Neuroborreliosis in children:

Epidemiological and clinical aspects

Dag Tveitnes

Contents

Acknowledgements

I was born in Hardanger, the beautiful fjord-landscape on the west coast of Norway. My father Alfred was born in 1919 and as a young boy worked as a shepherd in the mountains of Hardanger. He has told me how he enjoyed crushing the ticks that sucked blood off their cows, and watching the blood spray. However, he could not remember that he was ever told to be aware of tick bites himself.

My mother, Ovidia, came to Hardanger as a servant girl, and in 1949 they got married.

I grew up in a industrial, smoke-filled village, Ålvik, in which was based on heavy industry, sucking energy out of Bjølvefossen, once an impressive waterfall endlessly falling into the Hardanger-fjord.

Every weekend my parents took me and my siblings away from the polluted air, and we all went up in the mountain. I want to thank them for this, because it was here I became fascinated with natural science, and that early fascination later brought me on into environmental medicine.

As with ticks, the life circle of humans has stages. Now I have my own cabin in the mountains, bringing my children out into nature. During my PhD research my wife Marianne and my children Sofie, Johannes, Sigrid and Guro have all supported me enthusiastically. Marianne has been great help and I know that without her special pedagogical skills and love that this work hardly could have been completed. I caught the golden bird. Even my mother-in-law and colleague Solveig has contributed especially to the historical perspective of my thesis.

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Sigrid, 3 years old in 1995, got a primary role in this work. A winter morning in March her smile appeared asymmetrically, an alarming sign of something wrong with our happy daughter. She had gotten acute facial nerve palsy, and according to updated recommendations from Stavanger

University Hospital a lumbar puncture was performed. Neither meningitis nor neuroborreliosis were identified. However, this was a painful experience for her. Nevertheless, few days later her original smile shone.

In 1996, Jacob Havnen, then head of the paediatric department of SUS, employed me and very soon supported my growing interest of Lyme borreliosis in children. I am thankful to him and all my colleges at the paediatric department making this work possible, and will especially mention Dr Jonathan Bland, my supervisor in paediatric medicine and English proof-reader of the papers and thesis.

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Summary of thesis

Background: Lyme borreliosis (LB) caused by the bacteria Borrelia burgdorferi sensu lato (Bbsl) is the most common tick transmitted infection in Europe. LB is a multi systemic infection that develops when the bacteria disseminate from the local tick bite to different organs and tissues. Neuroborreliosis (NB) is the neurological manifestation of LB and may affect both the peripheral and the central nervous system. A variety of neurological symptoms are observed; aseptic meningitis (AM) and facial nerve palsy (FNP) are the major manifestations in children. The incidence of FNP and AM in children, as well as the rate of FNP and AM caused by LB is not well described and may vary between areas. Furthermore, it is not well documented how often FNP caused by LB is associated with AM.

Finally, the clinical presentation of NB and the result of diagnostic tests may differ between areas and even between children and adults. All these issues have been scarcely addressed in a population based setting in children.

Aim: The aim of this thesis was to study the epidemiology of childhood NB, FNP and infectious meningitis in an endemic area of LB in south western Norway. Further, to evaluate demographical, clinical and laboratory aspects of NB, FNP and infectious meningitis in children, and to study the interplay between these conditions.

Methods: In a population based study performed during 1996-2009, children up to 14 years old referred to Stavanger University Hospital with suspected NB, including acute FNP, were investigated by a standard procedure including a lumbar puncture. Except when bacterial meningitis (BM) was confirmed, Bbsl serological tests were performed in serum and CSF in all children with CSF pleocytosis. In paper I-III, NB was diagnosed in children with neurological symptoms compatible with NB and with positive Bbsl antibodies or recently EM. In paper IV, Lyme meningitis (LM) was diagnosed in children with CSF pleocytosis and symptoms suggestive of NB in combination with Bbsl antibody index (AI) (confirmed LM) or Bbsl antibodies in serum or CSF (probable LM).

Results: The annual incidence of NB in children up to 14 years of age was 21/100.000. A seasonal distribution was observed, all children with NB were diagnosed from April to December. The highest incidence of NB was found in the age group 6-7 years. Near all (98%) of children with NB had CSF pleocytosis, and FNP was observed in 69% of children with NB. The level of CSF inflammation, the proportion of children with positive Bbsl antibodies in serum and CSF, and Bbsl AI, all increased with the duration of symptoms before lumbar puncture. In addition, the level of CSF inflammation differed between clinical groups.

The incidence of FNP was 21/100.000, and NB was diagnosed in 65% of children with FNP. Tree quarters of children with FNP had CSF pleocytosis. No other cause than NB was diagnosed in children with both FNP and CSF pleocytosis. Children with FNP without NB were diagnosed through the whole year and were evenly distributed in all age groups, and differed from the age distribution of children with FNP and NB.

The incidence of infectious meningitis in children was 38/100.000 and 67% of these were caused by LM. Age, month of admission and clinical and laboratorial characteristics differed between children with LM, NLAM and BM. The positive predictive value for having LM if the child had FNP or meningism as the only symptom was 97% for both variables. The negative predictive value for not having LM if the child did not have a history of EM, or cranial nerve involvement or meningism as the only symptom was 95%.

Conclusion: In this population based study, the incidence of NB, incidence of FNP and proportion of FNP caused of NB were among the highest reported in children worldwide. Furthermore, nearly all children with NB had CSF pleocytosis. Our results suggest that in children with possible NB, the duration of symptoms and clinical characteristics must be included in the interpretation of laboratory results. Finally we found LM to be the major cause of infectious meningitis. In children with CSF pleocytosis, distinct clinical characteristics distinguished the majority of children with LM from NLAM.

List of papers

This thesis is based on the following papers, referred to in the text by their roman numerals:

I   Øymar K, **Tveitnes D**. Clinical characteristics of childhood Lyme neuroborreliosis in an endemic area of northern Europe. Scand J Infect Dis 2009; 41: 88-94.

II   **Tveitnes D**, Øymar K, Natås O. Laboratory data in children with Lyme neuroborreliosis, relation to clinical presentation and duration of symptoms. Scand J Infect Dis 2009; 41: 355-62.

III   **Tveitnes D**, Øymar K, Natås O. Acute facial nerve palsy in children: how often is it Lyme borreliosis? Scand J Infect Dis 2007; 39: 425-31.

IV   **Tveitnes D**, Olav Natås O, Skadberg Ø, Øymar K. Lyme meningitis; the major cause of childhood meningitis in an endemic area: a population based study. Archives Dis Child 2012; 97: 215-20.

Abbreviations

AM	Aseptic meningitis
B	Borrelia
Bbsl	Borrelia burgdorferi sensu latu complex
CMV	Cytomegalovirus
CDC	Center for Disease control and Prevention
CNS	Central nerve system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
EBV	Epstein Barr virus
ELISA	Enzyme-linked immunosorbent essay
EM	Erythema migrans
FNP	Facial nerve palsy
I	Ixodes
Ig	Immunoglobulin
LB	Lyme borreliosis
LC	Lymphocytoma
LM	Lyme meningitis
LP	Lumbar puncture
NB	Neuroborreliosis
NPV	Negative predictive value
NLAM	Non-Lyme aseptic meningitis
OD	Optical density
Osp	Outer surface protein
PCR	Polymerase chain reaction
PPV	Positive predictive value
SUS	Stavanger University Hospital
VlsE	Vmp-like sequence expressed
Vmp	Vacuole Membrane Protein
WBC	White blood count

1. Introduction

Zoonotic infections are human diseases acquired from animals. Frequently, the infection is transmitted from animal hosts to humans via an arthropod vector. Lyme borreliosis (LB) is a tick-borne infectious disease and is the most common zoonosis of the northern hemisphere. The European vector tick, Ixodes ricinus, is able to feed off different animal species including humans. During the tick bite the subcutaneous tissue and the bloodstream may be contaminated so the tick and the host exchange microbes. Many different tick-borne infections are known in human medicine, however in the veterinary medicine it is an even more challenging area. Furthermore, by cooperation between human and veterinary medicine, the contribution of environmental sciences has developed the research into a complex and fascinating ecological medicine.

Since the discovery 30 years ago of the Bbsl spirochete as the cause of LB, the infection has been intensively studied. However, the complexity of Bbsl distribution and the variety of manifestations caused by different genospecies of the Bbsl complex make community based studies important. The LB clinical picture seems to be different even between children and adults, making specific studies of children essential to improve management of LB.

1.1 Lyme borreliosis

1.1.1   History

In October 1975, two mothers, Polly Murray and Judith Mensch from the community of Lyme, Connecticut, USA, contacted the Connecticut State Health Department to report their worry concerning a high number of children in the neighbourhood who simultaneously were diagnosed with what was suspected to be juvenile arthritis (1). The investigation initiated by these observant women ended in the etiological discovery of LB. In 1977, Steere and colleagues published the paper "Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities" (2). They described characteristic skin lesion preceding the arthritis, observed a peak incidence of new cases during summer and early fall, and reported a positive effect of

treatment with antibiotics. They suggested that an unknown infectious agent was transmitted by an arthropod vector. In 1982, Willy Burgdorfer reported the observation of a spirochete in the gut of Ixodes ticks that was probably the cause of LB (3). Further, in 1983 the spirochete was isolated from blood in patients with LB (4).

Interestingly, more than hundred years earlier, the first bacteria ever observed in human blood were reported of the young German physician Otto Obermeyer in 1873 (5). The bacteria he discovered was the spirochete B recurrentis, a representative of the second branch of B spirochetes and the nearest and only genetically relative to Bbsl. B recurrentis is transmitted by human body louse, and are still a significant problem in poor environments when causing relapsing fever (1).

Furthermore, European cases of LB have probably been described in several reports through the whole of the 19th century (6-11). All these reports described dermatological or neurological manifestations, later confirmed to be the main manifestations of LB in Europe (12). This was in contrast to the observation of LB in US where the majority of disseminated cases were Lyme arthritis (13). Three major human pathogenic Bbsl genospecies were identified worldwide. These Bbsl genospecies: B afzelii, B garinii and Bb sensu stricto were all found in European Ixodes tics. In contrast, only Bb sensu stricto were identified in US, and this difference was thought to explain the unique difference in clinical characteristics of LB between Europe and US (13-16).

The final important step in the history of LB was taken when Steere and colleges confirmed that antibiotic treatment was an effective therapy for LB (17). However, for many years, single Scandinavian patients with erythema migrans and meningoradiculitis (Bannwarth syndrome) following tick bites had already been successfully treated with antibiotics (6, 9).

Interestingly, in 1962, Brennaas and Ræder reported the observation of 13 patients with meningoencephalomyeloradiculitt occurring from May to November in the area of Sunnhordland, the Norwegian coastal region north of the area studied in this thesis (10). Ten of the patients had reported tick bite of I.

ricinus prior to the illness, and six of these patients developed an erythema during the course of infection. The authors concluded that this was undoubtedly erythema migrans (EM), today known as the patognomonic manifestation of early LB. This was twenty years before the etiological cause of LB was discovered, and the authors proposed that this probably was an I. ricinus transmitted endemic viral infection. Retrospectively, it is most probably that they described patients with neuroborreliosis (NB), the neurological manifestation of LB (18). Brennaas and Ræder also reported that half of the patients admitted to their local hospital with aseptic meningitis (AM) from 01 July 1959 to 31 December 1961 seemed to have a tick transmitted endemic infection. None of the patients were treated with antibiotics, and the authors thereby provided important clinical information about the natural course of untreated NB. All patients complained of dorsalgia, except a 5 years old girl who was the only child in the study. The adult patients had intense radicular pain lasting for up to 5 months. Nearly all patients had peripheral or cranial nerve paresis. Seven out of 13 patients had sign of cerebral affection, and about half of the patients had persistent pareses two months after the start of symptoms. Even seven month after the start of symptoms, two out of 13 patients had remaining low-grade pareses in the upper-extremities. The authors concluded that compared to the patients with other causes of AM, the patients with tick transmitted meningoencephalomyeloradiculitis had a painful and prolonged illness. As reported elsewhere in the literature, some patients probably even got permanent sequalae (19).

Today, patients diagnosed with NB are effectively treated with specific antibiotics. However, the diagnostic process is still challenging. Furthermore, even the small study from Sunnhordland points to differences in clinical characteristics between adults and children with NB, which was later confirmed by others (20, 21). During the next decades, the majority of studies of NB have been performed in adults, and the results from such studies may not be representative for children with NB. Therefore, more clinical studies in children with NB were clearly needed.

1.1.2 The vector tick

Ticks are obligate hematophagous arthropods that parasitize every class of vertebrates in almost every region of the world. There are 2 major tick families. The Ixodidae, or "hard ticks," so called because of their sclerotized dorsal plate, are the most common and most medical important. Hard ticks feed only once in every stage of their life cycle, in contrast to the fast feeders, the Argasidae, or "soft ticks" (22). Ticks may have appeared 225 millions years ago, when they parasitized on reptiles (23).

Currently, only mosquitoes are more important than ticks as vectors of human infectious diseases in the world, but in North America and Europe ticks are most important (22).

I. ricinus is the most important of the Ixodes species transmitting Bbsl in Europe. I. ricinus is the vector for several different human pathogenic micro-organisms, and LB is by far the most frequent tick transmitted infection in Europe (24). Tick borne encephalitis caused by a flavivirus is the second most common tick transmitted disease in Europe. There are a few cases of TBE reported every year at the Southeast coast of Norway, however so far not observed in persons living in the study area of this thesis (25). Finally, co-infection in LB patients with Anaplasma phagocytophilium should be considered in cases with recurrent fever and bone marrow depression (26). Anaplasmosis in animals is a prevalent disease in the study area; however, no children have been diagnosed with human anaplasmosis in the study period. This may be caused of to low awareness of the disease (27).

I. ricinus ticks are found in coastal areas south of the polar circle. Norway forms a part of the northern distribution border of I. ricinus in Europe. There are local areas and regions with high endemic tick populations (28). LB is also geographically localized, and occurs only in foci with optimal conditions for the ticks and animals involved in the circulation of Bbsl. However, a number of events can disturb these associations, including macroclimatic changes, urbanization, and deforestation (22).

I. ricinus develop through four stages, i.e.; egg, larva, nymph and adult tick (figure 1). A new developmental stage is initiated when the tick takes a meal of blood on host animals (figure 3).

Most of the time during nonparasitizing phases, the tick is on or near the surface of the soil. The tick is sensitive to variation in temperature and humidity. Thermohygrometric stress influences both the survival of the tick during diapause in winter and during questing activity (29). The tick becomes active when the temperature exceeds 4-6 $^{\circ}$C (30). Desiccation is an important threat to the tick, and this limits the tick in host-seeking activity in climbing vegetation. Exposed to the sun, the tick must descend to the moist conditions at the base of the plant to restore its fluid content by uptake of atmospheric water (24, 31). In a Swedish study, the mean questing height for nymphal ticks was 30-39 cm in low vegetation and 50-59 cm in high vegetation. All stages of ticks were found between 10-140 cm over the ground (32).

In addition to climate conditions, the day length is known to affect the activity of I. ricinus, however Ixodes tics are frequently hunting for a host during night (24, 33). During the last decades, the number of I. ricinus ticks seems to have increased in northern Europe, partly due to climatic changes, increased roe deer population and changes in habitat structure (34).

Larvae and nymphs feed on mammals, reptiles and birds. Adult ticks feed on large mammals (figure 3). Human beings can be incidental hosts for all three stages of the I. ricinus lifecycle. However, questing larval ticks are not usually infected and adult ticks rarely feed on humans, and consequently the nymph tick is the most likely cause of transmission of Bbsl to humans (24). The infectivity of the animal called a reservoir host, the tick infestation rate and the host density are major variables determining the epidemiology of tick-transmitted diseases (22).

Nymphs suck blood for 4-5 days and increase in size to about 10 -20 times their unfed body weight (35). The transmitting process of Bbsl from the tick gut to the human skin usually takes more than 24 hours; however, cases have been reported where it has taking less time (36-38). Bbsl is connected by specific outer surface

proteins (Osp) to specific proteins in the tick gut during the non-parasiting phase. However, these protein genes are turned off when blood enters the tick bowel. The spirochete moves to the salvia gland of the tick and there it is joined to specific tick saliva proteins, and finally the Bbsl seems to be secreted into the victim. The transmission from infected nymphal ticks of one cohort to larval ticks of another via reservoir hosts is largely responsible for LB spirochete maintenance in nature (35). Furthermore, there are indications that nymphal ticks infected with Bbsl have survival advantages compared to un-infected Ixodes tics (29).

1.1.3   The host

I. ricinus is able to parasite a wide range of vertebras with the purpose of sucking the critical meal of blood. Regarding Bbsl, hosts are divided into groups of Bbsl reservoir-competent or not competent (figure 3). It is believed that this difference of host properties is due to difference in the host immune systems. A reservoir competent host will let the invading Bbsl spirochete multiply and be transmitted in good condition to the next parasiting ticks (35). Different Bbsl genospecies seem to have different reservoir-competent animals. Rodents and squirrels are the main reservoir for B afzelii, and birds are important reservoirs for B garinii in Europe, as shown in figure 3 (26). Roe deer and moose on the other hand, are believed not to be a reservoir-competent host, however deer have a unique host role for adult female ticks. They feed off deer before getting fertilized and thereafter producing 2000-3000 eggs (39).

In humans, a study from US showed that children are more frequently bitten by Ixodes ticks than adults (40). In a Scandinavian epidemiological study the location of tick bites were reported. Children had about half of their bites in the head and neck region, in contrast to only two percent in adults (12). This difference may be the reason for the difference between children and adults regarding the clinical manifestations of LB.

Figure 1. I. ricinus ticks named in order by increasing size: larvae, nymph, adult male and adult femal. Printed with permission.

1.1.4   The Borrelia burgdorferi sensu lato

Borrelia species are gram-negative microaerophilic mobile spirochetes. Among borreliae, Bbsl is the longest and narrowest (20–30 x 0.2– 0.3 µm) and has the least flagella (figure 2). Uniquely, the flagella are periplasmatic localised inside the outer membrane of Bbsl, in contrast to extra cellular flagella in most other bacteria. As a skeleton the flagella in Bbsl makes the shape and directs the swimming movement. The asymmetric rotation of the periplasmatic flagella-bundles generates backward moving waves along the cell body that propels the Bbsl forward. The unique movement enables them to swim efficiently through highly viscous media, such as connective tissue, where the movement of other bacteria is reduced or inhibited (35, 41). The top speed of Bb measured in vitro seems to be faster then the speed of the phagocyting human cells (42), indicating

possible clearance difficulties in humans. Bbsl may migrate extracellular and chemotactic mechanisms may guide the spirochete (43).

Presently, 17 different Bbsl strains are known worldwide (37). Eleven genospecies of Bbsl have been observed in Europe, among them the three main pathogenic species; B afzelii, B garinii and Bb sensu stricto. In addition, B spilimanii and B bavariensis are found capable of developing the multisystemic infection of LB. B bissettii, B lusitaniae and B valaisiana are rarely detected in patients, but are not recognized as important pathogens (26, 44). However, B valaisiana was recently identified for the first time in Norway (45).
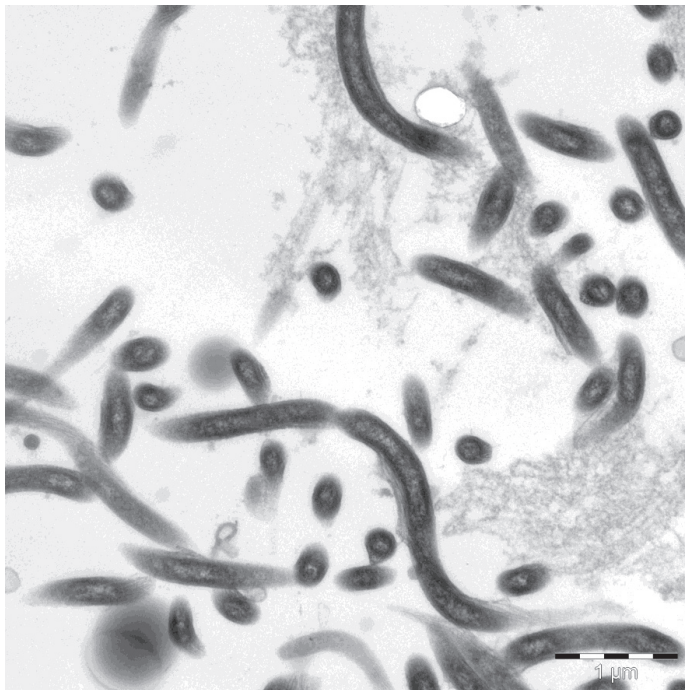
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Foto: Sverre-Henning Brorson,

Figure 2. Borrelia burgdorferi sensu lato (transmission electron microscopy)**. Printed with permission.**

Furthermore, multiple molecular mechanisms helping the spirochete to survive the immunological system of the host have been observed. Different Osp are

expressed on Bbsl living in ticks and in mammals. Even spatiotemporal variation of Bbsl Osp`s within the host or tick has been observed. The genome of Bbsl consists of a small linear chromosome and most unusual, 9 linear and 12 circular plasmids. The plasmid genome encodes different surfaces exposed lipoproteins, allowing for extensive antigen variation and helping the spirochete to survive. Nevertheless, once Bbsl is discovered in the body, the innate immune system initiates the neutralizing of the spirochete (46). However, the spirochete has mechanisms to neutralise the immune system of the host as well. A diversity of tick salivary proteins is believed to interact with and reduces the efficiency of the host immune system. The knowledge in this area is growing because it is a focus of research in order to develop a vaccine against LB (35). No toxins produced by Bbsl have been found, however the spirochetes invasiveness is uncommonly effective. The manifestations of tissue pathology in LB are therefore believed mainly to be due to host inflammatory reaction (1).

Different human pathogenic Bbsl species seem to have certain organ specific preferences and consequently different clinical manifestations and outcomes. B. afzelii is dermatotropic, B. garinii appears to be the most neurotropic agent, and Bb sensu stricto, is the most arthrotropic genospecie (47). However, all three genospecies are capable of invading the nerve system causing NB (48, 49). Usually, infectious agents enter the CNS by the haematological route. In addition, Bbsl is probably able of entering CNS by following peripheral nerves from the site of the tick bite (50).

Recently, the prevalence and genotypes of Bbsl infection in Ixodes ricinus ticks were studied in southern Norway, in a neighbouring county of the study area in the thesis (45). The overall percentage of nymphal ticks being infected with Bbsl was determined to be 24.5%, which is a high number compared to previous Scandinavian studies (51). The most prevalent Bbsl genospecie identified was B. afzelii (61.6%), followed by B. garinii (23.4%) and Bb sensu stricto (10.6%).
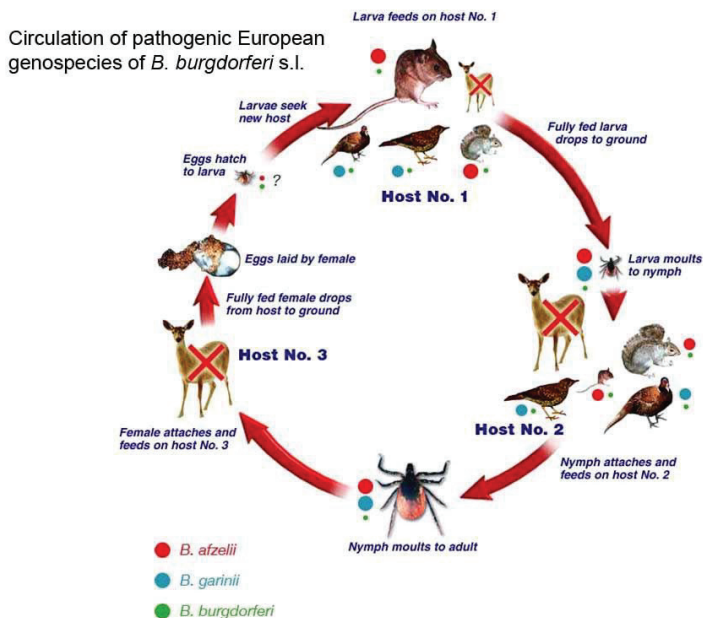
Figure 3: Life cycle of I. ricinus and infectious cycle of the European Bbsl genospecies. The size of the animal indicates the relative importance of the host. The size of the circles indicates the relative involvement of the different vertebrate reservoirs for the different genospecies. Cross indicates a non-reservoir host (37). Reprinted with permission from EUCALB representative, J.Gray.

1.1.5 The clinical picture of LB

LB is a multisystemic infection (13). The development of LB can only occur after a bite by Bbsl infected Ixodes ticks. However, in the majority of humans invaded by the spirochete, the immunological reaction and the production of Bbsl antibodies are probably sufficient to halt the development of LB. This is an interpretation from studies in endemic areas of LB, where the seroprevalence of Bbsl antibodies is much higher then the incidence of patients diagnosed with LB (52-54).

If a human is infected, the course of LB may develop through three following stages (13, 15, 26, 55-57). *The first stage of LB* usually develops as a *local* skin

infection. Days to weeks after the tick bite, a slowly expanding skin lesion, erythema migrans (EM), may develop from the site of the tick bite (figure 4). EM may be misdiagnosed. However the erythema of cellulites, insect bites, ringworm, granuloma annulare or nummular eczema do not have the steady enlargement of an inflammatory ring commonly leaving the central zone pale, as developed when Bbsl disseminate from the bite location (58). Flu-like symptoms sometimes accompany the local infection. Borrelia Lymphocytoma (LC) is a subacute skin manifestation developing in areas with loose skin, like the earlobe and nipple. LC is an uncommon manifestation of LB compared to EM, which is the overall most frequent manifestation of LB. However, in Europe, children develop LC more often than adults, corresponding with the higher frequency of tick bites in the ear region in children than in adults (12, 59). In Europe, B. afzelii is the major genospecie causing skin infection (60, 61). Correspondingly, B afzelii is also the most frequent Bbsl genotype found in I. ricinus in Scandinavia (45).

Foto: Jostein Førsvoll

Figure 4. Erythema migrans., Printed with patients permission.

In the second stage of LB, developing within weeks to months after a tick bite, the spirochete has disseminated to different areas of the skin or to other organs. In a German epidemiological study in children, the interval from a tick bite to the onset of symptoms of NB was ranging from two to 12 weeks, with a median time of five weeks (62). In addition to the skin and nerve system, Bbsb has been observed in myocardium, spleen, liver, muscle, bone and retina. Consequently, a variety of signs and symptoms may accompany the early stage of disseminated LB (57, 61).

Despite an active immune response, the spirochete may survive in localised niches in the human body for months and years (50). *The third stage*, late LB, is uncommon but develops in untreated cases more than six months after a tick bite. Late NB or Lyme arthritis may develop in European children, but the late manifestation in skin, acrodermatitis atrophicans (ACA), is observed predominantly in adults (26, 63). Late NB with encephalopathy is rare, especially in children. It should not be diagnosed in the absence of laboratory evidence of Bbsl infection. The symptoms include memory loss, depression, sensory polyneuropathy or spastic paraparesis, probably caused by direct infection of the nervous system.

Some manifestations of Lyme encephalopathy may resemble those of chronic fatigue syndrome or fibromyalgia. This condition may also be triggered by a preceding Bbsl infection (37). A multi-authored international review of 'chronic' LB concluded that despite strong advocacy by some physicians and patient groups, the "assumption that chronic, subjective symptoms are caused by persistent infection with Bbsl are not supported by carefully conducted laboratory studies or by controlled treatment trials. Chronic Lyme disease, which is equated with chronic Bbsl infection, is a misnomer, and the use of prolonged, dangerous, and expensive antibiotic treatments for it is not warranted" (64).

1.1.6 Treatment and prognosis

All stages and organ manifestations of LB are efficiently treated with antibiotics (61, 63). However, tissue damage secondary to the inflammatory process may lead to long lasting sequelae.

In-vitro studies have shown that Bbsl is susceptible to tetracyclines, most penicillins, many of the second- and third-generation cephalosporins, and macrolides. Parenteral administration has been preferred in patients with meningitis and radiculitis. Oral treatment has been reserved for patients with uncomplicated facial palsy (26). A recent multicentre, double-blind, randomised trial done in Norway have provided convincing evidence that oral doxycycline is as effective as parenteral ceftriaxone for any of the primary manifestations of early NB in adults (65). However, doxycycline is contraindicated in children younger than 8 years of age. In a retrospective study of Swedish children with NB, the outcome of treatment with intravenous beta-lactam antibiotics or oral doxycycline for 10 days was reported and no differences regarding outcome were found between the groups (66).

Most patients with LB have an excellent prognosis. Although most manifestations of LB seem to resolve spontaneously without treatment, antibiotic treatment may speed the resolution of symptoms and signs, and will prevent the progression to the later stages of the disease (26).

No vaccine against Bbsl for humans is presently available (26). Furthermore, antibiotic prophylaxis after a tick bite is not recommended, supported by a recent Swedish study. In 3 month follow up, only four out of 397 persons who removed a biting tick developed Bbsl seroconvertion, and only one person developed clinical LB (67). If living in tick habitat, regular inspection of the skin and prompt removal of any tick is effective in the prevention of Bbsl transmission (37).

1.1.7 Epidemiology of LB

Epidemiology is "the study of the distribution and determinants of disease frequency in human populations." In addition, it is "the branch of medical

science dealing with the control of epidemics," which has an important role in disease prevention at a population level (68). The Greek physician Hippocrates has been called the father of epidemiology. He is the first person known to have examined the relationships between the occurrence of disease and environmental influences. He connected the terms epidemic, for diseases that are seen at some times but not others, and endemic, for diseases usually found in some places but not in others (69).

Experimental studies provide stronger clinical evidence than observational studies do, however observational studies may provide unique information about exposure in conditions and populations where ethical considerations limit the possibility for experimental design of studies (68). There are different types of observational studies, including cohort studies. In population-based cohort studies, the cohort is the entire or a section of the general population with specific characteristics, such as age. Specific exposure or characteristics in the population without the disease of interest at baseline are followed up over time (70). Interpretation of specific observations depends on the properties of performed tests (sensitivity and specificity) and the prevalence of the condition in the studied population, defining the positive predictive value (PPV) of an observation (71). Based on observations in population studies, the properties of a disease can be calculated. In the first paper describing Lyme disease, the condition was described as a probable epidemic disease (2). However, nowadays LB is usually termed as an endemic infection found closely related to the distribution of Ixodes ticks, although the seasonal activity of the tick cause epidemic clusters of patients with LB during the summer and autumn.

The main exposure in the epidemiological studies of LB is the bite of a Bbsl infected Ixodes tick. The frequency of such bites varies with the size of the local tick population, the tick activity and the proportion of ticks infected with different pathogenic Bbsl genospecies (31, 35, 36). Furthermore, the activity and behaviour of the human population in the tick endemic areas, the primary and secondary prevention habits, e.g. avoiding tick bites will influence on the

number of persons getting a tick bite. Finally, early removing of ticks has an impact on the incidence of infected persons.

The different determinants of LB are variable studied. In vitro studies on ticks, the Bbsl spirochete and animal models of LB have revealed the complex interaction between causal factors (1, 50). The epidemiology studies of LB are closely interconnected to clinical studies, because the diagnosis of LB depends on the appearance of suspicious clinical signs and laboratorial results including serological tests. These findings are then the basis of the diagnostic definitions (63). Thereby, clinical studies are dependent on epidemiological knowledge.

There are few epidemiological studies from Europe involving all stages and manifestations of LB. In Norway, like in many European countries, only disseminated and late LB cases are notifiable (72). However, in a comprehensive Scandinavian study from an area of the southern part of Sweden comparable to the study area of this thesis, an overall annual incidence of LB of 69 pr 100.000 inhabitants was reported (12). They demonstrated that parts of the southern Scandinavia are among the areas with the highest incidence of LB in Europe. However, the incidence varied between geographical regions which have been demonstrated to be influenced by local variation of climate (73-75). In addition, Berglund and co-authors demonstrated that the incidence varied with age; the youngest and oldest persons had the highest incidence of LB (12). In the study, 77% of 1471 patients with LB had local infection presenting as EM. However, the most frequent manifestation of disseminated LB was NB, observed in 16% of all LB cases. Furthermore, the incidence of NB was higher in children than in adults.

The incidence of LB in Rogaland County is reported to be close to the median compared to other Norwegian counties (72). However, due to the dense population in Rogaland, this county contributed to the second most NB cases in Norway the last ten years (76). Strikingly, in a study from all parts of Norway, 20 % of healthy sheep in Rogaland were found to have positive Bbsl antibodies, the highest reported incidence in the study (77).

In addition to geographical distribution, the occurrence of LB seems to have specific age related and seasonal distribution. A follow up study of a defined population and with a study period for several years may provide important information about these specific properties of LB.

1.2 Neuroborreliosis

1.2.1 Clinical picture

NB is known as a great imitator of multiple neurological diseases (78). As a consequence of the imprecise diagnostic tests of NB and the high frequency of tick bites in the general population in endemic areas, the diagnostic process concerning NB is challenging. In patients with NB, EM has been recognised only in a minority of cases, both in children and in adults (12, 62, 79). Meningoradiculitis, either with paresis or as a radicular pain syndrome named Bannwarth syndrome, is the major manifestation of NB in European adults (8, 11, 20). A Danish epidemiological study reported that the clinical picture of NB in children differed significantly compared to adults, and that the incidence of NB was significantly higher in children than in adults (21). Children infrequently had radical pain syndromes. The occurrence of a monosymptomatic paresis (usually facial paresis) without concomitant pain, fever or neck stiffness was only reported in children. In a comprehensive prospective multicentre study in German children, it was confirmed that acute peripheral facial nerve palsy (FNP) and aseptic meningitis (AM) were the predominating manifestations of NB in European children (62). Furthermore, in children with NB they observed individual cases with other cranial neuropathies and children with several different cranial neuropathies occurring simultaneously. CNS affection was infrequent, but cases of focal encephalitis, Guillain-Barre syndrome, acute transverse myelitis, pseudotumor cerebri and acute ataxia were described. Most studies evaluating the epidemiology and clinical picture of NB in children are from earlier periods when newer and more sensitive and specific antibody

tests were not available, and few population based studies of European NB have been performed in children. Furthermore, no studies have evaluated the clinical characteristics of childhood NB in the endemic areas of Norway.

1.2.2 Facial nerve palsy

Facial expression is important for a child's communication. FNP is therefore a clear sign of illness and difficult to ignore (figure 5).

Figure 5; **Facial nerve palsy on the right side.**   Foto: Jostein Førsvoll

Since Dr Charles Bell, nearly 200 years ago, described the facial nerve and how the facial palsy developed when the nerve was injured, the aetiology of "Bell`s palsy" has been studied (80). Historically, the most common identified cause of FNP in children has been otitis media (81). For other cases, an infectious aetiology was suspected, and the connection between FNP and meningitis was observed in Scandinavia studies prior to the recognition of LB (82, 83). Furthermore, multiple cranial neuropathies were described simultaneously (84).

A microbiological cause of FNP was discovered with the description of NB. In the first study from US reporting neurological manifestations in Lyme disease, cranial neuropathy was found to be common, and the facial nerve was the most frequent peripheral nerve involved (85). This was later confirmed in European studies (86-89).

LB has been identified as the causative agent in different proportions of children with FNP, ranging from only a few percent to 60% (62, 88, 90). Furthermore, the

frequency of FNP associated with meningitis varies considerably between studies (13, 56).

In a study from the Northern Norway, a non endemic region of Bbsl and with a low occurrence of LB, the low incidence of FNP in children as compared to adults, has been confirmed by other authors (91, 92). A more recent study from the most Southern part of Norway which is endemic for LB, a slightly higher incidence of FNP in adults was found compared to the study from Northern Norway (93). In this area, 10% of adults with FNP were found to have NB. Similar studies in children in areas endemic for LB are lacking.

Studies in non-human primates have discovered that the inflammation of Bbsl are primarily localised to nerve roots, dorsal root ganglia, and the leptomeninges (16). As mentioned above, it has been proposed that Bbsl in addition to the haematogenous pathway may invade the CNS by migrating along peripheral nerves (50). This is partly based on the observations that FNP in NB more often occur at the same side as the tick bite (62).

Some authors have argued that a CSF evaluation should be performed in all children with FNP in LB endemic areas, to establish or exclude the diagnosis of NB, however, this has been debated (94, 95). In addition, the treatment recommended for FNP caused by LB has been different depending of whether CSF pleocytosis was detected or not (15, 88, 95-97). Outcome studies of FNP in childhood LB shows mild to moderate impairment of the facial nerve in up of 20 % of children 2 years after treatment with antibiotic (98). However, treatment does not seem to have impact on the prognosis of FNP, and the main reason to treat with antibiotics is to prevent further development of NB manifestations (88, 95). No study has been performed in children with FNP in an endemic area of LB in Norway. Furthermore, even though there is evidence of a high incidence of meningitis in children with FNP in general, few population based studies have systematically evaluated children with FNP by a lumbar puncture (LP).

1.2.3 Meningitis

Microbes crossing the blood – brain barrier activate the innate immune system in CNS. The production of chemokines regulates and directs the migration of leukocytes from the peripheral immune system into CSF. The immune response may thereby limit the CNS infection. However, the immune response may be a two-edge sword. Neuron dysfunction and damage may develop as a consequence of the pro-inflammatory response (99).

Quincke performed the first LP in 1891 to relieve increased intracranial pressure in children with tuberculous meningitis (100). Today, LP is essential for the diagnosis of CNS infections. The most common use of LP is to diagnose or exclude meningitis in patients presenting with the combination of fever, altered mental status or meningism, the sign of irritation of the meninges. Meningism usually are defined as the triad of neck stiffness, headache and photophobia. The term meningitis therefore consists of the finding of meningism and CSF pleocytosis, the state of elevated number of CSF WBC. In BM, all these signs are usually found. However, in AM and NB these findings are not observed consistently.

As mentioned in the pervious chapter, there is an ongoing discussion on whether a LP should be performed in children with a clinical picture suspicious for NB (94, 101). However, if LP is performed and CSF pleocytosis is discovered, differentiating LM from other forms of AM in children is a common dilemma in LB endemic regions (102, 103). There are no studies from Norway reporting the epidemiology of childhood infectious meningitis or studies comparing the clinical and laboratorial characteristics of different groups of childhood meningitis. Furthermore, to our knowledge, no European studies have compared the clinical and laboratorial characteristics of LM and AM in children in a population based context. Finally, studies comparing these characteristics in BM and LM in children are missing.

1.2.4   Diagnosis of neuroborreliosis.

Identification of the causative microbe is usually essential for the diagnosis and the decision of management in an infectious disease. This could be of special importance in all stages of LB due to the heterogeneity of clinical symptoms. In Europe, LB is caused by different genospesies of Bbsl, that give different clinical presentations of LB in general and probably in NB (49). However, culturing Bbsl is time-consuming and labour-intensive, and performed only in a few specialist microbiological laboratories. Furthermore, PCR has low sensitivity for demonstrating Bbsl DNA in CSF of patients with NB (104, 105). Therefore, the detection of antibodies against Bbsl is presently the keystone in the diagnosis of LB (104).

The adaptive immune system produces specific antibodies against microbe antigens. Identification of antibodies against infectious agents is an indirect method for the diagnosis of infectious diseases. In general, IgM antibodies against a microbe are usually produced during early phases of an infection. Later during the infection, IgG antibodies are detected and the production of IgG may last for years after the infection has disappeared (56). However, the antibody production may be delayed during early phases of a clinical disease, and the sensitivity of an IgM or IgG test may therefore be poor at this stage. In the case of a present infection, a retest some weeks later may discover a rise in antibody titre indicating active disease. In addition, antibiotic treatment of a bacterial disease may influence on the production of antibodies throughout the course of disease (96, 106).

The specificity of antibody tests to detect an infection may also be lowered by several reasons. First, the detection of IgG antibodies may be false positive due to earlier exposure or infection, which may have been asymptomatic. Second, during viral infections polyclonal B-cell activation with a high degree of cross-reaction of polyclonal IgM antibodies between different etiological agents is observed (107). Finally, "natural IgM antibodies" may be found without an active infection being present (108).

Enzyme-linked immunosorbent essay (ELISA) is the recommended serological tests for diagnosing LB in Europe. However, Bbsl expresses numerous immunogenic proteins, and Bbsl genospecies expressing different proteins are variable distributed in geographical areas. Furthermore, the Bbsl spirochete is able to change the proteins expression through time and stages of LB. This makes the serodiagnosis of LB challenging

The rate of discrepancy between different serological assays is significant. Ideally, a validation of the chosen serological Kit should be performed in each laboratory (53, 96). However, the VlsE protein is found highly immunogenic in all LB phases and contains a protein sequence common to all Bbsl species and is therefore recommended when diagnosing early NB (106, 109-112).

When early disseminated LB like NB is suspected, then both Bbsl IgM and IgG antibody tests are indicated (112, 113). However, IgM antibodies may be negative during early phases of NB, both in serum and in CSF. A study in patients with early NB, where Bbsl antibodies have been negative and Bbsl has been identified by PCR, demonstrates that false negative Bbsl serology may be a problem in the diagnosis of NB during early phases (114). Furthermore, antibiotic treatment in the early phases of LB may affect the antibody response, and the disappearance of antibodies soon after treatment has been observed (96, 106).

Over time, in an endemic area of LB, a large proportion of the population over time is at risk of having tick bite with the inoculation of Bbsl, and thereby the induction of Bbsl antibody production. The seroprevalence in blood donors for Bbsl in a nearby region was 15 - 20 % in the adult population (93). No study of Bbsl seroprevalence in children has been performed in Norway. However, in comparable European regions the Bbsl seropositivity in children was found to be less than 4% (54, 62).

EBV and CMV are the most common causes of false positive Bbsl IgM antibody stimulation; however they are an unusual cause of CSF pleocytosis and neurological manifestations in children (115). The clinical implication of such antibodies may therefore be small in children. Finally, natural IgM antibodies

(polyspesific immunoglobulins) to flagella p41 are demonstrated in our region in 1.5 % of the adult population (108).

In US, when an ELISA test is positive, a Western Blot test is used as a confirming test to avoid false positive diagnosis (116). This is a laborious and expensive test. Although the addition of the 2 step test increases the specificity of Bbsl diagnostics, the test also lowers the sensitivity of Bbsl serological testing. This is especially true in Europe were the Bbsl genotypes and thereby the antibodies are more heterogenic than in US. This test strategy cannot therefore be recommended in European LB diagnostics (63), and was not used at the start of the present studies (19).

In Europe, examination of the CSF is advocated in patients suspected of having NB, in order to confirm CSF inflammation and to detect specific intrathecal Bbsl antibody production. The CSF/serum Bbsl antibody index (AI) test is evaluated to be superior when compared to Western Blot test in the diagnosis of NB (63, 107). A positive Bbsl AI is a unique hallmark of NB, and may be observed already in the first week after onset of neurological symptoms in children with NB (62, 96, 117). However, studies both in adults and children suggest that the detection of intrathecal antibody production also may be delayed during early phases of NB (66, 105, 118, 119).

The reliability of performed Bb serological tests has had great impact on clinical and epidemiological studies in NB. However, the positive predictive value can be improved by strict case definitions (pre-test probability) (71).

The updated European clinical definition of confirmed NB in children requires clinical symptoms suggestive of NB with cranial neuropathy as the main manifestation, in addition to CSF pleocytosis and a positive Bbsl AI (63). According to this definition, CSF evaluation is necessary for the diagnosis of NB, also in children with FNP. In US, the case definition of NB is not built on CSF evaluation and usually lumbar puncture is not performed or not reported (81, 120). Instead, the diagnosis is made by the two titre serological serum testing, making it difficult to compare European and US studies and the conclusions from these.

No NB case definition today is 100% sensitive or specific, but by using validated serological tests and accepted case definitions of NB the accuracy of diagnosis has improved, and the quality of epidemiological and clinical research is assured.

2. Aims of the study

The overall objective was to study epidemiological, clinical and laboratory characteristics of childhood NB in a LB endemic area. Furthermore, we aimed to study causes and characteristics of all children having FNP or meningitis in a LB endemic area, to evaluate differences between children with non-Lyme and Lyme disease. The overall aim was to improve the care of children with NB.

The specific aims of the thesis were therefore:

I   To study the epidemiology and the clinical characteristics of NB in children in a LB endemic area.

II   To study the relation between different clinical symptoms and laboratory results in children with NB, further, to study the levels of antibodies in serum and CSF in children with NB, and how the results of CSF examination are influenced by the time from debut of symptoms to the time when diagnostic procedures are performed.

III   To study the epidemiology of FNP in children in an area endemic for LB, how often FNP is caused by NB and the occurrence of meningitis in children with FNP.

IV   To study the epidemiology of acute infectious meningitis in childhood in an area endemic for LB. Furthermore, to study demographic, clinical and laboratorial characteristics of BM, non-Lyme AM and LM, to see how these characteristics may discriminate between children with different types of meningitis.

3 Materials and methods

3.1 Study area

Rogaland is the southern County of western Norway. The latitude of Rogaland (58° N to 59° N) is at the same latitude level as Alaska (51° N to 71° N). However, the Golf stream passing the western Norwegian coast provides a climate distinct different from other sub artic areas in the world. In addition, the combination of high mountains and the Golf stream makes the western part of Norway into one of the rainiest areas of the planet outside the tropic zone. The climate of the costal area of Rogaland is therefore suitable for I. ricinus, and the tick vector of LB is found in abundance in many areas. The coastal area of Rogaland is both the main distribution area of I. ricinus and the main residential area in Rogaland. Furthermore, the areas of outdoor activity of children and adults often are overlapping with the ticks natural habitat.
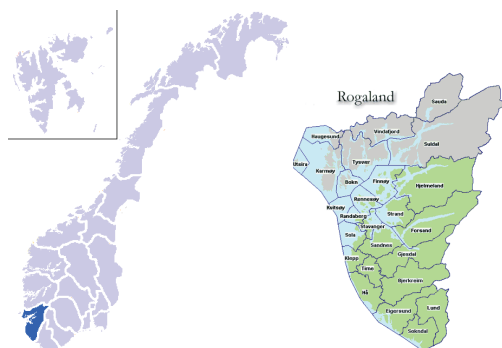
Figure 6; Rogaland county (blue), study area of South Rogaland (green).

The northern and southern region of Rogaland is divided by a fjord (Boknafjorden). The communities south and partly east of Boknafjorden; Egersund, Lund, Sokndal, Sandnes, Stavanger, Bjerkreim, Hå, Klepp, Time, Gjesdal, Sola, Randaberg, Strand, Hjelmeland, Finnøy, Rennesøy, Kvitsøy and Forsand together constitute the area of South-Rogaland, which is the study area (figure 6). Children from all these communities may be exposed to tick bites

through the period of year when the tick is active. The epidemiological differences between communities or sub-areas were not studied.

3.2 Study population

Stavanger University Hospital (SUS) is the only hospital with a paediatric department in the study area. The paediatric department receives all hospital admissions for acute childhood disease in the study area, for children up to 14 years of age. Consequently, all children with suspected meningitis, NB and acute FNP will be referred to this hospital.

Only children living in South Rogaland were included in the present studies. Annual community-based demographic data of the population were obtained from the Official Bureau of Statistics. The population of the South Rogaland region included approximately 62,000 children up to their 14th birthday throughout the whole study period. Norway has a public health system and all treatment of children is free of charge.

3.3 Testing procedures

During 1995 the paediatric department at SUS came to a consensus concerning the diagnostic procedures and treatment of children with possible NB. These recommendations were used through the whole study period. Throughout the whole period, children with possible NB have been discussed in plenum regarding diagnosis and treatment.

During the autumn 1995 all general practitioners and ear-, nose-, and throat (ENT) -consultants in the region received a written recommendation that all children less than 14 years of age with acute FNP should be admitted without delay to the paediatric department at SUS. This has been a standard clinical practise during the study period.

From January 1996 and through the whole study period, we aimed to investigate all children admitted with symptoms suggesting possible NB using the same procedure.

NB was suspected in children presenting with symptoms of FNP or other cranial neuropathy, radicular pain, meningismus (neck stiffness and neck pain), headache or cerebellar ataxia. The procedure in these children included a medical history, clinical examination, blood tests and a lumbar puncture. The clinical symptoms, age, month of appearance of symptoms, number of days from debut of symptoms until hospitalisation, and results from blood tests and lumbar puncture were registered.

In **paper I/II** data regarding demographical, clinical and laboratorial findings were retrospectively collected from the medical records of children suspected of NB in the study period from January 1996 to December 2006. In **paper III** the data were retrospectively registered from medical records of all children with FNP in the study period from January 1996 to December 2004. Finally, in **paper IV,** all children with CSF pleocytosis from the age of 3 months up to their 14th birthday were included, and data were retrospectively registered from the study period from January 2001 to December 2009.

During the whole study period, serum and cerebrospinal fluid (CSF) were analysed for IgM and IgG antibodies against Bbsl using an ELISA (Enzygnost Lyme borreliosis test) (Dade Behring, Marburg, Germany). The test was performed on a Bep III ELISA processor and an optical density (OD)_cut off_0.100 was regarded as positive. IgM- and IgG-positive controls were routinely used for test evaluation. From July 2006 Enzygnost Lyme link VlsE/IgG (Dade Behring, Siemens, Germany) was used. This test is comprised of a mix of whole cell detergents extracts and recombinant VlsE obtained from all three genopathological Bbsl species.

From 1999, the IDEIA Lyme neuroborreliosis capture ELISA was used for detection of intrathecal IgM and IgG antibodies (Dako, Glostrup, Denmark). The antibody index is a semi-quantitative measure of intrathecal antibody production. Optical density (OD) is used as a measure of antibody concentration, and the index formula specific for this test is $OD_{CSF}/OD_{serum}\_(OD_{CSF}-OD_{serum})$. The test was considered positive when the index for either IgM or IgG antibodies or both was > 0.3, indicating neuroborreliosis. CSF white blood cells (WBC)

(Fuchs Rosenthal chamber) and protein (turbidimetric technic (Roche, Basel, Switzerland)) were measured. Serum C-reactive protein (CRP) (immunoturbidimetric technic (Roche, Basel, Switzerland)) and blood WBC were measured in all children. When CSF pleocytosis were demonstrated, CSF was cultured by conventional methods. The decision to perform other microbiological tests was taken by the physician on duty, depending on the clinical presentation of the child.

3.4 Diagnostic criteria and definitions

In **paper I – III** children were diagnosed with NB if, in addition to suggestive neurological findings, either IgM or IgG antibodies for Bbsl were identified in serum and/or CSF. If serology was negative, but a thorough medical history revealed a probable erythema migrans during the past 8 weeks prior to submission, the child was considered to have probable NB. These criteria were consistent with the present European recommendations at the start of the study (19, 117). CSF pleocytosis was diagnosed when the lumbar puncture demonstrated CSF WBC $\geq$ 5 x 10^6/L. However, as the aim was to evaluate the occurrence of CSF inflammation in children with NB, CSF pleocytosis was not a part of the NB case definition.

FNP was defined as an acute palsy involving the facial muscles in both upper and lower parts of the face, either unilateral or bilateral.

In **paper IV** NB was defined according to updated European NB case definitions (63). Only children with CSF pleocytosis were included in this study. Therefore, in this study NB is identical with Lyme meningitis (LM). Confirmed LM was defined in children with CSF pleocytosis and neurological symptoms suggestive of NB without any other obvious cause, in combination with intrathecal Bbsl antibody production (positive AI). Probable LM was defined in children with CSF pleocytosis and suggestive symptoms of NB cases with specific Bbsl antibodies or with recent or concomitant EM. Furthermore, in **paper IV,** CSF pleocytosis was defined as CSF-WBC counts > 7 X 10^6/L in accordance with

updated guidelines for children from 3 months to 16 years of age, published in 2006 by Clinical and Laboratory Standards Institute.

In children with CSF pleocytosis confirmed BM was defined if bacterial culture in CSF or blood were positive combined with sudden onset of fever, neck stiffness, altered consciousness or other meningeal signs.

Children with CSF pleocytosis were defined having NLAM when BM, LM and non-infectious meningitis were excluded.

3.5 Statistical methods

Incidences were compared by logistic regression. Differences between groups were first analysed by the Kruskal-Wallis H-test when appropriate, and if significant, differences between groups were further analysed by the Mann-Whitney U-test. Results were presented as median and quartiles if not otherwise stated. Variance was tested for by Levene's test for equality of variances, and Spearman's test was used for correlation between variables. To study the differences in cell counts and protein counts between the groups, Poisson regression analyses were performed. In these analyses, in addition to the group variables, days from debut of symptoms were included as a covariate to adjust for the effect of this factor. Differences in categorical data between groups were analysed by chi-square tests. All tests were 2-tailed. Data were analysed using the latest versions of SPSS Statistical Package.

3.6 Ethical issues

The data used in study I-III this were registered anonymously. Therefore, there was no obligation to given notification or to obtain a separate ethical licence for the study, since no personal data were used. The studies were approved by the department management. For study IV, a link key to personal data was established, and the study was approved by the Privacy protection supervisor of the Hospital established in 2011.

4. Summary of results

Paper I

A total of 143 children were diagnosed with NB. All cases were diagnosed from April to December, with the majority from June to November. The average annual incidence of children with NB was 21/100.000 for the whole study group. The incidence varied significantly by age, with the highest incidence of 52/100.000 in the age group 6-7 years. The most common clinical presentations in children with NB were symptoms of mild meningism (75%) and/or facial nerve palsy (69%). Based on the clinical symptoms children could be divided into three major groups; A: those with isolated cranial neuropathy without other symptoms (n=36; 25%), B: those with both cranial neuropathy and other neurological symptoms (n=67; 47%) and C: those with neurological symptoms without cranial neuropathy (n=40; 28%). Children with FNP were younger than children without FNP. Radicular pain, for all children in the head and neck region, was present in only 7% of children with NB and in 2% other cranial nerve involvement were reported. Significant general symptoms such as fatigue and anorexia were present in 6 % of children with NB, none of these had cranial nerve involvement. EM preceded the neurological symptoms in 27% of the children with NB.

Paper II

In this study 146 children with NB were included. They were divided into 3 clinical groups: A (n = 37): only cranial neuropathy; B (n = 68): both cranial neuropathy and other neurological symptoms; C (n = 41): neurological symptoms without cranial neuropathy as described in paper I.
In group A, 3 children did not have CSF pleocytosis and in 2 children a lumbar puncture was not performed. The rest of children in group A and all children in groups B and C had LM which constituted 98% of all children with NB.

Levels of white blood cells (WBC) and protein in CSF correlated significantly to numbers of days with symptoms in all children with NB. Levels of WBC and protein in CSF as well as the proportion of children with antibodies in serum and CSF were generally lowest in group A, intermediate in group B and highest in group C. The proportion of children with antibodies in serum and CSF and a positive antibody index was also related to duration of symptoms; the antibody index was present in 51% of children with symptoms $\leq$ 7 days, and in 80% of children with symptoms > 7 days. Furthermore, the number of days from debut of symptoms to a LP was different in the three groups. However, the differences in levels of cells and protein in CSF between the groups were also significant after correcting for number of days with duration of symptoms, with higher levels of cells and protein in group B compared to group A, and higher in group C compared to group B

Paper III

A total of 115 children with acute FNP were included in the study. The annual incidence of children less than 14 years with FNP was 21 per 100.000 children. The incidence was 43/100.000 per year for children aged 6 years, and 11/100.000 for each year in children aged 12 and 13 years. Among all children with FNP, 65% were diagnosed as LB, all of these occurring from May to November. CSF pleocytosis was demonstrated in 78% of children with FNP and was present in all but one of the children with FNP diagnosed as LB. In children with FNP and CSF pleocytosis, the level was higher in children with LB than in children without LB. Nine children had CSF pleocytosis but negative Bb antibody titres in both serum and CSF and no history of EM. However, LB could not be excluded in these children, as no other infections were diagnosed.

The median age of children with LB did not differ significantly from those without LB but the age distribution differed significantly between the 2 groups. Whereas most children with LB were 4 - 7 years of age, those without LB were spread more evenly throughout all ages.

The monthly occurrence through the year differed significantly between those with LB and those without. During the months from December to April none of the children with FNP had LB, whereas during the months from June to October, 77% of the children had LB. No children with FNP had CSF pleocytosis during the months from December to April.

Paper IV

Infectious meningitis was diagnosed in 211 children (annual incidence 38/100.000). LM was identified in 142 children (67%), NLAM in 46 children (22%) and BM in 23 children (11%). Forty-six children were diagnosed with NLAM, and in 23 of these, a microbiological agent was identified. Consequently, an aetiological agent was found in 89% of children with infectious meningitis.

The age distribution was different in children with LM compared to children with BM and NLAM. The median age of children with LM was higher than for children with BM. Thirty-nine per cent of children with BM but only one child with LM (aged 23 months) were younger than 2 years of age. Furthermore, the month of admission differed between groups. BM and NLAM were diagnosed throughout the year. LM was only diagnosed from April to December. The duration of symptoms in days before admission was significantly longer in LM compared to BM and NLAM. FNP was reported in 104 (73%) children with LM. No children with BM or defined NLAM had cranial nerve involvement, but three children with NLAM without an identified aetiological agent had FNP. Symptoms of cerebral involvement or signs of a systemic inflammation were rare in children with LM compared to children with NLAM.

Headache and/or neck stiffness (meningism) as the only symptom was present in 20% of children with LM. However, no children with BM or confirmed NLAM had meningism as the only presenting symptom. The PPV for having LM if the child had FNP or meningism as the only symptom was 97% for both variables. The NPV for not having LM if the child did not have a history of EM, or cranial nerve involvement or meningism as the only symptom was 95%.

Levels of CSF WBC and CSF protein were higher in LM compared to NLAM, but lower in LM compared to BM. A predominantly mononuclear CSF WBC was found in 99% of children with LM, more frequent than in BM and NLAM. The level of CSF glucose was lower in LM compared to NLAM, but higher in LM compared to BM. However, for all these variables, levels were overlapping between children with LM, BM and NLAM.

5. General discussion

The four papers included in this thesis are among the largest clinical studies of childhood NB, FNP and infectious meningitis from a European LB endemic area. The study area and the study population are of a moderate size, but the study period is as long as13 years. Epidemiological changes have been reported by us during the study period; however these results were not included in this thesis (121). Moreover, the population based design of the studies increases the importance of the results. Altogether, we consider that the studies give valuable contributions to epidemiological and clinical aspects of NB in children, and the overlapping conditions FNP and infectious meningitis.

These studies were performed when the awareness of tick transmitted infections and LB was relatively high both in the population and among health workers. Consequently, children in the general population with LB may have been diagnosed early during the course of disease, usually in the local stage of LB and treated with appropriate antibiotics immediately. This may have had impact on the frequency of development of disseminated stages of LB. Therefore, we consider the epidemiology and the clinical characteristics observed in children with NB in this thesis to be largely affected by the early management of children exposed to bites of Bbsl infected ticks in our region.

5.1 Methodological considerations

The design of the present studies is principally retrospective. Patients were identified from hospital records, and data were collected retrospectively from medical files and registered in a database. The quality of data may therefore have some limitations compared to a fully prospective study. This will especially be the case for clinical data, such as duration of symptoms. However, epidemiological data as well as demographical and laboratory results are mainly independent of the retrospective design.

Moreover, prior to the study period, the department came to a consensus regarding the diagnostic procedures and treatment both for children with possible NB and for children with FNP. In addition, all medical doctors in the area were

informed prior to the study period that all children with FNP should be sent to the department without delay. Consequently, during the whole study period, all children in South Rogaland with symptoms suspicious of NB or with FNP were hospitalised in one paediatric department at SUS for investigation using a standard protocol including a LP.

The recommended serological tests including Bbsl AI were, with few exceptions, taken from children with symptoms susceptible for NB, FNP, and other cranial and peripheral neuropathies, as well as in children with AM. During the study period there has also been focus on the current practice and the diagnostic procedures for these patients among the paediatric staff. Even though data were not registered prospectively, the quality of data may therefore be close to what could have been achieved if they had been registered prospectively.

In children with symptoms suspicious of infectious meningitis, a LP will normally be taken in any department. However, the recognition in our department before the study started that FNP, other cranial neuropathies as well as mild signs of meningism may be caused by NB led to the rigorous investigation of CSF in all children with these symptoms. This included children in good general health, but with long lasting headache as the only symptom. No other studies worldwide have described an identical approach to identify all cases of childhood NB in a defined area. The focus on this approach may also have brought more children with FNP and infectious meningitis without NB to our department, and the number of children diagnosed with these conditions may be more correct than in other studies without this approach.

However, except for those with FNP, our studies included children with cranial neuropathy or other symptoms severe enough to warrant hospitalization. For those being hospitalized we had a high awareness of possible NB and of performing a LP throughout the study period, but it is possible that children with NB with minor symptoms and without cranial neuropathy were not hospitalized, and therefore not diagnosed. Furthermore, in children with FNP, the suspicion of NB was obvious and other cranial or peripheral nerve involvement was probably not systematically searched for, resulting in some underestimation of

simultaneously peripheral nerve involvement. Even partial bilateral FNP may have been overseen due to our study design.

South-Rogaland has a unique advantage for population based studies of paediatric disease. All children in the county with acute childhood disease will be referred to this department. The population of the area is stable. There are no costs for parents when a child is admitted to hospital for acute disease. Except for children with mild symptoms not seeking medical help, all children within the study groups should have been included in the studies. Compared to many other studies, epidemiological data should therefore have a high reliability.

5.2 The diagnosis of NB

Several of the conclusions in our studies will depend on the accuracy of different diagnoses, especially for the diagnosis of NB. To diagnose NB may be challenging, due to the dependence on imprecise and indirect serological Bbsl tests. Furthermore, the relevance of LP and CSF analysis are debated, and finally, NB is reported to present with a variety of neurological signs and symptoms. Therefore, we suspect that the clinical characteristics incorporated in to case definitions are difficult to determine, when attempting to increase the serological pre-test probability.

In order to evaluate the incidence of CSF pleocytosis in childhood NB, this parameter was not included in the case definition in paper I-III (19, 117). The case definition of NB used in **paper I-III** was based on suggested clinical characteristics, and Bbsl serological tests in serum and CSF. From 1999 and through the whole study period, Bbsl AI was included. However, in **paper IV**, current guidelines recommended in European case definitions from 2010 were employed (63, 122).

5.2.1 Clinical characteristics

The basic clinical characteristics in children with NB specifically mentioned in the European updated case definition are meningitis and FNP (63). In accordance

with these guidelines, we found in **paper I** that ¾ of children with NB had signs of mild meningism, the most frequent clinical finding in children with NB. Cranial nerve involvement was observed in 2/3 of children with NB, and FNP was the major manifestation. All children diagnosed with NB in our studies presented with at least one of these two symptoms.

In **paper IV**, we evaluated the clinical differences between NB/LM and NLAM, and how clinical variables could aid in the early diagnosis of NB. We found that in children with CSF pleocytosis, the presence of cranial nerve involvement or meningism with headache/neckstiffness as the only symptom, both were symptoms with near 100% PPV for having NB.

Furthermore, as presented in **paper IV**, we found that in children with CSF pleocytosis without EM, cranial nerve palsy or meningism as the only symptom, the NPV of not having NB was 95%. Others have published different predictive diagnostic models for having NB in children with CSF pleocytosis, but they are so far not evaluated in prospective studies in regions with different Bbsl genospecies (123).

5.2.2   CSF Inflammation

In **paper I and II**, when using a definition of NB without incorporating CSF pleocytosis, we found that all but 2 % of children with NB had CSF pleocytosis. In this overwhelming majority of children with NB, nearly all had predominantly lymphocytic CSF pleocytosis, as also reported in **paper IV**. Lymphocytic CSF pleocytosis represent an important property of NB in children, and this is in accordance with recent European NB cases definitions where CSF pleocytosis is incorporated as an essential evidence of NB. The minority of children with NB without CSF pleocytosis were diagnosed as NB with acute FNP lasting less than 48 hours, and with positive Bbsl serology in serum. In the literature, especially from the United States, a major group of children with NB are reported to have FNP without CSF pleocytosis (124). This difference may depend on the different distribution of Bbsl genospecies between Europe and US. Moreover, in our study it seems that the small group of children with FNP and negative CSF consists of

children diagnosed early with only peripheral involvement of both the immune system and the nerve system. Another possibility is that this group consists of children with FNP with false positive serology of LB, and therefore do not have NB. However, in **Paper II** we presented results showing how the levels of CSF WBC and CSF proteins increase with the duration of symptoms of NB before LP are performed. Therefore, these few children with FNP without CSF pleocytosis may be diagnosed at the very start of the intrathecal immunological activation initiated by the Bbsl invasion.

Finally, all children in our study were diagnosed within the first 6 months of NB manifestations, defined as early NB (105). However, in NB studies in adults, a significant proportion of NB patients have late NB, and a higher proportion of patients with late NB seem to have a normal CSF WBC count (122).

5.2.3 Serological tests

The laboratory investigations in these studies did not include attempts to detect Bbsl by cultivation or by Bbsl PCR tests in serum or CSF (19). Furthermore, we did not performed Western blot tests, that are recommended by Centers for Disease Control and Prevention (CDC) as the confirming serological second titre tests in LB diagnostics (116).

Different Bbsl ELISA tests based on different Bbsl antigens with different validation qualities are commercially available (96, 107, 125). The predictive value of each serological test is depended on the proportion of different Bbsl genotypes in the study area (104). The Enzygnost Lyme borreliosis test performed in our study is considered to have a good predictive value in all human pathogenic Bbsl genospesies in Europe and is recommended in European NB guidelines (63, 105, 107, 111, 122).

The Bbsl seropositivity in the healthy population may affect the interpretation of a positive Bbsl antibody test. The seropositivity of children in Scandinavia is low, but cannot be ignored (54). However, maximising the pre-test probability of NB by incorporating clinical and laboratory findings when interpreting the Bbsl antibody titre, may increase the PPV of a positive Bbsl serological test (26, 71).

Therefore, recent published European guidelines recommend that only patients with symptoms and signs suggestive of NB should be tested for positive Bbsl serology. In addition, analysis of CSF should be performed (63, 94, 105). Generally, both the IgM and the IgG antibody levels change during the course of an infection. In the early phase of an infection, a significant proportion of patients have negative serological tests, both for IgM and IgG antibodies. This is reported to be true also for Bbsl antibody tests performed during early NB (105). The consequence of this may be that some children with early NB have negative Bbsl antibody tests and are not diagnosed as NB. In our studies, this may have been the case for some children with FNP presented in **paper III,** and in some children with NLAM presented in **paper IV**. Therefore, the estimated proportion of NB in children with FNP and NLAM presented in **paper III and IV** may be to low. Consequently, the annual incidence of children with NB and LM presented in **paper I and IV** may have been underestimated.

In **paper II** we studied how the frequency of positive Bbsl antibodies in children was dependent on the time from the start of symptoms to diagnosis. In 27% of the children diagnosed as NB with CSF pleocytosis, neither IgM nor IgG Bbsl antibodies were present in CSF. Nevertheless, as many as 76% of the children with NB were diagnosed within the first week of NB symptoms. Furthermore, children without detectable IgM and IgG Bbsl antibodies in serum were diagnosed after a short duration of symptoms compared to the rest of children with NB. These results suggest that negative antibody titres after just a few days after debut of symptoms must be interpreted with caution when NB is suspected. Furthermore, as presented in **paper II**, 64 % of all children with NB had a positive Bbsl AI when diagnosed. Only 51% of the children diagnosed with NB had a positive Bbsl AI if tested during the first week of symptoms, compared to 82% when symptoms had lasted for more than 1 week. This is in accordance with the results of Ljøstad et al. in adult patients with NB in the neighbour region to the study area. They found a diagnostic sensitivity of 79% in the Bbsl AI test when diagnosed, 74% when symptom duration was <6 weeks, and 100% with symptom duration >6 weeks (126). This time limit is incorporated in the recent

published case definition presented by one of the authors (105). After 6 weeks of NB symptoms, all children in our study also had positive Bbsl AI (result not presented in the paper). However, only a few children with NB are diagnosed after 6 weeks duration of symptoms. Thus, the Bbsl AI has far less than 100% sensitivity in diagnosing early NB in children, and the duration of symptoms must be taken into account also when interpreting the antibody index in children with suspected NB.

Moreover, as reported previously by others, in 8 children (5%) with NB presented in **paper II**, both IgM and IgG Bb antibodies were negative in serum, whereas antibodies and a positive Bbsl AI were demonstrated in CSF (127). Therefore, NB cannot be excluded in a child with symptoms suggestive of NB and negative Bb antibodies in serum.

One of the most comprehensive studies of children with NB was published by Christen and his co authors in 1993 (62). This epidemiological and clinical study of children with NB, AM and FNP is an important reference for our studies. The LB study by this German group is among the pioneer research in the field. Their study was performed within 10 years after the discovery of Bbsl, and therefore prior to the recognition of the three human pathogenic Bbsl genospecies present in Europe (14). The serological tests used in the study, were among the first generation of Bbsl serological tests with lower accuracy than the tests used in our study. Furthermore, as commented by the authors, their NB case definition was conservative, that could lead to underestimating of the incidence numbers. These aspects limit the possibilities to compare our studies to the work by Christen el al. However, they investigated many questions related to childhood NB not studied by us, and some of their results are needed to be confirmed.

5.3 Epidemiological results

5.3.1 Neuroborreliosis:

As reported in **paper I,** the incidence of NB in South Rogaland in children up to 14 years of age was estimated to 21/100.000. This is probably one of the highest

observed incidences of NB in children worldwide (119, 128). However, few published investigations have evaluated the incidence of NB in children in a strict population-based study design as in this thesis. A comprehensive prospective study from Germany discussed earlier estimated the incidence of NB in children aged 1 to 13 years to be 5.8 /100.000 (62). In a Norwegian national survey the incidence of disseminated LB was 8/100.000 in children aged 5 - 9 years (72). However, the highest incidence of LB in Europe is probably found in Central Europe (36).

Comparing incidences from different studies must be done with caution. The true incidence of NB varies greatly between areas in Europe. In addition, as commented by the authors, the German study used a conservative and narrow NB case definition, diagnosing children with NB only if Bb IgM antibodies where present in CSF. This has probably contributed to a lower estimate of the incidence than if they had uses the same criteria for NB diagnosis as in our studies (62). Furthermore, new generation of Bbsl serological tests used in our study have superior sensitivity and specificity qualities compared to those used in the German study from 1993. The high incidence found in our study may of course be the result of a truly high occurrence of LB in our region. However, in addition to the strict population based design, it is also important to emphasize the thorough inclusion criteria for children with suspected NB in our studies as described above, consequently, we think we are close to the true incidence of NB in our area.

It is known from several studies that the incidence of NB in children is higher than in adults (12, 21, 72). The most southern part of Norway may have an even higher incidence of LB than in our area, however, the total incidence of NB there in adults was reported to be 10/100.000 (72, 129).

The incidence of NB in children as reported in **paper I-II** were not evenly distributed throughout childhood, different from the age distribution of both non-Lyme FNP and NLAM presented in **paper III and IV**. In children with NB, a substantially peak in incidence was found in the age group 6-7 years, in accordance with other studies (21, 62, 72). This age dependent incidence in

childhood NB has implications for the total incidence reported in other studies. Incidence also depends on the age groups included in a study. In the German article, children younger than one year and older than 13 years were not included. NB are extremely rare in children younger than one year and low in children above over 13 years of age, so the exclusion of these age groups compared to our study group (0-14 years of age) may have overestimated the general incidence of childhood NB (62).

The age distribution of the incidence of all LB stages in all ages is bimodal, with a peak in childhood, and an even higher incidence in adults over 50 years (12, 130) The peak incidence in children with NB, higher than in any other age group is therefore interesting and may partly be caused by specific properties for the development of NB in children (62). As mentioned in the introduction, one study has shown that children more frequently have tick bites than adults (40). Furthermore, the most common site for tick bites in children is the head and neck region, which is uncommon in adults (12). The authors postulated that this could contribute to the high frequency of NB in children. Furthermore, the facial nerve may be the cranial nerve most vulnerable to Bbsl affection due to its narrow intracranial pathway (92). However, FNP is an easily observed manifestation of NB, which is almost impossible to ignore compared to other manifestations. This makes it likely that the identified proportion of all children with early NB may be higher compared to the same proportion of adults diagnosed with NB having different NB manifestations.

Why do ticks bite children so often in the head and neck region? As described in the introduction, ticks are vulnerable to dehydration and do not climb high in the vegetation to seek their victim, (32). When playing, children expose their upper part of body and the head and neck area to questing ticks hanging on grass and bushes more frequently than adults. The mean length of the leg of a child exceeds 60 cm when children pass the age of 6-7 years. This is also the top level of mean questing height of the tick (32). A tick attaching to the leg of a child will less probable climb to the head and neck region, than if it attaches to the upper abdomen or thorax. Ticks always climb upwards and successfully hide around

the ear or on the scalp covered with hair of the child. In addition, the awareness of tick bites is low in children, leaving the tick undisturbed for a longer time and therefore increases the risk of being infected. We support the theory that the height and the activity habits in children may be important causes for both the peak incidence of NB in 6-7 years old children, and for the high proportion of children with NB presenting with FNP (62).

Similar to other studies we observed a seasonal distribution of children diagnosed with NB (12, 36, 62, 130). All cases were diagnosed from April to December, and with almost 90 % of cases from June through October. With about a month's delay due to the incubation period of early NB, the seasonal distribution of NB in children is shaped by the seasonal activity of the tick and the seasonal activity of children i.e. playing outside in light clothing. The knowledge of this specific annual incidence variation makes the physician better prepared during summer time to be aware of different manifestations of NB in children. Furthermore, parents and caretakers of children should be focused on finding and removing biting ticks after outdoor activities.

5.3.2 Facial nerve palsy:
In **paper III** we estimated the incidence of acute FNP in our region to be at least 21/100.000 children. Although few population-based studies on FNP from areas endemic of LM have been published, the incidence reported in our study is high compared to other studies (62, 91, 92, 131). However, the childhood incidence of non-Lyme FNP in our study was comparable to non-endemic areas in Norway (91). Therefore, the high incidence of FNP seems to be due to a higher number of children with FNP caused by LB. Recent published papers referring our results have not found any other study demonstrating that LB accounts for as much as 65% of acute facial palsy in childhood (81, 88, 132). Moreover, during the summer months, as many as 77% of the children with FNP in our region were diagnosed with LB. As discussed in the previous chapter, the incidence of children with FNP caused by LB may even be underestimated because of false

negative Bbsl antibody titre during early NB (96). CSF pleocytosis was demonstrated in 78% of children with FNP, and no other aetiology was identified in children with FNP and CSF pleocytosis. Supporting this theory that all children with FNP and CSF pleocytosis had NB, we found in our study that all children with FNP and CSF pleocytosis simultaneously were diagnosed from May to November independent of Bbsl serological findings. This is consistent with the occurrence of early dissemination of Bbsl within a few weeks after the primary infection with Bbsl, which is also found in other studies (56, 62, 88, 133). In contrast, we observed that children diagnosed with FNP without LB was more evenly distributed through the years compared to children with NB. This is consistent with earlier observations of an even distribution of Bell's palsy through the year (88). Furthermore, no cases of FNP occurring during winter months were associated with CSF pleocytosis, again suggesting that FNP in these cases may be caused by other infectious agents not associated with meningitis, witch are more common during winter months (131).

FNP caused by LB was most common in the age group from 4 to 7 years, with an incidence as high as 43/100.000 in 6 year old children. This is consistent with the high rate of NB in children we found in **paper I**, but significantly different from the age distribution of children with FNP without NB (91).

In a survey of adults from the neighbouring region to the study area, an annual incidence for FNP of 29/100.000 was demonstrated, which is in accordance with other epidemiological studies of general populations (92, 93). However, LB was identified as the cause of FNP in only 10% of adult patients. This further demonstrates that LB is a much more common cause of FNP in children compared to adults, even in high endemic areas, and underlines the differences in the aetiology of FNP between adults and children (134).

 In the study reporting the incidence of FNP in Northern Norway, a non endemic area for LB, the total incidence for all age groups were 19/100.000 and lowest in children (<10 years : 4/100.000) (91). Comparing these results with ours, suggests an increasing incidence of FNP from north to south in Norway, parallel to the distribution of ticks and the occurrence of NB (72). This is also in

accordance with results from a Swedish multicentre study (133). In the total population of that study, the frequency of FNP caused by LB varied between 1 and 16% and was highest along the southeast coast of Sweden. No cases were reported from the northern part of the country. LB was observed more common among children with FNP than among adults.

5.3.3 Infectious meningitis:

Paper IV is to our knowledge, the only European study reporting the specific incidence of LM in a population based manner. According to updated European NB case definitions, CSF pleocytosis is mandatory for the diagnosis of NB (63). Therefore, NB and LM described in **paper IV** may be considered as almost identical conditions, which are also supported by the results in **paper I.** It is therefore not surprising that we found a high incidence of LM of 26/100.000 in children aged from 3 months to 14 years. This is corresponding to the incidence of childhood NB (0-14 years) found in **paper I**. Furthermore, the study presented in **paper IV** is also the first to demonstrate that LM is the major cause of infectious meningitis in children. 67 % of all children with infectious meningitis and 75% of all children with AM had LM,. This may be the result of a high incidence of LM in our area. However, we also demonstrated a low incidence of both BM and NLAM (135, 136). As many as 89% of all infectious meningitis cases were diagnosed according to aetiology, which is a high proportion compared to a recent study (137). Compared to studies reporting the incidence of AM in LB endemic areas, we found a low incidence of meningitis caused by enterovirus (62, 102, 138). This may be because we did not observe any epidemic clusters of cases during the study period, in contrast to the majority of studies demonstrating a higher incidence of enterovirus infections (139).

5.4 Clinical and laboratorial results

In **paper I**, mild meningism was found in ¾ of children and was the most frequent presentation of NB. In contrast to other studies, nearly all children with NB had CSF pleocytosis (62). Surprisingly, ¼ of children with CSF pleocytosis did not have clinical signs of meningitis, but only signs of acute FNP. Children with NB and FNP but without meningism were diagnosed earlier than children with meningism only. This may suggest that cranial nerve involvement is an earlier neurological affection than meningeal inflammation in NB. Furthermore, in **paper III and IV**, the concurrent observation of FNP and CSF pleocytosis was only observed in children diagnosed with NB. Finally, as children with NB may present with FNP as the only symptoms, this supports the argument that in LB endemic areas, NB should be suspected and evaluation of CSF should be performed in all children presenting with acute FNP (140, 141). However, during the winter from December to April, no children with acute FNP had CSF pleocytosis and LB was not identified as the aetiological cause in any of these children. Furthermore, no other findings with impact on management on these children were made by LP in these children.

Cranial nerve involvement, mainly FNP, was found in more than two-thirds of the children with NB. In adults, studies have demonstrated that Bannwarth syndrome is the most common presentation of NB in Europe (16, 20, 129). This is a painful meningoradiculitis with or without paresis, and it has been suggested that Bannwarth syndrome is a hallmark of infection with B Garinii (16). In our study, nearly all children presented with CSF pleocytosis, but painful symptoms were reported in only a few children all in head and neck area. No studies have reported a difference in the Bbsl genospecies affecting children and adults, and such a difference is unlikely. However, a difference in the manifestations of NB between children and adults is clearly observed (20, 21). This difference is partly explained due to the difference observed in the localisation of tick bites (12). As described in the introduction, in addition to the common haematogenous pathway used by other microbes invading the CNS Bbsl may enter the CNS by following peripheral nerves (50). Bbsl entering the skin in the head and neck

region may more frequently migrate along the facial nerve, causing ipsilateral peripheral nevritis as shown in animal studies and reported in clinical studies (62, 142). The facial nerve consists mainly of motor fibres, so FNP is therefore the main clinical symptom when peripheral neuropathy develops. Radiculitis developing after tick bites in lower part of the body mainly on adults may however involve thoracic and lumbar nerve roots affecting both sensorial and motor fibres. Consequently, pain may be the major manifestation (143). Furthermore, the cranial nerve involvement is probably part of a more widespread multifocal patchy inflammation, mononeuritis multiplex (144). In **paper I**, we observed a few children with FNP and peripheral pain syndrome, possibly a manifestation of mononeuritis multiplex.

In **paper II** we observed that the degree of CSF inflammation measured by WBC and protein in CSF differed significantly between groups with different clinical presentations. These differences were present even when corrected for the number of days from debut of symptoms to diagnosis, suggesting a pathophysiological link between the clinical presentation and the level of CSF inflammation. Children without cranial nerve involvement had higher levels of CSF pleocytosis and CSF protein than children with FNP. It has been speculated that the process of dissemination in NB may differ between meningoradiculitis and more diffuse LM (50). Both Bannwarth syndrome and FNP may be the result of a local invasion of the spirochete in the nerve root, whereas more diffuse meningitis may be the result of haematogenous spread (16, 50). The different mechanism may give both different symptoms and different laboratory results as demonstrated by us. Additionally, different Bbsl genospecies seem to present with different clinical manifestations of NB. In Europe, the most frequently Bbsl genospecie causing NB is reported to be B.garinii, usually associated with distinct clinical manifestations, CSF pleocytosis, peripheral neuropathy and further with positive Bbsl antibody titres and typically diagnosed as early NB in adults (49). However, B. afzelii is the second most frequent cause of NB in Europe, gives a less distinct clinical presentation which may be more

difficult to diagnose (49). We do not know the proportion of different Bbsl genospecies in our study. However, the results from others discussed above suggest that different genospecies may be unevenly distributed in the different clinical groups of NB in our studies.

In clinical practice, LM and NLAM may be the two main groups of childhood meningitis which are difficult to distinguish during early phases. In **paper IV** we found significant differences when comparing clinical and laboratory data of children with LM and NLAM. Cranial neuropathy was a common and highly specific finding in children with LM, 95% of the children with cranial neuropathy having FNP. Few other microbiological agents are reported to cause both CSF pleocytosis and cranial neuropathy in children. In a comprehensive study from US, a subgroup of children with FNP underwent LP. In these children, 59% had CSF pleocytosis and 98 % of these had proven LB. In patients without CSF pleocytosis, no aetiological agent was identified (81). In **paper IV,** all but three children with CSF pleocytosis and FNP were diagnosed with LM, and as previously discussed, it is highly possible that these children had early LM as well (145). Furthermore, headache and/or neck stiffness as a single symptom was a specific finding in children with LM, as recently described by others (128).

In summary, children with NLAM frequently had signs of involvement of both the meninges and the cerebral parenchyma in addition to a systemic inflammation. In children with LM however, signs of meningeal and peripheral neural inflammation dominated. This is an important and specific property of NB emphasized in a recent comprehensive overview (144).

Five previous studies have compared characteristics in children with LM and NLAM in order to develop predictive models for the early diagnosis of children in the two groups (102, 103, 115, 138, 146). In accordance with our results, they all report a high frequency of cranial neuropathy in children with LM and no aetiological agent was identified in the few children with cranial neuropathy in those diagnosed with NLAM. However, in these studies, different definitions of

LM were used, and the proportion of children with an identified agent in the NLAM group differed significantly from our results, mainly because of a higher proportion of children found to be infected with enterovirus. Furthermore, age of inclusion, the procedure for inclusion and the routines for the evaluation of children susceptible for NB differ between the studies, and this may affect the demographical, clinical and laboratorial results. Finally, our demonstration in **paper II** of how both the duration of symptoms and the clinical characteristics in children with NB may affect the level of CSF inflammation and the production of Bbsl antibodies, makes it more complicated to compare and generalize from such studies.

6. Conclusion:

The results of this thesis are based on a thorough work up including a lumbar puncture, in all children with symptoms suspicious of neuroborreliosis in an area endemic for Lyme borreliosis. The impact of the results is increased by the population based design of the study.

One major result is the high incidence of LB of 21/100.000 children, among the highest incidences ever presented. The highest incidence was shown for the age group of four to seven years, and most patients presented between June and October. Two thirds of children presenting with FNP had NB, which is the highest proportion presented worldwide.

The typical clinical presentation of NB in children was FNP with or without symptoms of mild meningism. However 1/5 of children with NB had only mild meningism with headache or neck stiffness without peripheral or cranial nerve involvement.

The number of children with NB having antibodies in serum of CSF, and the level of inflammation measured in CSF depended on the clinical presentation and duration of symptoms. These aspects must therefore be included in the evaluation of children with possible NB.

Another important result was that nearly all children with NB had lymphocytic CSF pleocytosis. Furthermore, pleocytosis was also present in nearly all children with FNP diagnoses as NB. Other causes of FNP with CSF pleocytosis were rare. The results underline that in areas endemic for LB, a lumbar puncture should be performed in all children with possible NB, including those with FNP as the only symptom.

Finally, this is the first study to report that Bbsl is the most common infectious agent causing meningitis in children in a defined area. Simple clinical variables enabled us to distinguish LM and NLAM during early phases of childhood meningitis.

7. Future perspectives

For the near future one of the major challenges related to NB in children will still be diagnostic accuracy. Today, both the sensitivity and specificity of available tests are limited, with the possibility of both under – and over-diagnosis, especially during early phases of disease. While the studies presented in this thesis were underway, newer diagnostic possibilities have emerged. Among these is the discovery of the important pathophysiological role of the cytokine CXCL13, a chemoattractant (50). The NB immune response seems to be orchestrated by innate immune cells in CNS producing CXCL13 acting as a selectively chemotactic protein for B-lymphocytes. Furthermore it seems to have specific properties in the immune response in spirochete meningitis and may achieve an important diagnostic role in NB in the near future. This may particularly be the case for children with early manifestation of NB, when CSF pleocytosis and Bbsl antibody production has not yet started. In children with NB, CXCL13 seems to be specifically elevated in CSF compared to serum, so a lumbar puncture seems to be an inevitable procedure even in future when evaluating children with possible NB.

It is a challenge that children with NB may have specific clinical and laboratorial constellations related to the proportion of different Bbsl genotypes present in the region. In our region, we have observed that children with NB may present with distinct clinical differences. We do not know the cause of this, but it may be related to differences in patient immunity, different Bbsl genospecies causing the disease, or even patient-spirochete interaction. In the future, NB diagnostics may hopefully include novel methods which are able to discriminate between different Bbsl genotypes. This may provide a potentially important base for genotype specific management of patients with NB.

To possibly answer these questions, our research group at Stavanger University Hospital has designed and initiated a prospective study, aiming to study aspects related to the diagnostic properties of CXCL13 and the relation of different Bbsl genospecies to clinical symptoms in children with NB. In addition, the study area with its stable population has the necessary qualities for epidemiological studies

into LB. New knowledge about childhood NB with regard to interventional studies and the consequences of climate change may well be possible from Stavanger University Hospital and the surrounding county.

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9. Errata

Paper IV
Abstact; Setting: should read A paediatric department serving all children
(62 000) in a coastal LB endemic region of southwestern Norway.