genospecies varies in different reservoir animals in Europe and Asia. *B. valaisianae* and *B. garinii* occur preferentially in birds (Humair et al. 1998), *B. afzelii* in small mammals (Humair et al. 1995; Humair et al. 1998) and *B. burgdorferi* sensu stricto and *B. garinii* in squirrels (Humair et al. 1998). In addition to reservoir hosts, which are capable of transmitting *B. burgdorferi* to ticks, there are hosts that either have a zooprophylactic effect by destroying spirochetes in the ticks feeding on their blood (Lane et al. 1998) or serve as inadequate hosts by favoring certain genospecies, as is the case with birds, which harbor *B. garinii* and *B. valaisianae*, but not *B. afzelii* (Kurtenbach et al. 1998).

Infestation of *I. ricinus* with *B. burgdorferi* sensu lato

All of the *I. ricinus* populations that have been examined in Europe have harbored *B. burgdorferi* sensu lato. A critical analysis of 79 European studies on the infestation rates of ticks with *B. burgdorferi* showed rates of 2.8% (n=3 210), 13.8% (n=31 288) and 21.1% (n=27 421) for larval, nymphal and adult ticks, respectively (Hubalek et al. 1998). In the northeastern part of the United States, 25-50% of the nymphal and more than 50% of the adult *I. scapularis* ticks harbor *B. burgdorferi* (Bosler et al. 1984; Piesman et al. 1986). Determinations of the prevalence of *B. burgdorferi* in *I. ricinus* in Finland showed these spirochetes to be present in all the tick populations studied. All three human pathogen genospecies are found in Finnish ticks; however, *B. burgdorferi* sensu stricto has been isolated only from the Åland islands in the southwestern archipelago of Finland (Junttila et al. 1994; Tuomi et al. 1995).

Transmission of *B. burgdorferi* by *I. ricinus* to humans

Tick bites are common among humans and LB affects both males and females in all age groups, although

the clinical picture of LB is different in children and adults. The bimodal seasonal activity of the *I. ricinus* corresponds with the seasonal variation of early clinical cases. The painless tick bite often remains unnoticed, and only about 30-50% of the patients with clinical LB recall having been bitten (Weber et al. 1983; Weber et al. 1986). Although nymphal ticks are less frequently infected than adult ticks, they are more numerous, smaller and more easily unnoticed and therefore seem to be the main vector of human LB.

B. burgdorferi resides in the midgut of the unengorged tick. Penetration of the gut epithelium and dissemination into other tissues take place during early feeding and reach maximum in 1-2 days (De Silva et al. 1995). The transmission of *B. burgdorferi* seems to occur from contact with the saliva of the tick (Ribeiro et al. 1987) rather than by regurgitation of the gut content (Burgdorfer 1984). The transmission of *B. burgdorferi* into laboratory animals usually occurs 36-48 hours after tick attachment (Piesman 1993; Shih et al. 1995). The prompt removal of tick from the skin can thus diminish the risk of infection (Kahl et al. 1998). On the other hand, the possibility of rapid trans-

mission has also been suggested (Piesman 1995). The best method for tick removal remains controversial (Kahl et al. 1998). Despite the high mean infestation rates of ticks with *B. burgdorferi*, the risk of developing LB after a tick bite is low, less than 1% in Europe (Paul et al. 1989) and from 1% to 5% in the United States (Magid et al. 1992; Shapiro et al. 1992).

2.4. Pathogenesis of Lyme borreliosis

Lyme borreliosis in animals

The investigation of the pathogenesis of LB has been hampered by a lack of an animal model that adequately mimicks the stages of human disease (Hu et al. 1997). Numerous animals, including mice, rats, gerbils, hamsters, rabbits, dogs, cows and monkeys have been used to develop an animal model for human LB. Animals can be infected naturally by letting them be bitten by infected ticks or inoculating spirochetes either intradermally, subcutaneously, intravenously or intraperitoneally. The susceptibility of laboratory animals in developing clinical infection after *B. burgdorferi* inoculation varies considerably. Infection can also be

present in an animal without clinical signs of the disease (Barthold et al. 1988).

Proliferation and dissemination of *B. burgdorferi* in humans

B. burgdorferi spreads readily from the site of primary infection to other parts of the body, either via blood circulation (Berger et al. 1994) or directly by tissue invasion (Christen et al. 1993). The progression from local skin infection to disseminated LB can occur already in the early phase of the disease. For example, *B. burgdorferi* has been isolated from the blood 4 days after the tick bite (Steere et al. 1983) and two days after the appearance of EM (Benach et al. 1983). The cultivation of *B. burgdorferi* from the blood is rarely successful, and therefore a rapid clearing of the spirochetes from circulation is suggested (Galbe et al. 1993).

Host pathogen interaction in Lyme borreliosis

After entering the human body, *B. burgdorferi* elicits a series of events in the host, which attempt to destroy the invader or limit its spread to other parts of the body. These events include accumulating inflammatory

cells around the skin lesion and inducing immune response. The central paradox in the pathogenesis of *B. burgdorferi* is its ability to persist despite the defense mechanisms of the mammalian host. Tick's saliva contains factors that impair a range of proinflammatory responses in the host and promote the infectivity of *B. burgdorferi* (Zeidner et al. 1996).

B. burgdorferi is able to bind *in vitro* to various receptors present on numerous cells, including certain proteoglycans, glycosphingolipids and glycosaminoglucans, which may allow specific tissue tropism. All *B. burgdorferi* genospecies can cause similar clinical symptoms, but *B. afzelii* is found to be predominant among European skin isolates, and *B. garinii* prevails among isolates recovered from patients with neurological symptoms (Saint Girons et al. 1998; Picken et al. 1998).

In the absence of its own enzymes, *B. burgdorferi* can utilize host enzymes in spreading through the skin and other tissues. The spirochete is capable of binding human plasminogen and its activator urokinase (Fuchs et al. 1996; Coleman et al. 1997). These enzymes can then enhance its penetration through endot-

helial cell layers (Szczepanski et al. 1990) and enable the spirochetes to disseminate into various organs (Garcia-Monco 1998).

Although *B. burgdorferi* resides clearly in the extracellular compartment of the mammalian host, it has been found also inside endothelial cells, fibroblasts, synovial cells and macrophages (Ma et al. 1991; Klempner et al. 1993; Montgomery et al. 1993; Girschick et al. 1996). Treatment failures have been proposed to be due to either an intracellular location of the spirochete or to the location of the organism in "a protected site", such as the central nervous system (CNS) (Hu et al. 1997).

In tick midgut the major outer surface protein of *B. burgdorferi* is OspA, whereas in the mammalian host the production of OspA is down-regulated and the production of OspC is up-regulated (de Silva et al. 1996; Montgomery et al. 1996). This antigenic modulation may help the pathogen to evade the host immune system.

Immune response to *B. burgdorferi*

Humoral immunity appears to be the host's most effective defense against *B. burgdorferi* (Hu et al. 1997). The

development of specific antibodies follows the classical pattern of IgM response preceding IgG and IgA responses. During the first 2-4 weeks in early, localized infection (EM) less than half of the patients produce measurable levels of antibodies to *B. burgdorferi*. IgM antibodies rise commonly during the 3rd week, peak after 4 to 6 weeks, and disappear a couple of weeks later (Craft et al. 1984). IgG antibodies appear after 6 weeks of infection and can increase for months to years. IgG antibodies often persist for years or decades. Sometimes even IgM antibodies can persist for months to years. Antibody response to *B. burgdorferi*, however, can (but rarely) be weak or absent at different stages of the disease in some patients (Dattwyler et al. 1988; Steere 1993; Oksi et al. 1995).

The humoral immune response in LB is characterized by the initial recognition of a limited number of antigens, followed by a marked expansion in the repertoire of antigens recognized later in the course of illness (Craft et al. 1986). The earliest antibody responses are usually directed against the 21 kDa OspC protein, the 41 kDa flagellar antigen, a 39 kDa antigen and the 58 kDa antigen (Simpson et al. 1990; Dressler et al. 1993). In the United States, the

Centers for Disease Control and Prevention (CDC) (1996) recommends Western blotting as a confirmatory test for positive results of borrelia antibodies in ELISA. However, in Europe generally accepted interpretation criteria have not yet been established for Western blotting (Hauser et al. 1997).

A specific T-cell response is detectable early in the course of LB, often preceding the humoral immune response (Dattwyler et al. 1986). The use of cell-mediated immune responses in the diagnosis of various clinical manifestations of LB remains, however, controversial. T-cells reactive with the antigens of *B. burgdorferi* may cross-react with many other antigens (Volkman et al. 1991), and cell proliferation assay is not considered as sensitive tool as antibody measurement in the diagnosis of LB (Dattwyler et al. 1988).

2.5. Clinical spectrum of Lyme borreliosis

Description of Lyme borreliosis by stages

The clinical picture of LB varies considerably in individual patients. However, epidemiological observations have led to the concept of the

development of LB in three different stages, including early stages I and II and late stage III (Steere et al. 1984; Åsbrink 1991). The hallmark of stage I, and of the disease in general, is EM, the pathognomonic expanding erythematous rash on the site of the tick bite. Stage II includes early disseminated infection with a broad spectrum of symptoms. The stage III is the late phase of infection with chronic neurological, dermatological or inflammatory joint symptoms. The three stages overlap considerably, however, and are often difficult to differentiate. A more practical and pathophysiologically valid staging of LB into early localized and disseminated disease has been proposed (Halperin 1995).

Erythema migrans and other early signs and symptoms

EM, previously called erythema chronicum migrans, starts as a macula or papule expanding over a period of days to weeks (Figure 5.). In 30-50% of the cases EM is preceded by a recognized tick bite and rarely by a sting of a flying insect (Weber et al. 1983; Åsbrink et al. 1988; Oksi et al. 1994). The tiny and easily unnoticed nymphal tick is probably the responsible vector in cases without any his-



Figure 5. Slowly expanding rash, Erythema migrans is the hallmark of early Lyme borreliosis. (Photo: Department of Dermatology, Helsinki University Central Hospital)

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 tory of previous tick bite. An EM lesion is often asymptomatic, but the patient can, however, experience pain, burning, heat or mild itching (Weber et al. 1993). The shape and outlook of EM varies. The initial lesion is usually homogeneous, red or bluish red with a round or oval shape. About 70% of the patients with EM present with constitutional symptoms like fatigue, headache, fever, arthralgia and myalgia (Weber et al. 1986). With the exception of regional lymphadenopathy and fever, objective findings are rare in these patients (Weber et al. 1993; Nadelman et al. 1995). The enlargement of EM varies considerably, and, if left untreated, the lesion commonly exceeds a diameter of 15 centimeters or

more. EM usually resolves spontaneously in a few weeks or months, but without treatment it can reappear later (Weber et al. 1993).

In Europe 4-8% (Weber et al. 1993) and in North America up to 50% (Steere et al. 1983) of the patients with LB develop multiple EM lesions as the sign of disseminated disease.

Borrelial lymphocytoma and acrodermatitis chronica atrophicans

Borrelial lymphocytoma is a rare skin manifestation, and it has been reported in connection with LB only in Europe (Hovmark et al. 1993). It is a bluish-red, tumorlike skin infiltrate, usually 1-5 centimeter in diameter. Lymphocytoma is usually located on the earlobe, nipple or scrotum, and it occurs more frequently in children. Untreated lymphocytoma resolves spontaneously, but it may take up to 1 year and result in sequelae (Hovmark et al. 1993).

ACA is a late skin manifestation of LB and has been described mainly in Europe. It is a bluish-red, atrophic lesion, that is sometimes complicated by sclerodermic changes. It typically starts in later decades of life and occurs predominantly in women (Åsbrink 1993).

The predilection sites of ACA are distal extensor surfaces of the limbs. Extracutaneous manifestations, such as peripheral (Kaiser 1972) and central neuropathy (Sandström et al. 1989) and joint manifestations (Hovmark et al. 1986), are not uncommon concomitantly with ACA.

Musculoskeletal manifestations

Arthritis is a relatively frequent manifestation of LB. However, the incidence figures from various countries and from studies representing various specialities are not always comparable because of bias caused by patient selection (Herzer 1993). Lyme arthritis (LA) is characterized by recurrent brief attacks of objective joint swelling in one or a few joints, occasionally progressing to chronic arthritis (Cimmino et al. 1998). In the United States about 60% of the patients with untreated EM later develop episodes of LA (Steere 1989). *B. burgdorferi* can only rarely be isolated from the joints of patients with LA. In most cases the pathogenesis is, however, associated with the presence of spirochetes in the joints (Nocton et al. 1994). In some patients with chronic, treatment-resistant LB, autoimmune mechanisms can play a role in the pathogenesis of the disease. Antibodies to OspA and OspB have been lin-

ked to the development of chronic arthritis (Kalish et al. 1993). The concept molecular mimicry, whereby bacterial antigens share epitopes with host proteins, has been proposed as one mechanism of the chronic inflammation in patients with chronic LB (Sigal et al. 1988; Garcia-Monco et al. 1993; Sigal 1993).

Arthralgia and myalgia occur in most patients with early LB in North America, but only in every fifth patient in Europe (Steere et al. 1983; Weber et al. 1986). These symptoms alone, however, are not criteria for the musculoskeletal involvement of LB (Stanek et al. 1996). Other musculoskeletal manifestations associated with LB include myositis (Schmutzhard et al. 1986; Reimers et al. 1993) and osteomyelitis (Jacobs et al. 1986; Oksi et al. 1994). LB can trigger fibromyalgia (Dinerman et al. 1992), which, however, not rarely seems to be misdiagnosed as LB (Sigal et al. 1992; Steere et al. 1993; Hsu et al. 1993).

Neurological and otoneurological manifestations

Early neurological manifestations of LB include meningitis, cranial and peripheral nerve neuropathies and

Bannwarth's syndrome, which is characterized by painful meningoradiculoneuritis with lymphocytic pleocytosis in the CSF with or without peripheral paresis and with or without cranial nerve affection (Pfister et al. 1993). Patients with Lyme meningitis in Europe usually do not suffer from meningeal symptoms (Garcia-Monco 1992). In the United States, however, most of the patients have meningeal symptoms, such as headache and mild neck stiffness (Reik et al. 1979; Pachner et al. 1985). The clinical picture of Lyme meningitis resembles acute aseptic meningitis, but it can also take a long-term or relapsing course (Stiernstedt 1985; Pachner et al. 1985).

Cranial neuropathies develop in 47% to 82% of LB patients without antibiotic treatment (Pfister et al. 1993). FP accounts for 70% to 80% of cranial neuropathies that occur in LB (Reik 1991) and it is the most common single neurologic symptom of LB.

Chronic CNS abnormalities are rare in LB, and they develop months to years after the initial infection (Hansen et al. 1992). Late CNS LB includes all forms of encephalitis, encephalomyelitis, meningoencephali-

tis and radiculitis (Kristoferitsch 1991; Hansen et al. 1992; Martin et al. 1993). Chronic disease often affects peripheral nerves also. As much as 40% to 63% of patients with ACA suffer from concomitant chronic peripheral neuropathy (Åsbrink 1985; Kristoferitsch et al. 1988).

Cardiac manifestations

Lyme carditis is estimated to occur in 8% of patients with LB in North America (Steere et al. 1980). In Europe the frequency of cardiac manifestations has not yet been established (van der Linde et al. 1993). Lyme carditis can occur as rhythm or conduction abnormalities, endomyocarditis and pericarditis either with or without heart failure (van der Linde et al. 1993). An atrio-ventricular block of fluctuating degree is the most common sign of Lyme carditis, but also total atrio-ventricular block occurs (Steere et al. 1980; Olson et al. 1986). The role of *B. burgdorferi* in chronic heart disturbances is unclear, but the significantly higher prevalence of positive levels of antibodies to *B. burgdorferi* in patients with cardiomyopathy than in patients with coronary heart disease or healthy blood donors suggests that there may be an association between LB and chronic

heart disease (Klein et al. 1991; Stanek et al. 1991).

Ocular manifestations

In LB the eye can be affected either by the infection and inflammation of ocular tissue or by the involvement of extraocular tissues. Like syphilis, LB seems to be a great imitator of various ocular inflammations. Neurophthalmic and ocular manifestations of LB include cranial neuropathies, optic nerve disease, meningitis with papilledema, neuroretinitis, conjunctivitis, keratitis, uveitis, vitreitis, and other forms of posterior segment inflammatory disease (Lesser 1995; Karma et al. 1996; Balcer et al. 1997; Mikkilä et al. 1997; Mikkilä et al. 1997; Zaidman 1997). Intraocular inflammations caused by *B. burgdorferi* are rare and difficult to diagnose (Schönherr et al. 1993). In endemic areas LB is not an extreme rarity among patients with uveitis or posterior segment inflammations of unknown etiology and it should be taken into account in the diagnostic work-up (Mikkilä 1998).

Other manifestations

B. burgdorferi disseminates hematogenously, and several organs can be

infected. Subclinical hepatitis with elevated serum transaminases (Kazakoff et al. 1993), splenomegaly (Cimmino et al. 1989; Nelson et al. 1992), orchitis, testicular swelling, microscopic hematuria, and proteinuria (Steere 1989) have been reported in patients with LB.

2.6. Diagnosis of Lyme borreliosis

Typical signs and symptoms of the patient are the cornerstone of the diagnostics of LB. If pathognomonic symptoms, such as EM, are present, the diagnosis is straightforward and usually no laboratory tests are needed. However, many of the numerous symptoms of LB are not specific for the disease, and laboratory methods are necessary to support or confirm the diagnosis. These laboratory methods fall in two categories: indirect methods (including the assessment of antibodies against *B. burgdorferi* and the lymphocyte stimulation test) and direct methods (including cultivation, microscopy and staining, antigen detection and PCR).

Serological methods

The first serological tests introduced for the diagnosis of LB were based on indirect immunofluorescence (IFA)

technology (Wilkinson 1984). Later, ELISA has become the most widely used screening method in LB diagnostics. Western blotting is recommended for use in the confirmation of positive results obtained by ELISA. The antigen preparations used in these tests include whole cell preparations, sonicated cell suspensions, purified flagellin protein and recombinant proteins of *B. burgdorferi* (Hansen et al. 1988; Burkert et al. 1996). The genetic variability of *B. burgdorferi* makes the selection of appropriate antigens for the serological tests a challenging task. The diagnosis of LB by serological testing suffers from poor standardization of the methods, which has become evident in interlaboratory comparisons (Tilton 1994; Bakken et al. 1997; Guy et al. 1998).

Indirect immunofluorescence

IFA is a rather simple procedure and does not need very sophisticated laboratory equipment. In experienced hands the method has also been accurate (Magnarelli et al. 1984; Russell et al. 1984). The interpretation of IFA results can vary, however, because of the subjectivity of the reading process. In addition, IFA is less sensitive than ELISA, too laborious for high-volume testing and prone to

variability in manufacturing processes (Stiernstedt et al. 1985; Golightly 1993; Tilton 1994).

Enzyme linked immunosorbent assay

ELISA is the most widely used method for assessing antibodies against *B. burgdorferi*. This method, as well as other serological tests is hampered, however, by the occurrence of cross-reacting antibodies and a lack of sensitivity, especially in the early phases of LB (Bruckbauer et al. 1992). ELISA tests are available as both in-house assays and commercial kits.

Numerous antigens have been tested in attempts to improve the efficacy of ELISA. Sonicated bacteria are widely used for screening purposes, usually combined with purified or recombinant antigens for confirming positive results (Burkert et al. 1996; Guy et al. 1998). Immunological and molecular biological investigations have revealed and characterized a variety of borrelial antigens, including p83/100, p66, p41 (flagellin), p39 (BmpA), p17 and outer surface proteins OspA, OspB, OspC, OspD, OspE and OspF. In early infection, the immune response is mainly restricted to p41 (Craft et al. 1986) and OspC (Wilske et al. 1986). Later, du-

ring the course of prolonged infection, the immune response is directed against tens of different antigens. This phenomenon can best be demonstrated by Western blotting with which sera from patients with late LB can show as many as 40 immunoreactive bands (Hauser et al. 1997). Several reports have been published, however, on seronegative patients with definite LB (Dattwyler et al. 1988; Guy et al. 1989; Schutzer et al. 1990; Oksi et al. 1995). ELISA, as well as other serological methods, are more sensitive in late than early stages of the disease. In the diagnosis of late European LB, the most specific diagnostic antigens for ELISA include p83/100, p58, p43, p39, p30 OspC, p21, p17 and p14 (Hauser et al. 1997). Several important proteins are immunologically variable in different genospecies of *B. burgdorferi*. Thus the use of purified antigens does not solve the problem caused by antigen polymorphism. In the future, carefully designed antigen cocktails should be constructed for ELISA to cover different *B. burgdorferi* genospecies as well as possible.

Western blotting

Ideally, all positive ELISA results should be confirmed by Western

blotting (Tilton 1994). In the United States the CDC recommend a two-step protocol for the evaluation of the sera. ELISA should be performed for screening, and positive results should be confirmed by Western blotting assay according to the criteria accepted by the CDC (Centers for Disease Control and Prevention 1995). In Europe, however, generally accepted criteria have not been established. The three known *B. burgdorferi* strains pathogenic for human and at least eight different serotypes present in Europe make the definition of Western blotting criteria more complicated. Attempts have recently been made to establish European criteria, however (Hauser et al. 1997).

Cultivation

B. burgdorferi requires a special medium for growth. BSK-II medium was the first described for this purpose; later several modifications of it have been developed (Barbour et al. 1983; Preac Mursic et al. 1986). *B. burgdorferi* is a slowly growing organism, and sometimes cultivation has to be continued for weeks to obtain detectable growth. Although *B. burgdorferi* has been isolated from specimens obtained at all stages of LB, isolation is more successful at early stages than later.

B. burgdorferi has been isolated from a variety of body tissues, including skin (Steere et al. 1983; Åsbrink 1985; Stanek et al. 1985) blood (Benach et al. 1983; Viljanen et al. 1992), CSF (Steere et al. 1983; Karlsson et al. 1990), synovial fluid and synovial membrane (Snydman et al. 1986; Schmidli et al. 1988), tendon (Haupt et al. 1993), myocardium (Stanek et al. 1990), iris (Preac Mursic et al. 1993), and subcutaneous fat (Viljanen et al. 1992). Skin biopsies from patients with EM have been culture positive in 60% - 86% of the cases (Wormser et al. 1992; Berger et al. 1992; Mitchell et al. 1993). Furthermore, *B. burgdorferi* has been cultured from the sera of 25% of the patients with EM (Wormser et al. 1998). The sensitivity of cultivation is much lower for tissues other than EM skin. The yields of cultivation from the blood of patients with EM and from the CSF of patients with neuroborreliosis have been 5% or less and 7%-10%, respectively (Wilske et al. 1993; Wormser et al. 1998). Thus, a negative result in cultivation does not exclude LB, and the value of this method in the diagnosis of LB is considered rather limited.

Microscopy

Various specimens have been stained by Giemsa, silver impregnation and

immunohistochemical techniques for the microscopical observation of *B. burgdorferi* (de Koning et al. 1993). However, microscopy is time consuming, lacks both specificity and sensitivity and is not suitable for the routine diagnosis of LB.

Polymerase chain reaction

PCR is a powerful method for detecting small amounts of nucleic acids. It has been used to detect *B. burgdorferi* in ticks (Persing et al. 1990) and in a wide variety of human and animal specimens (Picken et al. 1997). PCR has many advantages, including high sensitivity, specificity and rapidity. The extreme sensitivity can, however, cause false positive results because of contamination. This problem can generally be controlled by careful attention to controls and laboratory procedures. On the other hand, false negative results can be caused by inhibitors in the specimens or simply by the fact that not a single bacterium has reached the minute proportion of the specimen subjected to PCR analysis. In practice, the reports on PCR from human tissues and body fluids show controversial results (Schmidt 1997). Neither the ideal type of specimen nor the selection of primers for the optimal de-

tection of borrelial DNA in patients is known. In optimized conditions PCR from EM and ACA skin biopsies have reached sensitivities of 100% (Schempp et al. 1993; Moter et al. 1994). In practice the sensitivity of PCR for body fluids and tissues is much lower and varies considerably depending on the specimen obtained and PCR method used (Schmidt 1997). The routine use of PCR in the diagnosis of LB is far from being generally accepted, but it is a promising method for a more reliable and faster confirmation of LB.

Lymphocyte proliferation test

B. burgdorferi infection usually also induces cell-mediated immune response, which can be detected in vitro by the lymphocyte proliferation test (Dattwyler et al. 1986). This test can be helpful in the diagnosis of late LB in the small subset of patients with no or weak antibody responses (Dressler et al. 1991). However, the test has several limitations. It is considered less sensitive than the measurement of antibodies, and it can produce false positive results because of cross-reactive immunity against microbes antigenically resembling *B. burgdorferi* (Dattwyler et al. 1988). Thus a negative result in a lympho-

cyte stimulation test does not exclude LB.

Antigen detection

There is both in vitro and in vivo evidence that *B. burgdorferi* sheds vesicles containing DNA from its surface membrane, and also surface-associated proteins (Barbour et al. 1986; Garon et al. 1989). *B. burgdorferi* antigens have been detected in urine, CSF, blood, and tissue specimens with ELISA, Western blotting and electron microscopy (Benach et al. 1988; Garcia-Monco et al. 1990; Dorward et al. 1991; Coyle et al. 1993). Although promising, antigen detection tests are not available for routine use. Their limitations include a lack of standardized reagents and a lack of controlled clinical studies on sensitivity and specificity (Coyle 1993).

2.7. Medical treatment of Lyme borreliosis

Antibiotic susceptibility of

B. burgdorferi

Antibiotics have been used in the treatment of LB since the late 1940s (Bianchi 1950; Thyresson 1950; Hollström 1951). Initially the disease was treated

with penicillin, later with various other antibiotics. Current knowledge about the efficiency of individual antibiotics is based on several *in vitro* and *in vivo* studies (Johnson et al. 1984; Johnson et al. 1987; Luft et al. 1988; Johnson et al. 1990; Johnson et al. 1990; Wormser 1990; Preac-Mursic 1993). The *in vitro* growth of *B. burgdorferi* is inhibited by several antibiotics, such as amoxicillin, ampicillin, azithromycin, cefotaxime, ceftriaxone, cefuroxime, clarithromycin, doxycycline, erythromycin, imipenem, penicillin, roxithromycin and tetracycline (Wormser 1990). The *in vitro* effect of erythromycin is one of the strongest, whereas the effect of penicillin is rather weak. *B. burgdorferi* is resistant to aminoglycosides, rifampin and trimethoprim-sulfamethoxazole. These *in vitro* results should be viewed cautiously because the methods used are not well standardized and there is no proof that the results correlate with the clinical effectiveness of the drugs (Nowakowski et al. 1993). For example, the good *in vitro* efficacy of certain macrolide antibiotics contrasts with the results of clinical trials in humans and animals, where these drugs have had an activity lower than that of penicillin and tetracycline (Steere et al. 1983; Johnson et al. 1990; Hansen et al. 1992; Luft et al. 1996).

Antibiotic treatment of erythema migrans

There is evidence that patients with early LB and even with meningoradiculoneuritis or meningitis can recover spontaneously (Pfister et al. 1993). Antimicrobial therapy is, however, always indicated for EM to prevent the development of the more serious later manifestations of LB (Weber et al. 1994).

EM disappears usually within a few days after the start of antimicrobial therapy. Numerous studies have been conducted to compare the efficacy of different antibiotic agents in the treatment of EM and the prevention of late symptoms of LB. In an early study by Steere et al., oral penicillin and tetracycline, but not erythromycin, reduced the duration of EM (Steere et al. 1980). In a subsequent study by the same group, tetracycline was superior to oral penicillin and oral penicillin was superior to erythromycin in preventing minor late complications (Steere et al. 1983). As other researchers later, Steere et al. showed a positive correlation between the severity of initial symptoms and the outcome of LB (Steere et al. 1983; Weber et al. 1987; Weber et al. 1990).

In comparative studies oral penicillin, tetracycline and amoxicillin/clavulanate (Weber et al. 1988), amoxicillin/probenecid and doxycycline (Dattwyler et al. 1990), doxycycline, amoxicillin/probenecid and azithromycin (Massarotti et al. 1992), cefuroxime axetil and doxycycline (Nadelman et al. 1992), azithromycin and penicillin (Weber et al. 1993), azithromycin and doxycycline (Strle et al. 1996) and penicillin and minocycline (Breier et al. 1996) have been found equally effective in the treatment of EM.

Ceftriaxone was as effective as oral penicillin in the treatment of EM in a study which also noted that ceftriaxone was superior to penicillin in a subgroup of patients with more than one symptom prior to therapy (Weber et al. 1990). In the study conducted by Strle (Strle et al. 1992), azithromycin was superior to doxycycline and oral penicillin in terms of the resolution of EM-associated local and systemic symptoms. In another study amoxicillin was superior to azithromycin (Luft et al. 1996). Even though antimicrobial therapy has been used for EM, mild or severe other manifestations of LB can develop (Weber et al. 1994). Most, if not all, antibiotics used thus far have

been associated with treatment failures in patients with EM (Weber 1996). *B. burgdorferi* has also been cultivated from the site of EM after antimicrobial treatment (Preac Mursic et al. 1989; Strle et al. 1993).

Antibiotic treatment of disseminated Lyme borreliosis

The beneficial effect of penicillin G in the therapy of neurological symptoms associated with EM was known in Europe already before the etiology of LB was clarified (Hollström 1951; Weber 1974). Most of the patients with acute borreliosis meningitis and meningoencephalitis can be cured by intravenously administered penicillin (Kristoferitsch et al. 1987; Sköldenberg et al. 1988; Pfister et al. 1989; Mullegger et al. 1991; Karlsson et al. 1994). However, there are reports of progressive disease despite penicillin therapy (Diringer et al. 1987; Pal et al. 1988; Gourmelen et al. 1989). Lipid soluble doxycycline with the ability to cross the blood-brain barrier (Karlsson et al. 1996) has been shown to be equally effective as penicillin G in cases of neuroborreliosis (Karlsson et al. 1994). Penicillin-resistant neuroborreliosis has successfully been treated with ceftriaxone (Dattwyler et al. 1987), chlo-

ramphenicol (Diringer et al. 1987) and cefotaxime (Pal et al. 1988).

Little information is available about the antibiotic treatment of late borreliac encephalomyelitis and cerebral vasculitis (Weber et al. 1994). Symptoms and sometimes signs of late peripheral neuropathy have improved after therapy with intravenous penicillin or intravenous cephalosporins (Weber et al. 1994).

LA has a tendency to resolve spontaneously over the years (Steere 1989). Antibiotic therapy is, however, always indicated. Benzathine penicillin and, especially, intravenous penicillin can cure LA (Steere et al. 1985; Huaux et al. 1988). A study comparing intravenous penicillin and intravenous ceftriaxone in the treatment of patients with late LB, including patients with arthritis and arthralgia, suggested the superiority of ceftriaxone (Dattwyler et al. 1988). From one-third up to two-thirds of the patients with LA have responded to oral treatment with penicillin (Schaad et al. 1986), doxycycline (Schaad et al. 1986; Liu et al. 1989) or amoxicillin/probenecid (Liu et al. 1989).

Recommendations for the antimicrobial treatment of Lyme borreliosis

The optimal treatment of various forms of LB is unknown. Most of the therapeutic trials have drawbacks if evaluated critically, and their results have to be interpreted with caution (Weber et al. 1993).

The recommendations for the treatment of EM and associated symptoms (Stage I of LB) include oral phenoxymethylpenicillin (1.5 million IU three times a day), amoxicillin (500mg three or four times a day) and doxycycline (200 mg a day) for 2-4 weeks (Rahn et al. 1991; Sigal 1992; Weber et al. 1994). For patients with intolerance to penicillin or tetracycline, cefuroxime axetil or azithromycin may be an alternative (Nadelman et al. 1992; Weber 1996). Roxithromycin and erythromycin seem to be ineffective in the treatment of EM (Weber 1996). Intravenous ceftriaxone is recommended for patients with EM accompanied by severe associated symptoms (Weber et al. 1993). In early disseminated LB without meningitis oral doxycycline is an alternative to intravenous ceftriaxone (Dattwyler et al. 1997).

Patients with Bannwarth's syndrome and meningitis are usually recommended to be treated with intravenous antimicrobial therapy. Ceftriaxone (2 g once daily) or other third-generation cephalosporins seem to be appropriate therapy for early neuroborreliosis (Weber et al. 1994). Oral doxycycline has also been used successfully in the treatment of neuroborreliosis (Karlsson et al. 1994). The duration of the intravenous therapy for late LB is often 2-3 weeks, and many authorities consider therapies longer than 4 weeks rarely indicated (Steere 1989; Rahn et al. 1991; Sigal 1992; Weber et al. 1994).

Penicillin, amoxicillin, azithromycin and tetracycline have been used successfully for the treatment of borreliac lymphocytoma. (Hovmark et al. 1986; Strle et al. 1996). However, conclusive data on the efficacy of these drugs in this respect are not available (Weber et al. 1993; Strle et al. 1996). The recommendations for the antimicrobial therapy of EM are widely considered valid also for the therapy of borreliac lymphocytoma.

For several decades, ACA has been successfully treated with penicillin and later also with tetracyclines. Penicillin or doxycycline courses of 30

days were superior to a 2-week course of intravenous ceftriaxone in the eradication of *B. burgdorferi* in patients with ACA (Aberer et al. 1996).

2.8. Prevention of Lyme borreliosis

LB is, to a large extent, a preventable infection. Avoidance of heavily tick-infested areas, personal protection with proper clothing, and prompt removal of attached ticks remain the most effective protective measures. Many other preventive measures are available and could be efficiently used to reduce the abundance of vectors. However, since the ecology of *B. burgdorferi* varies greatly between different localities, it may be necessary to apply different combinations of control methods in different endemic regions (Jaenson et al. 1991).

The risk of borreliac infection after a single tick bite is low (1%-3.5%) (Warshafsky et al. 1996). Several trials with antimicrobial prophylactics after *Ixodes* tick bites have been conducted in endemic areas (Costello et al. 1989; Magid et al. 1992; Shapiro et al. 1992; Agre et al. 1993; Warshafsky et al. 1996; Sood et al. 1997; Fix et al. 1998). No trial, however, and not even a meta-analysis of published trials, has statistically significantly

demonstrated the efficacy of antimicrobial treatment in preventing LB after tick bites. Even though antimicrobial therapy is not recommended by scientists as a prophylactic measure, a considerable percentage of physicians use it (Eppes et al. 1994; Ziska et al. 1996). A recent study showed that tick identification and the measurement of engorgement can be used to identify a small, high-risk subset of persons who may benefit from the immediate administration of an antibiotic (Sood et al. 1997). The awareness of the local infestation rates of ticks with *B. burgdorferi* may help to identify the high-risk areas.

Vaccination for Lyme borreliosis

Animal models have shown that both passive and active immunization can protect against infection by *B. burgdorferi* (Johnson et al. 1988; Fikrig et al. 1992). Both human and veterinary LB vaccines are needed in areas endemic for the disease. A recombinant OspA vaccine has been licensed for the prevention of LB in dogs (Edelman 1991). Two extensive efficacy studies of vaccines for the prevention of LB have recently been completed in the United States (Sigal et al. 1998; Steere et al. 1998). Both vaccines based on recombinant OspA and showed an efficacy

of 49-68% during the first year and 76-92% during the second year after three-stage vaccination on days 0, 30 and 360. CDC licensed in United States an OspA vaccine for humans in December 1998. Unfortunately OspA vaccines, because of the variability of OspA in local *B. burgdorferi* sensu lato strains, hardly are of any use in Europe.

2.9. Neurootological manifestations of Lyme borreliosis

Facial nerve paralysis

Acute peripheral FP was first described in 1821 by Sir Charles Bell, who defined the motor function of the seventh cranial nerve (Bell 1821). The reported incidence of FP varies between 11.5 and 40 annual cases per 100 000 of the population (Melotte 1961; Gregg 1961; Lagerholm et al. 1971; Hauser et al. 1971; Mair et al. 1974; Adour et al. 1978; Peitersen 1982; Katusic et al. 1986; Yanagihara 1988). The annual incidence of FP in childhood is less than half of the corresponding incidence of FP in adults (Adour et al. 1978; Peitersen 1982; Katusic et al. 1986; Christen et al. 1993). In up to 75% of the cases the etiology has remained obscure, and the symptom has been called Bell's palsy (Adour 1982).

Several factors have been suggested for the etiology of FP, including genetic, metabolic, autoimmune, vascular, entrapment and infectious causes (Bauer et al. 1996). Diabetes mellitus and pregnancy are known predisposing factors for FP (Adour et al. 1974; Hilsinger et al. 1975; Adour 1977). During the last two decades viral infections, especially the herpes simplex virus, have gained popularity among investigators as a potential cause of FP (Barringer et al. 1973; Adour et al. 1980; Morgan et al. 1992; Murakami et al. 1996; Adour et al. 1996). Thus far the evidence implicating HSV as a cause of FP is still incomplete. However, based on empirical evidence, acyclovir has been proposed for the treatment of FP (Adour et al. 1996). FP caused by the *Varicella zoster* virus forms its own entity.

Some bacterial diseases, including spirochetal infections like syphilis (Verduijn et al. 1982) and relapsing fever (Southern et al. 1969), are known to induce FP. *B. burgdorferi* also belongs to the group of spirochetes capable of inducing FP. In a study of almost 1000 patients with LB, FP occurred in 10% (Clark et al. 1998). Cranial neuropathies develop in about 60% of the patients in early

neuroborreliosis and in 45% of the patients in late neuroborreliosis (Reik 1991). Up to 55% of pediatric patients with neuroborreliosis can incur FP (Christen et al. 1993). FP accounts for 70-80% of all cranial nerve neuropathies in early LB (Reik 1991) and about one-third of them in late LB (Ackermann et al. 1988).

LB, or serological evidence of it, has been found in 6-20% of adult patients with FP (Åsbrink et al. 1985; Olsson et al. 1988; Jonsson et al. 1990; Laurikainen et al. 1990; Puhakka et al. 1992; Roberg et al. 1991; Kuiper et al. 1992; Hyden et al. 1993). The incidence of LB is higher in pediatric patients than in adults with FP. In studies on pediatric FP, as many as two thirds of the patients have had LB (Dotevall et al. 1994). In a German multicenter study, LB was diagnosed in every third child with FP and in every second child during the warm season, and it was the most frequently verifiable cause of FP (Christen et al. 1993).

The spontaneous course of borreliac FP and neuroborreliosis is predominantly favorable (Kruger et al. 1989; Niemann et al. 1997). Although LB is often a self-limiting infection, antimicrobial therapy is widely accepted and used to prevent rare la-

ter complications. FP can be a manifestation of early LB without signs of spirochetal dissemination, but it can also be a manifestation of early or late disseminated disease. Whether acute disseminated *B. burgdorferi* infection should be treated differently from localized infection is not known (Dattwyler et al. 1997). Because of the small number of controlled studies, the optimal treatment of borrelial FP also remains unknown. FP caused by LB has been successfully treated with intravenous penicillin (Jonsson et al. 1987; Olsson et al. 1988; Engervall et al. 1995; Kindstrand 1995; Christen 1996), intravenous cephalosporins (Pfister et al. 1989; Christen 1996) and peroral tetracyclines (Dattwyler et al. 1997).

Sudden sensorineural hearing loss

SHL is a loss or impairment of hearing that develops during a period not exceeding a few hours or it is present on awakening. The annual incidence of SHL increases with advancing age and is reported to be between 5 and 20 cases per 100 000 of the population. (Byl 1984)

Since the first published group of patients with "idiopathic" SHL in 1944 (De Kleyn 1944), it has been

shown that this disorder has numerous possible causes, including infectious, traumatic, neoplastic, immunologic, toxic, circulatory, neurologic and metabolic factors (Hughes et al. 1996).

Publications concerning the relationship between LB and SHL are few. The eighth cranial nerve or the vestibulocochlear nerve is involved in less than 5% of cranial neuropathies in patients with LB. LB seems to affect the cochlear part of the nerve more often (Reik 1992), but the vestibular part can also be affected (Hanner et al. 1988). There are some reports of SHL in patients with both early and late LB (Mokry et al. 1990; Heininger et al. 1990; Quinn et al. 1997; Zajkowska et al. 1998). In patients with ACA brainstem response audiometry (BRA) has shown abnormalities indicating the involvement of central auditory pathways in chronic LB (Sandström et al. 1989). Serological evidence of LB has been reported in about one-fifth of patients with SHL (Hanner et al. 1989; Hanner 1995). Patients with a favorable effect from antimicrobial therapy on long-lasting LB-related hearing deficit have occasionally been reported (Lessaer et al. 1990; Goldfarb et al. 1994; Quinn et al. 1997; Zajkowska et al. 1998).

Vertigo

Vertigo, dizziness and different types of dysequilibrium are common clinical problems. Although, in the medical literature, the term vertigo is used to include a hallucination of turning or other motion separating it from dizziness and other forms of dysequilibrium, in this study it is used to describe all forms of a subjective perception of imbalance. The etiology of vertigo is multifaceted and includes various systemic diseases, disorders of the CNS and more specific disturbances in the equilibrium apparatus (vestibule, semicircular canals, vestibulocochlear nerve, vestibular nuclei in the brainstem and their temporal lobe connections and eyes). In neurootological clinics the most common diseases involving vertigo are Menière's disease, benign positional vertigo, vestibular schwannoma, vestibular neuritis, traumatic vertigo, sudden deafness with vertigo, inflammatory diseases, perilymphatic fistula, ototoxicity, benign recurrent vertigo, benign pa-

roxysmal vertigo of childhood, inner ear autoimmune disease, CNS tumors, brainstem ischaemia, neurovascular compression syndrome and epilepsy (Kentala 1996).

The literature on vertigo in relation to LB is sparse and limited to a few papers. Rosenhall et al. (Rosenhall et al. 1988) demonstrated serological evidence of LB in 10 of 73 (14%) patients with vertigo. Ishizaki et al. (Ishizaki et al. 1993) showed positive levels of antibodies against *B. burgdorferi* in 12 of 350 (3.4%) patients. Riechelmann et al. (Riechelmann et al. 1990) demonstrated that 8 of 45 (17%) patients with vertigo had positive levels of antibodies against *B. burgdorferi*, but they found the same rate of seropositivity in the control group. On the other hand, vertigo is not an uncommon symptom among patients with disseminated LB. In series of patients with LB the prevalence of vertigo has varied between 8% and 26% (Krejčová et al. 1988; Moscatello et al. 1991; Wahlberg et al. 1993).

3. AIMS OF THE PRESENT STUDY

The aim of the present study was to evaluate the prevalence and clinical picture of LB among patients from southern Finland presenting with neurootological symptoms. These symptoms included acute peripheral FP, SHL and vertigo. In addition, the endemicity rate of LB was studied in the main patient enrollment area.

The specific aims of the study were

1. to determine prospectively the prevalence of FP caused by LB in southern Finland and to study the characteristics of LB-related FP compared with FP unrelated to LB (I);
2. to study prospectively LB as the cause of pediatric FP and, in addition, to evaluate retrospectively the long-term prognosis of untreated FP caused by LB (II);
3. to study prospectively the prevalence of LB in patients with SHL and vertigo with special emphasis on signs and symptoms possibly predicting the occurrence of LB (III-IV) and
4. to estimate the density of *I. ricinus* in recreational areas in Helsinki and to assess the prevalence of *B. burgdorferi* in these ticks. Three different methods (DFM, cultivation and PCR) used in the detection of borreliae in ticks were compared.

4. PATIENTS AND MATERIALS

The patients for the present study were examined in the Department of Otorhinolaryngology (study I-IV) or in the Departments of Paediatrics and Otorhinolaryngology (study II) of the Helsinki University Central Hospital.

4.1. Study I

For study I, 503 patients with acute idiopathic lower motoneuron FP were consecutively examined from 1993 through 1994. On admission, the patients filled out a questionnaire concerning symptoms and signs related to LB, and their sera were screened for antibodies to *B. burgdorferi* by ELISA. For patients with a clinical suspicion of LB or positive levels of antibodies against *B. burgdorferi*, CSF and sera were analyzed by PCR for DNA of *B. burgdorferi*. The CSF specimens also were subjected to routine chemical (protein and glucose concentration) and cytological (leucocyte count) analyses to estimate the degree of CNS inflammation. The outcome of the FP was evaluated by another questionnaire (mean follow-up of 3 years) concerning recovery from FP and possible residual symptoms.

4.2. Study II

Study II consisted of an evaluation of 49 consecutively examined children (<17 years) with 50 episodes of FP from April 1994 through September 1996. The patients were carefully interviewed with special emphasis on tick bites and signs or symptoms of LB in their history, and their sera and CSF were analyzed for *B. burgdorferi* DNA and for antibodies against *B. burgdorferi*. Other laboratory tests included leucocyte count, sedimentation rate, C-reactive protein concentration in the blood and routine chemical and cytological analyses of the CSF. The CSF was also incubated in BSK-II medium for the cultivation of *B. burgdorferi*.

Study II included a retrospective analysis of 43 children who had had FP an average of 5 years earlier. These patients had not been previously examined for LB, nor had they been treated for FP with antimicrobial agents. On admission the patients completed a questionnaire, with special emphasis on possible signs and symptoms of chronic LB. The examination of the children included an otoneurological evaluation, screening for *B. burgdorferi* DNA and antibodies against *B. burgdorferi* in their serum and an assessment of leucocyte count, sedi-

mentation rate and C-reactive protein concentration in their blood. *B. burgdorferi* DNA, antibodies against *B. burgdorferi* and routine chemical and cytological parameters were also analyzed from the CSF. Sera and CSF were also incubated in BSK-II for the cultivation of *B. burgdorferi*.

4.3. Study III

In study III, 168 patients with idiopathic SHL during 1993-1994 were consecutively and prospectively studied for antibodies against *B. burgdorferi*. On their admission signs or symptoms possibly related to LB were given a special attention. For the seropositive patients, sera and CSF were analyzed for *B. burgdorferi* DNA, for antibodies against *B. burgdorferi*, and for signs of inflammation. The patients were given an audiological evaluation that included pure tone audiometry with air and bone conduction thresholds and brainstem auditory responses (Nicolet Spirit). The audiological outcome data included the pure tone average (PTA), the PTA recovery percent and the PTA improvement percent. In case of any suspicion of retrocochlear pathology, posterior fossa, including the internal acoustic meatus, was scanned by magnetic resonance ima-

ging (MRI) or computed tomography (CT).

4.4. Study IV

In study IV 2055 consecutive vertigo patients who entered to the department from 1993 through 1994 were examined for the prevalence of LB. The diagnoses of these patients included a wide range of neurootological, neurological and vascular causes of vertigo. The patients were interviewed with special attention being paid to possible signs and symptoms related to LB. The sera of patients were screened for antibodies against *B. burgdorferi*, and those with positive levels of antibodies were further analyzed by Western immunoblot and for *B. burgdorferi* DNA. The CSF specimens of the seropositive patients were analyzed for *B. burgdorferi* DNA and antibodies against *B. burgdorferi*. The neurootological tests included the evaluation of saccades, pursuit eye movements, posturography and the caloric test. The audiological evaluation included pure tone audiometry and BRA.

4.5. Study V

For study V, 726 *I. ricinus* ticks were collected by dragging a 1m² cloth

through the vegetation of five recreational areas in Helsinki. The density of ticks in the areas was estimated. The ticks were processed further by opening their shield and removing the midgut. So that the prevalence of *B.*

burgdorferi could be determined and the methods used for the detection of *B. burgdorferi* in the ticks could be compared, the midgut was cut in three pieces, one for DFM, one for PCR analysis and one for cultivation.

5. METHODS

The methods are presented in detail in original publications. The outlines are as follows:

5.1. Enzyme linked immunosorbent assay

IgM and IgG antibodies against *B. burgdorferi* were measured by a commercial flagellin-based ELISA kit (Dako, Glostrup, Denmark) modified by using titration of the antibodies as described earlier (Seppälä et al. 1994). Sera were diluted serially in three-fold steps for the test and applied to the plates for overnight incubation. The bound antibodies were detected by biotin-labeled goat anti-human IgM and IgG (Zymed, Los Angeles, CA, USA). An end point titer was obtained at an optical density level determined by a cut-off control provided by the kit. The titer limit for a positive IgG antibody level was 500, and for a positive IgM level it was 2500. This cut-off control material conformed with the level of the mean +3 standard deviations (SD) of the reference population living in central Finland (Seppälä et al. 1994). In Denmark and Sweden 98% of the population have antibody levels below this standard (Hansen et al. 1988). The modifications to the commercial

ELISA kit were made in order to allow measurement of serum and CSF antibodies in the same linear titration scale, thereby allowing the calculation of intrathecal antibody production in combination with immunoglobulin concentration data.

For the CSF the cut-off limits for both the IgM and IgG antibodies were 3.0 in the same scale as applied to the serum analysis. The IgG antibody titer in the CSF was divided by the total IgG concentration in the CSF. A corresponding ratio was also calculated for the serum. If the calculated ratio of the CSF was more than two-fold higher than the ratio of the serum, IgG antibodies to *B. burgdorferi* in the CSF were considered to be intrathecally synthesized.

5.2. Western blotting

In study IV the sera from patients with positive levels of antibodies against *B. burgdorferi* were further analyzed for IgG1 antibodies by Western immunoblot using Finnish *B. afzelii* strain KS1 as the antigen source. The immunoblotting was performed as described by Seppälä et al. (Seppälä et al. 1994). The sample was considered positive when at least four bands, out of those most regu-

larly found in Finnish patients with LB, were observed. These bands represented proteins with molecular masses of 17, 19, 21, 30, 41, 48, 52 and 83 kDa.

5.3. *Treponema pallidum* antibodies

In an attempt to rule out syphilis as a source of false positive serological results, in studies I, III and IV, sera were examined for *T. pallidum* antibodies by a commercial hemagglutination assay (Porton Cambridge, Newmarket, UK).

5.4. Polymerase chain reaction

PCR analyses were performed in all the studies (I-V). The method for DNA extraction and primers used in the PCR assay for ticks in study V differed from those used in clinical studies I-IV.

In studies I-IV, the serum or CSF specimens were first treated with SDS, and then DNA was extracted from them with phenol chloroform, precipitated with ethanol, and finally dissolved with water.

In study V, DNA was extracted from the tick midgut by InstaGene DNA extraction matrix (Bio-Rad Laborato-

ries, Hercules, CA, USA) according to the instructions of the manufacturer.

In studies I-IV the target of the PCR amplification was a segment of the gene encoding the 41-kDa flagellin of *B. burgdorferi*. The primers were modifications (He et al. 1994) from those described by Picken et al. (Picken 1992). Oligonucleotides were synthesized by an automatic DNA synthesizer (Model 391 PCR-Mate DNA Synthesizer; Applied Biosystems, Foster City, CA, USA) based on phosphoamidite chemistry. The PCR reaction consisted of 40 cycles carried out in a thermal cycler (HB-TR1, Hybaid Ltd., Middlesex, UK). After amplification, the reaction mixture was run in an agarose gel stained with ethidium bromide, and the PCR products were visualized and photographed under ultraviolet light.

In study V a nested PCR test based on the flagellin gene was used to demonstrate the presence of *B. burgdorferi* DNA in the tick midgut. Primers BBSCH31 and BBSCH42 were used in the first PCR that consisted of 25 cycles. Primers FL59 and FL7 were used in the second PCR with 35 cycles (Schmidt et al. 1996). The PCR product was visualized as already described.

The spirochetes grown from the ticks were identified to the species level by the PCR test based on amplification of the 16S rRNA gene (Marconi et al. 1992). The extracted DNA was amplified with four sets of primers specific for *B. burgdorferi* sensu lato (LD primers), *B. burgdorferi* sensu stricto (BB primers), *B. afzelii* (VS461 primers), and *B. garinii* (BG primers). For the LD primers, 40 cycles of denaturation at 94°C for 1 minute, annealing at 47°C for 30 seconds, and extension at 72°C for 1.5 minute were carried out. For the other primers, the PCR procedure was the same, except that the annealing was performed at 42°C. The resulting PCR products were visualized by agarose gel electrophoresis.

The samples giving ambiguous results in the PCR reactions specific for *B. afzelii* and *B. garinii* were further analyzed by PCR-based sequencing of the flagellin gene. A 277 bp product was obtained by using primers FL7 (biotinylated) and FL59. The biotinylated PCR products were rendered single stranded by streptavidin-coated Dynabeads according to the instructions of the manufacturer (Dynabeads M-280 streptavidin; Dynal AS, Oslo, Norway). Manual sequencing was performed using Sanger's

dideoxynucleotide chain termination method and Sequenase 2.0 (United States Biochemical Corp., Cleveland, OH, USA) (Soini et al. 1994). The obtained sequences were compared with the flagellin gene sequences of the type strains of *B. afzelii* Bo23 and *B. garinii* 387.

Rigorous measures were undertaken to avoid carry-over contamination and contamination caused by amplicon. Pre- and post-PCR stages of the process were carried out in physically separate rooms and by separate technicians. Each PCR run included a positive control containing DNA extracted from reference strain B31 of *B. burgdorferi* sensu stricto (ATCC 35210). Furthermore, every fifth or sixth tube of each run was used as a negative control and subjected to all sample treatment procedures.

5.5. Audiological evaluation

The audiological tests in studies III and IV included pure tone audiometry recorded at frequencies of 0.125, 0.250, 0.5, 1, 2, 4 and 8 kHz, including the assessment of air and bone conduction thresholds for both ears. A Nicolet compact auditory electrodiagnostic system (Nicolet Spirit, Nicolet Instrument Corporation,

Madison, Wisconsin, USA) was used for measuring the BRA.

5.6. Otoneurological tests (study IV)

The otoneurological tests included the evaluation of saccades, pursuit eye movements, posturography and the caloric tests.

5.7. Criteria for the diagnosis of LB

Special emphasis was placed on the critical evaluation of the diagnosis of LB. The following criteria were used for the diagnosis of LB:

Study I: in addition to seropositivity to *B. burgdorferi*, the presence of one or more of the following findings were required: 1) presence or history of untreated EM, 2) a positive level of antibodies to *B. burgdorferi* in CSF, 3) a positive PCR test and 4) CSF pleocytosis in children.

Study II: one or more of the following findings were required: 1) positive levels of serum or CSF antibodies to *B. burgdorferi*, 2) presence or history of untreated EM and 3) a positive PCR test.

Study III: in addition to seropositivity to *B. burgdorferi*, at least one of the

following findings were required: 1) history of EM or 2) a positive PCR test.

Study IV: in addition to positive serum levels of antibodies to *B. burgdorferi*, the presence of one or more of the following findings were required: 1) history of untreated EM, 2) a positive PCR test and 3) a positive serum immunoblot.

5.8. Tick preparation, dark field microscopy and cultivation of *B. burgdorferi* (V)

The midguts of the ticks were removed under a stereo microscope and placed in a drop of BSK-II medium using small sterile forceps, a disposable 28 G needle as a scalpel, and sterile insect preparation needles.

A part of the tick midgut was placed on a microscope glass in a drop of BSK-II medium, and the number of spirochetes in the sample was estimated by DFM (Leitz, Laborlux D, Nürnberg, Germany) by examining 100 fields with a magnification of 400x. Typical movement, morphology and size were used as the identification criteria for borrelia. Another part of the tick midgut was inoculated immediately after the preparati-

on into tubes containing BSK-II medium supplemented with rifampin (final concentration 100 mg/ml) and phosphomycin (final concentration 50 mg/ml) and incubated at 30°C for 8 weeks or until growth was detected. The growth media were examined by DFM every other week. If growth appeared, the cultures were passaged into new tubes containing BSK-II medium without antibiotics.

5.9. Statistical methods

In study I and III, the correlations between symptoms and signs in different subgroups of patients were estimated with the chi-square test or with an analysis of variance. Then potential risk factors for poor outcome and signs or symptoms possibly explaining the occurrence of LB were incorporated into logistic regression analyses. In study II, the numbers of children and the prevalence of different signs and symptoms possibly explaining the occurrence of LB were estimated with the Mann-Whitney U-test and Fisher's exact test. In study V, the chi-

square test was used to test the association of borrelia prevalence in ticks with factors possibly influencing the infestation rate of the ticks. Interactions between these factors and the putative confounding effect caused by the viability of ticks was tested with log-linear modeling. The independence of the occurrence of *B. garinii* and *B. afzelii* in ticks was tested with Fisher's exact test. The agreement of the three methods used for the detection of *B. burgdorferi* in ticks was studied with kappa statistics, and the statistical significance of the disagreement was obtained with McNemars' chi-square test. These statistical data were obtained by the use of Statistics 4.0 (Analytical Software) and Statview 4.5 (Abacus Concepts Inc.) programs.

5.10. Ethics

The study protocols (I-IV) were approved by the ethical committees of the Department of Otorinolaryngology and the Department of Pediatrics (study I) of the University Central Hospital of Helsinki.

6. RESULTS

6.1. Lyme borreliosis in patients with facial paralysis - a prospective analysis of outcome and predictive signs and symptoms (I)

Of the 503 patients enrolled to study I, 61 (12%) had positive levels of antibodies to *B. burgdorferi* in their serum. Eleven of these seropositive patients had definite LB. Three LB patients had history of EM. Positive levels of CSF antibodies were present in eight LB patients and CSF pleocytosis was found in five. The PCR test was positive in none of the serum specimens and in one of the CSF specimens.

FP caused by LB was significantly more common during the summer season (June-November) than the winter season (December-May) ($p=0.039$). Bilateral FP was rare in the whole material (2.4%), but significantly ($p=0.0015$) more common in patients with definite LB (18%) than in the rest of the patients (2%). Of the signs and symptoms preceding or coinciding with FP, fever ($p=0.0018$), headache ($p=0.012$), pharyngalgia ($p=0.015$), enlarged cervical lymph nodes ($p=0.0062$) and arthralgia ($p=0.036$) were more common in patients with definite LB. Swedish as a

native language was more common among the patients with positive levels of serum antibodies against *B. burgdorferi*. In the logistic regression analysis the best model to predict the occurrence of LB included the presence of enlarged cervical lymph nodes, the presence of arthralgia and the onset of symptoms during the summer season.

The outcome of facial paralysis (I)

The outcome of FP was evaluated by a dedicated questionnaire (mean follow-up 3 years) concerning recovery from FP and possible residual symptoms. Incomplete recovery from FP was more common among the patients with total paralysis ($p=0.002$), patients with recurrent FP ($p=0.0005$) and patients with arthralgia ($p=0.005$), vertigo ($p=0.02$) or nausea ($p=0.01$) at the time of FP. Incomplete recovery was more frequently reported by women than by men ($p=0.01$). Hyperacusis was also more common in the patients with incomplete recovery, but the difference was not significant ($p=0.06$). In the logistic regression modeling the factors in the model that best predicted incomplete recovery were the presence of total FP, recurring FP and hyperacusis.

6.2. Pediatric facial paralysis caused by Lyme borreliosis: a prospective and retrospective analysis (II)

Of the 49 children with 50 episodes of FP, 17 (35%) had LB. Eleven of the children with LB had positive levels of serum antibodies on admission, and two children seroconverted during the follow-up. Four children remained seronegative, and the diagnosis of LB in these patients was based on positive levels of CSF antibodies in one case, a positive PCR result in one case and a history of EM in two cases. Altogether nine children had a history of untreated EM, and one child developed EM four weeks after FP. Of the signs and symptoms coinciding with or preceding FP, headache ($p < 0.001$) and arthralgia ($p < 0.05$) were more frequent among the patients with LB. The patients with LB also recalled tick bites more often than the other patients with FP ($p < 0.01$). The occurrence of FP among patients with LB was more frequent during the summer season ($p < 0.001$). The incidences of bilateral FP and total paralysis were similar for the patients with and those without LB.

Abnormalities in the CSF analysis were observed for 12 of the 41 patients and exclusively in patients with

LB. The intrathecal production of antibodies to *B. burgdorferi* was present in 8 children, and 11 children had pleocytosis. The CSF protein concentration was elevated in five patients and the PCR analysis from the CSF was positive in one patient.

The mean follow-up of the patients was 28 months. The recovery was complete (House-Brackmann grade 1) for 34 of the 49 (69%) patients. The full recovery was more frequent for patients with LB, but the difference between the groups was not significant. Most of the sequelae in both groups were mild (House-Brackmann grade 2).

In the retrospective part of study II, the children had no signs or findings related to chronic LB. However, one 16-year-old girl had a positive serum level of IgM antibodies against *B. burgdorferi* coinciding with headache, fatigue and arthralgia on admission. During the follow-up of this patient, a four-fold rise in IgM antibodies was detected. Her history of three recent camping trips to an area hyperendemic for LB in the Finnish southwestern archipelago supports the concept that she had an acute borreliosis infection at the time of the investigation.

6.3. Lyme borreliosis, an etiologic factor of sensorineural hearing loss? (III)

In this prospective study 20 of 165 (12%) patients with SHL had positive levels of antibodies to *B. burgdorferi*, and 4 of them fulfilled the criteria for definite LB. Two of the patients with definite LB had a history of untreated EM, and two had a positive result in the Borrelia-PCR analysis in the serum. In the CSF analysis of the seropositive patients none had intrathecal synthesis of antibodies against *B. burgdorferi*, and all of them had a negative result in the Borrelia-PCR analysis.

The statistical analysis of audiological recovery and the prevalence of tinnitus and vertigo in seronegative patients (n=145), seropositive (probable LB) patients (n=16) and definite LB patients (n=4) revealed no significant differences between these groups. No significant differences were found, when the clinical data of the patients in different groups was cross-tabulated. Thus no specific signs or symptoms were found to predict the occurrence of LB in patients with SHL. The seasonal distribution of SHL was even in all the subgroups, with a slight accumulation of cases in early spring and late autumn.

The clinical data of all the patients were cross-tabulated, and the significance of single symptoms and signs as explanatory factors for recovery was tested with the chi-square test. Patients with a fair to poor recovery (Mattox et al. 1977) were more frequently older than 40 years ($p=0.013$) and had a high frequency or flat-type hearing loss more frequently ($p=0.0015$), when compared with those with good recovery. In the logistic regression modeling the factors in the best model explaining excellent to good recovery included young age, mid-frequency hearing loss and positive levels of antibodies against *B. burgdorferi*.

6.4. Lyme borreliosis as a cause of vertigo (IV)

Of the 2055 prospectively screened patients with vertigo, 41 (2%) had positive levels of antibodies against *B. burgdorferi* and 6 of them fulfilled the diagnostic criteria for LB. The incidence of seropositivity to *B. burgdorferi* in patients with vertigo did not differ from the corresponding incidence of the general Finnish population. Of the patients with LB, three had a history of untreated EM, and the seropositivity was confirmed with immunoblotting for all but one

of these patients. The serum *Borrelia*-PCR analysis was positive in two cases. The *Borrelia*-PCR analysis of CSF was negative in all cases. The CSF specimens of the 16 seropositive patients were analyzed for protein concentration and cell counts and found to be normal.

The vertigo of patients with LB was rotational in six patients and, positional in one; in addition one patient suffered from drop attacks of the Tumarkin type. Two patients showed evidence of CNS affection in the otoneurological tests. Five patients had symptoms resembling Menière's disease. Seven of the eight patients received antimicrobial therapy, and the response was estimated as good (vertigo disappeared during medication and patient had no symptoms at the end of the follow-up) in three cases and moderate (a considerably decreased vertigo after the medication) in four cases. After the treatment the mean follow-up of the patients was 30 months.

6.5. Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks in urban recreational areas of Helsinki (V)

Altogether 726 *I. ricinus* ticks were collected from five popular recreati-

onal areas in Helsinki to estimate the density of ticks and to assess the infestation rate of ticks with *B. burgdorferi*. The estimated number of annual visitors to these areas is 1.4 million (Helsinki 1997). The tick densities varied from 1 to 36 ticks for every 100 meters of flagging with a 1m² piece of fabric. All the tick populations were infected with *B. burgdorferi*, and the infestation rate varied from 19% to 55%. The chi-square analysis indicated that the infection rate was associated with the area from which the ticks were collected ($p < 0.001$), but not with the developmental stage ($p = 0.06$) or viability of the ticks ($p = 0.19$). A log-linear analysis, taking into account the possible confounding effects of a uneven distribution of developmental stage and the viability of the ticks in different areas, confirmed the significant difference between the infestation rates of the different areas.

Altogether 234 ticks were *Borrelia*-positive in the cultivation ($n = 144$), DFM ($n = 193$) or PCR ($n = 135$). Of the 142 cultivated and typable *B. burgdorferi* strains, 100 (70%) belonged to the subspecies *B. afzelii* and 37 (26%) to the subspecies *B. garinii*. Five strains were untypable; yet no *B. burgdorferi* sensu stricto strains were

found. Mixed infection with both *B. afzelii* and *B. garinii* was rare and found in two ticks only.

The DFM gave more positive results than either the culture ($p < 0.001$) or

PCR analysis ($p < 0.001$). The comparison of the three different methods with kappa statistics showed a fairly good agreement between them (kappa value between 0.53 and 0.59 in all the comparisons).

7. DISCUSSION

The present study evaluated the occurrence of LB in patients with neurootological complaints in an area endemic for LB. The results show the importance of LB in the diagnostic work-up of an otorhinolaryngologist. LB is a frequent diagnosable cause of FP in adult patients (I) and it is especially frequent in pediatric patients (II). It was also shown that vestibulo-cochlear dysfunctions, i.e. SHL (III) and vertigo (IV) can be caused by LB. The endemicity of the patient enrollment area was confirmed by demonstrating an abundance of *I. ricinus* ticks and their high infestation rate with *B. burgdorferi* in the area where most of the patients live. (V).

7.1. Laboratory diagnosis of Lyme borreliosis

Findings in cerebrospinal fluid

CSF abnormalities are frequently found in patients with LB. Lymphocytic pleocytosis and an elevated total protein concentration is a characteristic finding in the CSF of patients with Bannwarth's syndrome. LB-associated peripheral neuropathies including cranial neuropathies are often, but not always, accompanied by

lymphocytic meningitis (Halperin 1998). The analysis of CSF has been emphasized for selected patients with cranial neuropathy to verify borreliosis etiology (Kindstrand 1995). In the present study most of the patients with FP caused by LB (81% and 70% in studies I and II, respectively) had abnormalities in their CSF, whereas none of the patients with LB-related SHL or vertigo had any.

These CSF abnormalities seemed to be age-dependent. In study I (mean age of the patients 28 years), 45% of the patients had pleocytosis, and the same proportion also had increased protein concentration in their CSF. In study II (mean age of the patients 8.4 years) increased protein concentration was observed in 29% of the patients, whereas pleocytosis was present in 65% of them.

The findings show the importance of CSF analysis in the search of etiology of FP in areas endemic for LB. Patients with SHL and vertigo did not show CSF abnormalities, probably because of the long time that had passed since the initial infection. The results do not encourage routine CSF analysis of patients with these disease manifestations.

Culture

The gold standard for the diagnosis of LB is cultivation of the pathogen from body fluids or tissues. This study showed, again, that the isolation of *B. burgdorferi* from clinical specimens is difficult. The culture was negative in all the specimens studied. This low yield of culture is in accordance with the experience of others. The CSF from patients with neurological manifestations of LB has been positive in only 7-10% of patients (Karlsson et al. 1990; Wilske et al. 1993). A positive culture from the CSF is more frequent in manifestations of short duration (Karlsson et al. 1990). The clinical type of specimen most frequently positive (up to 70%) in cultures is the biopsy from EM (Wilske et al. 1993). However, in clinical practice the diagnosis of EM is straightforward without cultivation and other laboratory tests.

The cultivation of *B. burgdorferi* was first accomplished from *I. scapularis* ticks by Burgdorfer et al. in 1981 (Burgdorfer et al. 1982), and it has later been successfully used in studies on the prevalence of *B. burgdorferi* in ticks (Ackermann et al. 1984; Junttila et al. 1994; Strle et al. 1995; Humair et al. 1995; Solari Basano et

al. 1996). The present study also showed that *B. burgdorferi* can be cultivated much more effectively from the midguts of the ticks than from clinical specimens (V). Thus the negligible yield of cultures from clinical specimens could not be, at least solely, attributed to an inadequate culture procedure because the same procedure was used for all the specimen types.

There are at least two plausible explanations for the low yield of cultures of *B. burgdorferi* from clinical specimens. First, *B. burgdorferi* may occur only temporarily and in low numbers in body fluids and tissues during the course of infection (Pachner 1998). The problem of a small bacterial number can be solved only by introducing large volumes of a specimen to the culture medium. Such a procedure is not, however, always possible because of the low amount of specimen available. Second, host defense factors may impair the viability of *B. burgdorferi* not only in vivo but also later in vitro. The fact that the pathogen can be relatively easily isolated from ticks may be due to both of these reasons. The number of *B. burgdorferi* in ticks is rather large – the organism can easily be seen by light microscopy in

tick midgut preparations. It is also possible that the primitive defense mechanisms of ticks do not impair the viability of *B. burgdorferi* to the same extent as mammalian immune defense does.

A recent study showed that, by optimizing growth conditions, it may be possible to increase the yield of cultivation even for specimens from patients with chronic LB (Phillips et al. 1998). For the present, however, cultivation remains an impractical, but promising method for the clinical diagnosis of LB. For scientific purposes, the patients with culture-proven LB, however, are extremely valuable.

Antibody measurement

ELISA is the most suitable method for the screening of serum and CSF antibodies against *B. burgdorferi* in patients with symptoms suggestive of LB (Golightly 1993). The assessment of antibodies has its limitations, however. Some of the limitations of serologically testing LB are true for all serologic tests, and some are specific to this infection. In general the antibody response takes time to develop, and in the early course of LB a considerable proportion of the pa-

tients remain seronegative (Strle et al. 1996). Second, the antibody response may persist for a long time (Fahrer et al. 1998). Thus positive levels of antibodies may reflect both old immunity and active infection. Third, antibodies reactive with *B. burgdorferi* antigens can be elicited by other infectious agents because of cross-reactive antigenic molecules or epitopes (Seppälä et al. 1994; Burkot et al. 1997). Fourth, many illnesses can induce polyclonal B-cell expansion (e.g., infectious mononucleosis and subacute bacterial endocarditis), which can lead to false positive reactions in antibody determinations (Kaell et al. 1990). Fifth, individual antibodies are produced against molecular epitopes, not against specific bacteria, and cross-reactivity occurs between epitopes resembling each other. Sixth, the complexity of *B. burgdorferi* offers various approaches to antigen preparation, ranging from the use of sonicated whole cell lysates to highly purified or recombinant antigens (Magnarelli et al. 1996; Hauser et al. 1997). Usually however, these highly purified singular antigens improve the specificity of the assay at the cost of sensitivity (Oksi et al. 1995). For the moment, the choice of the best antigen(s) for ELISA remains unresolved.

There has been an avid discussion on seronegative LB. This is a natural state in the early phases of the disease, but it can definitely occur also at later stages of LB (Dattwyler et al. 1988; Guy et al. 1989; Oksi 1996). One reason for the seronegativity may be early antimicrobial treatment that can permanently abrogate the antibody response without completely eliminating the organism (Dattwyler et al. 1988). It is also possible that the immune system of some persons does not mount a response to borrelian antigens. The mechanisms behind this unresponsiveness remain to be clarified.

Although most of the patients with extracutaneous LB almost always have positive levels of serum antibodies to *B. burgdorferi*, in the early course of the disease some patients may have positive levels of CSF antibodies only (Tugwell et al. 1997). This phenomenon was also seen in the present study. One patient (study I) was seronegative, but proved to have positive levels of CSF antibodies to *B. burgdorferi*.

In this study, the intrathecal production of antibodies to *B. burgdorferi* was taken as one of the inclusion criteria for definite LB. This criterion

was met in 73% of the LB patients in study I (mean age of the patients 28 years) and 47% of the LB patients in study II (mean age of the patients 8.4 years). Although our findings show that the lack of CSF antibodies does not exclude LB, the analysis of CSF antibodies is important in the diagnosis of FP in areas endemic for LB. In contrast to FP, no intrathecal antibody production could be found for the patients with SHL and vertigo.

The cut-off titers of the flagellin-based ELISA used in this study conform with the antibody level of a mean +3 SD of the general population from nonendemic area in central Finland. This definition means that 1.25% of the healthy normal population provides a positive result in the antibody measurement. According to this definition, the patients with FP (I) and SHL (III) had a high prevalence (12%) of seropositivity against *B. burgdorferi*. This finding agrees with the results of previous studies, which have shown, that cranial nerve dysfunctions, especially the affection of the facial nerve, are frequent and well-documented manifestations of LB (Kindstrand 1995). In this study, 2% of the patients with vertigo (IV) had positive levels of antibodies to *B. burgdorferi*, which accords

with the seropositivity rate of the general population. The study demonstrates the rarity of LB as the cause of vertigo.

With the afore mentioned limitations of serological screening taken into account, the interpretation of positive test results must be made cautiously. In the present study special emphasis was put on the criteria of the diagnosis of definite LB, which was confirmed for 11 of 61 (18%), 4 of 20 (20%) and 8 of 41 (19%) seropositive patients with FP, SHL and vertigo, respectively. However, the positive serological results in the patients not fulfilling the criteria used for definite LB are not necessarily all false. It is probable that there are real LB cases among these patients, and better diagnostic tests are needed in the future to reveal them.

Although the application of strict criteria, while necessary to avoid vast overdiagnosing of LB, may allow some seropositive patients to be underdiagnosed, it can be considered to be a less serious problem than uncritical overdiagnosing (Sigal 1996). On the other hand, the analysis of only one specimen from each patient makes it possible for some single patients with LB to be missed because of

the early phase of the disease. In clinical practice, clinical and laboratory follow-up of the patients with symptoms that arouse suspicion of LB must be underlined.

Is it necessary to screen patients with neurootological symptoms for antibodies to *B. burgdorferi*? If the utmost importance of the history and clinical evaluation of the patient in the diagnosis of LB is kept in mind, the present study shows that the screening of serum antibodies to *B. burgdorferi* is helpful in the differential diagnosis of FP and SHL. However, the screening of vertigo patients for antibodies to *B. burgdorferi*, even in areas endemic for LB, seems not to be recommendable.

There is an obvious need for the further development of serological and other laboratory methods for the diagnosis of LB. The CDC in the United States recommends that positive or borderline serological results be confirmed with Western blotting (Ledue et al. 1996). The reliability of this two-test approach has been questioned (Tugwell et al. 1997). The use of Western blotting in Europe is difficult because of the heterogeneity of *B. burgdorferi* sensu lato. A recent evaluation based on a large panel of

European patient sera suggests that a method with sufficient sensitivity can be obtained using the *B. afzelii* strain Pko as a source of antigen (Hauser et al. 1997; Hauser et al. 1999). Each laboratory using this strain should confirm that their strain expresses the diagnostically important proteins. The problem of variable protein expression can be solved only by a centralized or commercial production of the antigen preparation for European Western blotting.

Polymerase chain reaction

PCR has opened a new era in the diagnosis of infectious diseases, and it is a promising method in the diagnosis of LB as well. While being sensitive in the detection of spirochetal DNA from the tick midgut, as also shown in this study (V), the results of PCR in the clinical diagnosis of LB have varied (Schmidt 1997). In the present study PCR was positive in one serum (II) and two CSF (I and II) samples from patients with FP and two serum samples from both patients with SHL (III) and with vertigo (IV). PCR testing can sometimes detect *B. burgdorferi* DNA in CSF of patients with acute or chronic neuroborreliosis, but the sensitivity of the current tests has generally been low

(Christen et al. 1995; Nocton et al. 1996; Issakainen et al. 1996). The low yield of PCR from sera and CSF may reflect technical limitations in the performance of PCR, but more likely it is caused by the minimal and transient occurrence of borreliel DNA in the CSF and blood during the course of LB (Pachner 1998). In diseases like LB, for which a minute amount of pathogens causes the symptoms, there is a need to capture the pathogen specifically from a large specimen volume in the small aliquot used for PCR. One way of achieving this is to use an immunomagnetic concentration of the pathogen (Stark et al. 1996). Despite its limitations, I advocate the use of PCR in addition to clinical assessment and other laboratory methods in the diagnosis of LB.

7.2. Lyme borreliosis in patients with neurootological symptoms

Cranial neuropathies are well documented manifestations of LB. The facial nerve is the most commonly affected, followed by the nerves to the extraocular muscles, the trigeminal nerve and the vestibulocochlear nerve (Reik 1991). The list of various causes possibly responsible for the dysfunction of the facial nerve is

long. Recent results by Murakami et al. (Murakami et al. 1996) suggest that reactivation of *Herpes simplex* virus infection is an important cause of idiopathic FP. However, these results remain to be confirmed by other research groups. FP can be one of several other concomitant symptoms in LB, but it can still be the single presenting symptom. In the latter case LB-related FP is not easy to distinguish from Bell's palsy. In the present study (II), the features of the clinical picture, signs and findings that best explain the occurrence of LB in patients with FP, were evaluated. The risk for LB as the cause of FP was highest in patients with enlarged cervical lymph nodes, arthralgia and the onset of the symptom during June-November. None of these factors is pathognomonic for LB, but in clinical practice an awareness of these risk factors may facilitate the differential diagnosis.

Bilateral FP has been shown to be associated with LB, the reported prevalences ranging from 29% to 71% (Pfiester et al. 1993). In the present study (I), bilateral FP was present in a fifth of the patients with definite LB, whereas only 2% of the seronegative patients had bilateral FP. However, in the study on pediatric FP (II) bilateral FP was

equally common in patients with and without LB (6%). In a series of Bell's palsy, bilateral involvement occurs in less than 1% (Adour et al. 1978). In the case of bilateral FP the clinician must be aware not only of LB, but also of other specific causes, as for example the Guillan Barré syndrome, malignant diseases and other infectious causes (Keane 1994).

The earlier information of the occurrence of LB in SHL and vertigo is scarce. However, both of these manifestations have been described as evolving in both early and late LB (Pachner et al. 1989; Reik 1991). The results of the present investigation show that LB should be taken into account as a causative factor of SHL. In contrast, LB plays a marginal role in the pathogenesis of vertigo.

T. pallidum, the causative agent of syphilis, belongs to the same order (Spirochaetales) as *B. burgdorferi*. Syphilis is a well documented cause of symptoms mimicking classic Meniere's disease (Pulec 1972). There are many similarities in the clinical course of syphilis and LB (Pachner 1989). An interesting observation in this study was that vertigo caused by LB can mimic the symptoms of Menière's disease.

7.3. Outcome of patients with Lyme borreliosis

The spontaneous course of neurological symptoms of LB is relatively well described in the literature from the era before the discovery of *B. burgdorferi*. These data include, however, limitations of retrospective analyses and must be interpreted with caution. In the United States, 55 untreated patients with EM were followed for several years, and 80% of them developed arthritis or musculoskeletal pain, 9% experienced recurrent EM, 8% developed neurological symptoms and 4% had cardiac abnormalities (Steere et al. 1987). Bannwarth's syndrome, which includes painful meningoradiculitis, cranial nerve affection - in most cases FP - and lymphocytic pleocytosis in CSF, has been a self-limiting manifestation in the majority of patients (Pfister et al. 1993).

The present results strongly support the favorable outcome of LB-related FP. In study II, 43 children with a history of FP were retrospectively evaluated for the outcome of FP and for symptoms or signs of late LB. In the light of the concomitant prospective study, there was a considerable reason to suspect that up to one-third

of these children had their untreated FP caused by LB. The outcome of FP in these children was good, and no signs or findings of late LB were found in these children. However, since the risks of adverse events due to antimicrobial medication are relatively low, patients with definite LB should be treated with appropriate antimicrobials.

7.4. Evaluation of the density of ticks and their infestation rate with *B. burgdorferi*

Knowledge of the epidemiology of the vectors of LB and their infestation rate is essential for understanding the risk of LB in a local setting. The present study (V) confirmed the endemicity of LB in the study area by demonstrating not only the high density of *I. ricinus* ticks, but also the high infestation rate of ticks by *B. burgdorferi* (average 32%).

With the exception of the most northern regions, the distribution area of ixodid ticks covers all of Europe. The density of ticks varies as a function of geographic area (e.g., proper habitat, density of host animals) and time (e.g., variation of humidity from year to year) considerably (Gray et al. 1998). In Europe the ticks have a bimodal

seasonal activity with activity peaks in early summer and early autumn. Usually, tick densities have been studied in rural areas, although there is growing evidence of the occurrence of ticks in urban settings (Steere 1994). Our results confirm that even very urban parks can serve as habitats for *I. ricinus* ticks. Study V demonstrated tick populations residing in abundance in the vicinity of the city center of Helsinki, the most densely populated area of Finland. Of special interest was the finding, contrary to previous reports, that dense populations of *I. ricinus* ticks can exist in an urban environment not populated with large mammals like the deer or elk. Our findings underline the necessity of inhabitants and local health care officials to be aware of the risk of contracting LB in city parks and other recreational areas.

B. burgdorferi spirochetes have been found in all the *I. ricinus* populations examined in Europe (Hubalek et al. 1998). The present study (V) confirms this finding. The published data on the infestation rate of ticks vary considerably according to the method used for the detection of the spirochete. Extensive comparative studies between different detection methods have not been available. The present

study (V) compared DFM, cultivation and PCR as methods of detecting *B. burgdorferi* in the midgut of *I. ricinus* ticks. The agreement between the performance of the three methods was fairly good. DFM provided a positive result for some of the specimens that remained negative both in culture and in the PCR analysis. These specimens generally contained very low numbers of spirochetes, a finding which suggests that DFM may be more sensitive than the other two methods. The specificity of DFM has been argued about, and the present study could not rule out the occurrence of an unknown organism morphologically similar to *B. burgdorferi* in the ticks positive only with DFM. However, in practice DFM is the method of choice for routine screening of tick infestation rates.

The three European isolates of *B. burgdorferi* are associated with different late clinical manifestations (Assous et al. 1993; Canica et al. 1993). In this context the finding that the *I. ricinus* ticks found in Helsinki harbor *B. afzelii* and *B. garinii* only is of clinical importance. The absence of *B. burgdorferi* sensu stricto in the ticks in Helsinki may explain the rarity of frank arthritis in the patients with LB in this study.

8. CONCLUSIONS

1. LB is an important infectious cause of FP (I) and especially in FP in children (II) in southern Finland. It is advisable to screen patients with FP for antibodies to *B. burgdorferi* in endemic areas like southern Finland. In the diagnosis of LB, special attention must be paid to the history of the patient and clinical signs and findings related to LB. Suspicion of LB in a patient with FP is an indication for the analysis of the CSF. Fever, headache, pharyngalgia, enlarged cervical lymph nodes and arthralgia were more frequent in patients with LB-related FP than in other FP patients. Suspicion of LB as the cause of FP must be especially high during late summer and autumn. Strict criteria must be applied to the diagnosis of LB to avoid overdiagnosis.

2. SHL can be caused by LB (III). The prevalence of antibodies to *B. burgdorferi* in patients with SHL was six-fold higher than the corresponding prevalence in the general population. No special signs or symptoms were found to explain the occurrence of LB in these patients. Serological screening for antibodies to *B. burgdorferi* is recommended in patients with SHL in areas endemic for LB.

3. LB can, although rarely, present with vertigo. Vertigo caused by LB may mimic the symptoms of Menière's disease. The prevalence of antibodies to *B. burgdorferi* in unselected patients with vertigo was equal to the corresponding prevalence in the general population. Therefore, the routine screening for antibodies against *B. burgdorferi* in patients with vertigo is not recommendable.

4. Although the neurootological manifestations of LB seem to have a favorable outcome even without treatment, patients with definite LB should be treated with appropriate antimicrobials.

5. The present study demonstrates that *I. ricinus* ticks are abundant in the vicinity of the city center of Helsinki. In contrast to previous studies, the occurrence of ticks was confirmed in the absence of large mammals like deer and elk. Of the three genospecies of *B. burgdorferi* causing LB in Europe, one, *B. burgdorferi sensu stricto*, was absent in the ticks in Helsinki. This finding may explain the rarity of frank arthritis in the patients with LB in the present study. Altogether 32% of the ticks in Helsinki harbored the spirochete *B. burgdorferi*.

This information from the popular recreational areas of a densely populated metropolitan area should alert the local health officials to inform the

population about the risk of contracting LB and about the need for preventive measures.

9. SUMMARY

Lyme borreliosis, although clinically known for more than a decade, is scientifically a rather new disease. The isolation of the causative agent of this multifaceted syndrome in 1981 opened an era of ever increasing intensive scientific work. Neurootological symptoms of Lyme borreliosis, especially the paralysis of facial nerve, have played a central role in the clinical picture of Lyme borreliosis. The purpose of the present study was to evaluate Lyme borreliosis as the cause of vertigo, sudden sensorineural hearing loss and facial paralysis. Additionally, the endemicity of Lyme borreliosis in our study area was assessed by studying the density of *Ixodes ricinus* ticks and their infestation rate by *Borrelia burgdorferi* in city parks of Helsinki.

During a two year period, 503 consecutive patients with acute idiopathic facial paralysis were prospectively screened for antibodies to *Borrelia burgdorferi*. In the same way we evaluated 49 children with facial paralysis, 165 patients with sudden sensorineural hearing loss and 2055 patients with vertigo. Clinical symptoms and signs related to Lyme borreliosis were evaluated.

Of the 503 patients with facial paralysis, 61 (12%) had positive levels of serum antibodies against *Borrelia burgdorferi* and, according to the clinical criteria used for the diagnosis of Lyme borreliosis, 11 of them had Lyme borreliosis. Of the 49 children with facial paralysis, 17 (35%) had Lyme borreliosis, and of the 165 patients with sudden sensorineural hearing loss, 20 (12%) had positive levels of serum antibodies against *Borrelia burgdorferi*, and according to the clinical criteria 4 of them had Lyme borreliosis. In patients with vertigo Lyme borreliosis was rare; 41/2055 (2%) had positive levels of antibodies against *Borrelia burgdorferi*, which did not differ from the incidence of seropositivity among the normal Finnish population. According to the clinical criteria eight patients (0.4%) were diagnosed as having Lyme borreliosis.

Altogether 726 *Ixodes ricinus* ticks were evaluated from five recreational parks in Helsinki. The density of ticks varied from 1 to 36 per 100 m along the tract on which the collection cloth was dragged. Ticks were dissected and the midgut of the ticks was analysed for the presence of *Borrelia burgdorferi* by dark-field microscopy, cultivation in the BSK-II me-

dium and polymerase chain reaction. The agreement between the three methods for detection of *Borrelia burgdorferi* was good. The rate of ticks infected with *Borrelia burgdorferi* sensu lato varied from 19% to 55%, the average being 32%. *Borrelia afzelii* was the most predominant genospecies in all the areas and no *Borrelia burgdorferi* sensu stricto isolates were detected.

In summary, the present study shows the importance of Lyme borreliosis in the differential diagnosis of patients with otoneurological complaints. Lyme borreliosis is a common identifiable cause of facial paralysis and sudden sensorineural hearing loss. As the cause of vertigo Lyme borreliosis is rare. In endemic areas, like major parts of Finland, Lyme borreliosis must be taken into account in the diagnostic workup of an otorhinolaryngologist or neurootologist.

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