

Lyme borreliosis in Hungary in the years 1984 through 1989

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Abstract: Lyme borreliosis (Lb) is one of the most important bacteriological and clinical discoveries of the last few decades. The first isolation of the pathogen called *Borrelia burgdorferi* (Bb) in 1982 started off a tremendous interest in this tick-borne illness. Recently, Lb has become the most frequently reported vector-borne infection in North America and Europe. The aim of the present study is to describe the clinical picture and epidemiology of Lb in Hungary. This work was inspired by the first recognition of Lb cases in 1984. By the end of 1989, 1175 cases had been found. Of them 44% had the characteristic skin lesion of erythema chronicum migrans (ECM), 30% had neurological involvement and 25% showed arthritis. The clinical, therapeutic and epidemiological features of Lb based on 1175 Hungarian cases are presented.

Key words: Lyme borreliosis, *Borrelia burgdorferi*, epidemiology, Hungary, *Ixodes ricinus*

INTRODUCTION

Lyme, Old-Lyme and East Haddam are little rural towns in Connecticut, U.S.A., where a cluster of juvenile rheumatoid arthritis cases was reported in 1975. Steere and his co-workers investigated the origin of this endemic and described the new disorder in 1975-1977.

Recently, Lyme disease is the most frequently reported tick-borne infection. The most important vectors are *Ixodes dammini* and *Ixodes pacificus* in North America, as well as *Ixodes ricinus* in Europe. The pathogen was first isolated by Burgdorfer and named *Borrelia burgdorferi* (Bb) (Photo 1). Nowadays, by the suggestion of Bózsik et al. (16) the disease is called Lyme borreliosis (Lb). After the isolation of the new spirochete, it became possible to prove that erythema chronicum migrans (ECM), acrodermatitis chronica atrophicans (ACA), lymphadenosis benigna cutis (LBC), and a group of meningoradiculitis. Bannwarth's syndrome (BS) are different manifestations of the same disease. Bb infection may also cause carditis, chronic arthritis and several other forms of neurological disorders. Most recently, otoneurological and ophthalmological complications as well as fetal injury have

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been reported. The disease is famous for protean, chronic, fluctuating manifestations resembling another spirochetal illness, syphilis and is a candidate for the award of the "great imitator".

Lb occurs world-wide. By WHO estimates, we have to face 300.000 new cases per year. Probably, Europe is the most infected area in the world; Lb has been reported by 19 of the 32 European countries (161).

The aim of the present study is to describe the clinical picture and epidemiology of Lb in Hungary. This work was inspired by the first recognition of Lb cases in 1984. It became obvious that the disease was not a rarity and after the introduction of serological testing for Bb in 1986, the number of identified cases has increased exponentially with time.

The clinical, therapeutical and epidemiological features of Lb based on 1175 Hungarian cases are described in this paper. The first isolation of Bb from ticks is also reported.

Abbreviations:

ACA – acrodermatitis chronica atrophicans,
Bb – *Borrelia burgdorferi*,
BS – Bannwarth's syndrome,
ECM – erythema chronicum migrans
CNSI – central nervous system involvement

Lb – Lyme borreliosis
LBC – lymphadenosis benigna cutis
PN – peripheral neuritis
IFA – indirect immunofluorescence assay

MATERIALS AND METHODS

Patients

The present study starts with the first recognized Hungarian Lb cases in July 1984 and finishes in the end of 1989. A total of 3549 patients were tested for Bb antibody during this period. Of them, 3304 were suitable for further investigation. Serum and/or CSF samples were sent by 851 physicians from 191 hospitals to our laboratory. Of the 436 patients seen by the author 167 cases were treated by him. Moreover 692 patients were examined in his institute, the Central Hospital for Infectious Diseases. Questionnaires were filled up for data processing in these latter cases.

Methods

Serology

Standard indirect immunofluorescence test (IFA) was used. We started by using as antigen *Borrelia burgdorferi* strain N 34, originated from a German neurological case of Lb, went on to a Swedish tick isolate, G 152, and since December

1986 antigen 297 have been used which was isolated from an American case of neuroborreliosis. In the serological reaction there was no detectable difference between the three antigens. Antigenicity was much more influenced by the culture conditions than by differences in the original antigenic structure. That is why a large amount of pooled antigen was produced in March 1987, and it was frozen in small portions. These portions were routinely tested, but a loss of antigenicity was never found. All samples were tested twice, in different series. In the case of discrepancy the test was repeated. IgG antibody was tested only.

Serum dilution of at least 1:128 was regarded as positive, which was equivalent to the 95th percentile cut-off level of 200 Hungarian blood donors. Every microscope slide contained three sample drops of control sera: one negative, one borderline and one positive, diluted 1:100. Figure 1 shows that no antibody titer above 1:256 was found among blood donors. This means that the predictive value above this titer is nearly 100%. These high titers were reported to clinicians as "strongly positive". If a titer of 1:256 was found, then the "positive" sign was used with a predictive value of 98.5%. Similarly, 1:128 dilution was equivalent to "weak positive" and 1:64 to "unequivocal" finding. The cut-off level in the CSF was established in the same way. The CSF was drawn from 93 patients subjected to myelography. Only one sample was found to have a titer higher than 1:4; this was accepted as the cut-off level.

Borrelia isolation from ticks

Thirty-one *Ixodes ricinus* (17 male, 14 female), five *Haemaphysalis inermis* (three female, two male) and two *H. punctata* (male) field collected adult ticks were prepared. Ticks were dissected under stereomicroscope in a drop of BSK medium. One part of the midgut diverticula was dropped into culture media, incubated at 31°C and checked weekly for borrelia. The remaining part was transferred to a microscope slide, covered and depressed with a coverslip and examined under dark-field microscope. The coverslip was then removed and the material was air-dried and fixed in acetone and tested by direct or indirect immunofluorescence. For direct immunofluorescence the preparations were flooded with fluorescein-isothiocyanate-conjugated antibody produced in hyperimmunized rabbits (kindly provided by R. Gustafson, Huddinge Hospital, Stockholm). A serum sample with extremely high antibody titer, drawn from an ACA patient was applied for the indirect immunofluorescence test. The antigenic structure was evaluated by Western blot. Three patients' sera were used as antibody: an American case of Lyme arthritis, a Hungarian arthritic case and a BS patient. All three patients had extremely high borrelia antibody titers. A negative control serum from a blood donor was also applied.

Statistical analysis

The statistical calculations were done by an ATARI 1040 ST computer with the EPI INFO software developed by the Centers for Disease Control. Fisher's exact test was used for statistical analysis.

In the statistical analysis some of the data were missing in most cases. That is why only a part of the patients are shown in the figures and tables. The number of cases available for actual calculations are indicated, but the missing data are not.

The categories of "Lyme-case" or "non-Lyme" will frequently be used in data processing.

A patient was regarded to have Lb if one of the criteria below was fulfilled:

1. If a typical picture of Lb was seen (ECM, ACA, LBC, BS) independently of the serological results.
2. If a significantly elevated specific antibody level was found in the serum and/or in the CSF.

The frequently seen manifestations of Lb will be called major symptoms. These major symptoms can easily be judged on the clinical examination.

1. ACA: The diagnosis was based on the typical dermatological picture.
2. Arthritis: Joint involvement with swelling was accepted only. Bone and joint pains were classified separately. The category of arthralgia was used in the latter case.
3. Bannwarth's syndrome: Lymphocytic meningitis accompanied by radicular pain and sensory disturbances.
4. Carditis: Was accepted only when ECG changes were documented.
5. ECM: The diagnosis was based on the typical dermatological picture. The category of "complicated ECM" is used if any extradermal symptom (fever, malaise, or major symptoms: e.g. facial palsy) were detected.
6. Facial palsy: Only the peripheral type is included in this category. Many cases of this group were enrolled by screening tests. All the Bell's palsy cases which had been seen in three hospitals were screened for Bb antibody in the last two years of the study.
7. Central nervous system involvement (CNSI): Patients with CSF pleocytosis were assigned to this category. Meningitis was accompanied by mild encephalopathy in almost every case. Therefore the two overlapping syndromes (meningitis and encephalitis) were not separated.
8. LBC: The diagnosis was based on the typical dermatological picture.
9. Peripheral neuritis: Patients were enlisted in this category with complaints of paraesthesia or limb weakness even if "objective" signs of neurological involvement could not be detected.

RESULTS

Clinical Manifestations

Dermatological involvements

Erythema chronicum migrans (ECM). ECM is the most typical sign of Lb. Inflammation of the skin usually begins within a month after tick bite. It showed a

fluctuating course during a year in some untreated cases. The lesion develops from a small papule at the site of tick bite (*Photo 2-6*). In our cases, the longest diameter of the slowly enlarging ring was 90 centimetres, the shortest 5 centimetres. Occasionally the erythema was accompanied by burning sensation or mild pain. Sixty-one out of 519 patients complained of definite general symptoms: myalgia, headache, malaise. Only six had fever above 38°C. Two-thirds of the ECM cases had no other symptom. Multiple ECM rings were observed in 16 of the 131 (12.2%) personally examined ECM patients. In those cases the skin lesion developed not only at the site of tick bite but in different areas of the body.

Lymphadenosis benigna cutis (LBC). LBC is a solitary tumour-like skin lesion. Nevertheless, by histological examination a benign lympho-plasmocytic infiltration is detected. LBC prefers the two coldest parts of the body: usually one of the nipples or ear lobes are involved. In all of our eight patients, the skin lesion was seen at the latter site. It was often accompanied by a bluish or reddish discolouration at the edge of the external ear. If ECM had preceded the LBC the ear was always involved at the same site. Similarly, all the noticed tick bite were close to the ear. In one patient, ipsilateral facial palsy was also seen. Five of the eight cases were seropositive.

Acrodermatitis chronica atrophicans (ACA). This unusual manifestation of Lb was diagnosed in 16 patients. The first signs were aspecific: bluish-red or cyanotic infiltration with pasty consistency on the distal parts of limbs, particularly on the extensor surfaces. Although the disorder usually appeared on both hands or both feet, it was never strictly symmetrical. If ACA was preceded by ECM, the latter had always involved the same region where ACA developed later. The involved skin gradually thinned, decolourated and wasted, while the inflammation spread on the edges, often in spots. Almost all of the patients had small joint arthritis in the region of ACA (84). Three patients had problems after antibiotic treatment: they had to buy new shoes because their feet became smaller again.

Carditis

Eighteen cases of Lyme carditis were diagnosed. Rhythm disturbances were the principal signs in all of them. Fluctuating tachycardia was registered by ECG in six, otherwise healthy patients. Repeated ventricular or supraventricular extrasystole was found in two cases. Atrioventricular block was the most typical sign of Lyme carditis: 1st, 2nd and 3rd degree AV block was seen in nine, two and three cases, respectively. The latter three required temporary pacemaker treatment (87, 88). Moderate cardiomegaly and mild pericardial effusion were demonstrated by echocardiography in one case only. Moreover nine seropositive and/or ECM cases had cardiac pain or palpitation, but ECG did not reveal any abnormality. These cases were not enrolled into this group. Spontaneous improvement of carditis was seen in 13 cases, but subsequently facial palsy developed in one and gonarthrititis in another patient.

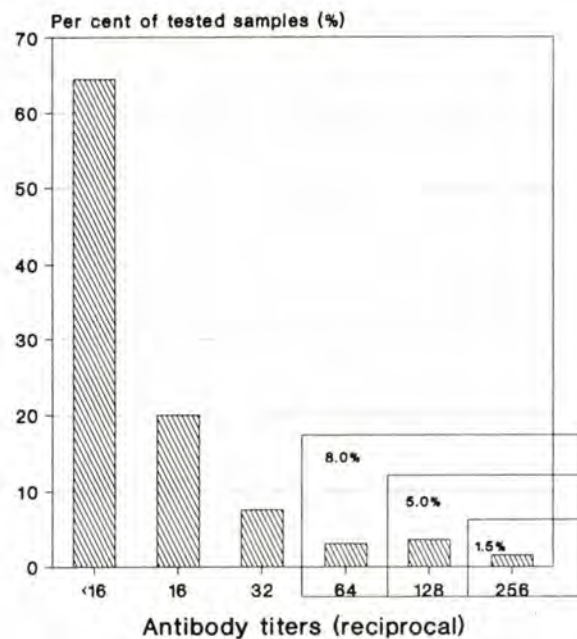


Fig. 1. *Borrelia burgdorferi* antibody titers in blood donors (n=200)

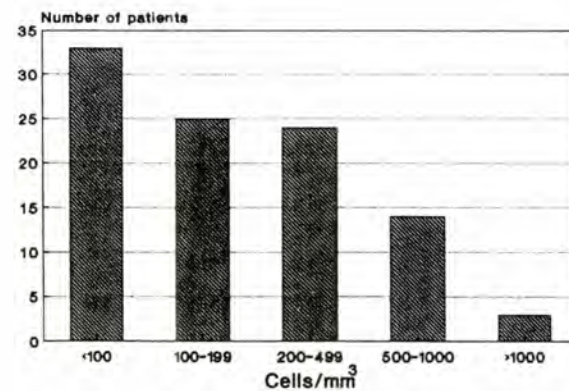


Fig. 2. CSF cell count in Lyme meningitis (n=104)

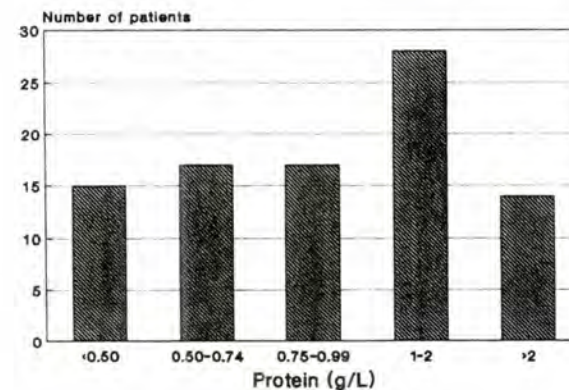


Fig. 3. CSF protein in Lyme meningitis (n=91)

Table 1
Frequency of meningitis in facial palsy

Etiology	Meningitis		sum
	no	yes	
non-Lyme	46	25 (35.2%)	71
Lyme	25	36 (59.0%)	61
Sum	71	61	132

$p < 0.005$

Neuroborreliosis

Bannwarth's syndrome (BS). Twenty-four typical cases of BS were diagnosed. The low number of patients in this group may be due to the strict diagnostic criteria. Only clinically unambiguous cases with CSF pleocytosis were enrolled. Usually the neurological examination found only mild organic signs, and nuchal rigidity was missing. In many cases, lumbar puncture was made only after receiving the positive serum antibody finding or after the more severe general symptoms or headache had developed (74). Fever was observed in about half of the paediatric patients but in a lower percentage in adulthood. Myelography was done in half of the patients and CT in almost all of them. CNS tumour or discopathy was the suspected diagnosis in almost every case. The clinical symptoms resembled multiple sclerosis in four patients.

Pleocytosis was mild (average: 267) in most cases of Lyme meningitis (Fig. 2), but in three cases more than 1000 cells/mm³ were observed. CSF cytology revealed 25-35 per cent granulocyte in two cases of Lyme meningitis. In nine of the 14 CSF samples where plasmocytes were looked for, at least 5 per cent were noted. The protein level was elevated in almost every Lyme meningitis case (average 1.17 g/l, SD:0.91) and in half of them it was extremely high (Fig. 3). The very high CSF pro-

Table 2
Frequency of bilateral facial palsy in Lyme borreliosis

Etiology	Facial palsy		sum
	unilateral	bilateral	
non-Lyme	470	18* (3.7%)*	488
Lyme	149	17 (10.2%)	166
Sum	619	35	654

$p < 0.005$

* 6/18 Guillain-Barré syndrome

Table 3
Relapsing facial palsy in Lyme borreliosis

Etiology	Facial palsy		sum
	single	relapsing	
non-Lyme	443	45 (9.2%)	488
Lyme	159	7 (4.2%) [0%]*	166
Sum	602	42	654

$p < 0.05$

* Multiple or single relapse after six months

tein level was even more typical of the Bannwarth's syndrome patients (Fig. 4). CSF sugar levels were usually found to be normal (average 2.98 mM/L, SD:0.89) however, low sugar levels were measured in significantly more cases of Lyme meningitis than in other diseases with CNS involvement (Fig. 5). The difference was striking in BS ($p=0.0005$). Four cases of typical BS were originally treated with antituberculous drugs because of the chronic lymphocytic pleocytosis, high protein and low sugar levels in the CSF. Culture for mycobacteria was negative in all four cases.

Facial palsy. Shortly after Bb antibody testing was started, the high frequency of peripheral facial paresis among Lb patients became obvious (76). Facial palsy was often associated with meningitis (Table 1) or, in many cases, it was just one symptom forming part of Bannwarth's syndrome. There were also many occasions when it presented by itself, thus fulfilling the diagnostic criteria for Bell's palsy. Bilateral presentation was more frequently found among Lb patients (Table 2). Of the seronegative patients with diplegia, six suffered from Guillain-Barré syndrome. The relapsing facial palsy often proved to be seronegative (Table 3). In the relapsing case of facial palsy caused by borrelia the second attack was seen within 6 months, while the symptom-free intervals were longer (usually several years) in the seronegative patients. In the idiopathic group multiple relapses were regularly seen, but never among Lb patients.

In a previous study, thirty well-documented and untreated Bell's palsy cases were analysed. We found that Bell's palsy caused by Bb has a better outcome in spite of the initially more serious paresis (102).

Central nervous system involvement. Patients with CSF pleocytosis but not fulfilling the criteria of BS were included into this group. In many cases, lymphocytic meningitis was found, clinically indistinguishable from those of viral origin. However, in cases of borrelia meningitis there was a strong tendency for longer recovery and more serious fatigue as compared to viral meningitis. More pronounced differences could be observed in the encephalitis. In Lb, symptoms started less dramatically and coma seldom developed. Convulsion appeared in three cases only. Serious fatigue and memory disturbances developed in almost every long-standing

Lb case. Slight mental deterioration was mainly observed in adults, but could sometimes be noticed in children as well.

Neuritis. In one patient coronarography was performed because of serious chest pain, another was operated on because of suspicion of gastric perforation, and in a third case surgery was performed because of symptoms resembling vertebral disc herniation. The pain – which was the leading symptom in almost every case – seldom was so serious. Migrating paresthesia, numbness, fluctuating headache, fatigue and sleeplessness usually rose the suspicion of neurotic syndrome in the physicians. Paresthesias were rarely accompanied by severe paresis. Complete paraplegia developed in three cases only. Reflex asymmetry could be demonstrated in half of the patients with neuritis. Cranial nerves other than the facial nerve were seldom involved. The eye movements were restricted in 17 cases.

Progressive borreliosis. Only one of our patients met the strict criteria of progressive borreliosis. The diagnosis was undoubtedly proved by the success of therapy (72).

Arthritis

In five cases, acute arthritis developed while ECM could still be seen. All had large joint involvement. In one patient gonarthritis preceded ECM. In the region of the involved knee a tick had been found ten days (!) earlier. ECM developed in the same region two weeks after the tick bite.

When arthritis developed after a short incubation time, mainly large joints were swollen. In those patients who had arthritis in two months after tick bite or ECM large and small joints were equally involved. In cases of a longer incubation period small joint inflammation predominated (Fig. 6). A significant difference was found in the median incubation time between large, mixed (large and small) and small joint arthritis. It was 66, 137 and 302 days, respectively. In 102 patients, arthritis was the only sign of Lb. Temporomandibular arthritis was observed in five cases.

The relapsing-remitting course of joint inflammation was especially characteristic of the large joint arthritis. The highest number of relapses (six) were observed in a patient who had chronic synovitis in the knee. He remained therapy resistant despite treatment with high doses of antibiotics (iv. penicillin, doxycycline, ceftriaxone). Occasionally, arthralgia preceded the frank arthritis. The time that elapsed between the onset of arthralgia and of frank arthritis was highly variable, the shortest being 3 days and the longest up to 3 years. In some cases arthralgia persisted as long as 5 years after the improvement of frank arthritis. In many cases – sometimes during, or shortly after the treatment – large joint arthritis was followed by small joint inflammation.

Pregnancy and borreliosis

Only three borrelia infections were diagnosed during pregnancy. ECM developed in the third trimester in two cases and facial palsy appeared in the second

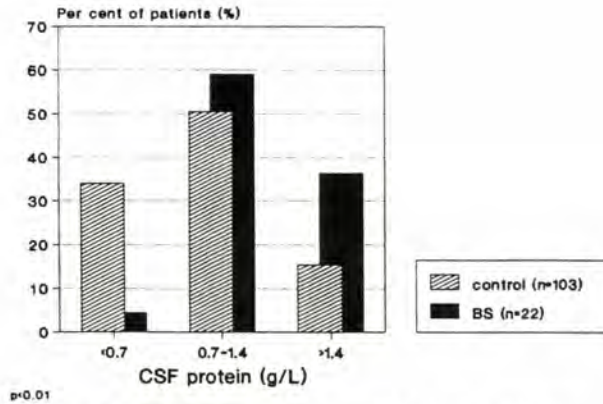


Fig. 4. CSF protein in Bannwarth's syndrome (BS) and in the control group

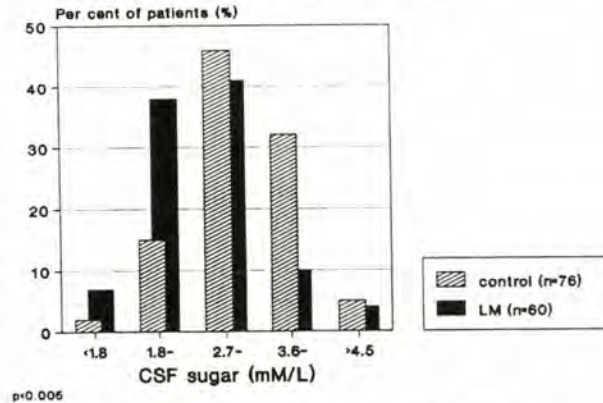


Fig. 5. CSF sugar in Lyme meningitis (LM) and in the control group

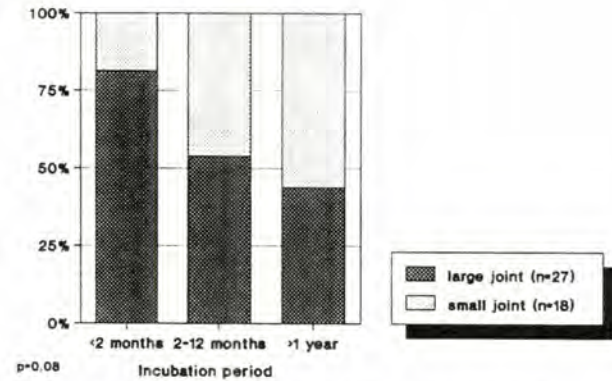


Fig. 6. Ratio of small and large joint arthritis as a function of the incubation period (n=45)

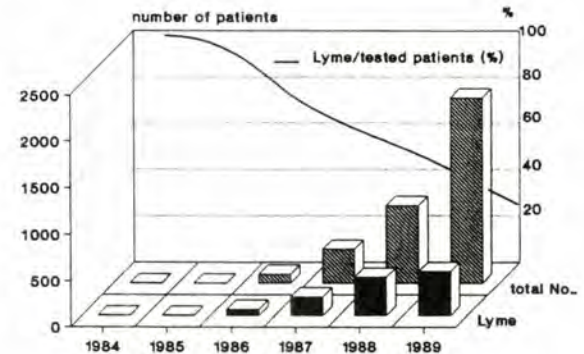


Fig. 7. Ratio of Lyme disease cases (n=1175) to all patients tested (n=3304) 1984-1989.

month of pregnancy in the third woman. Two newborns' sera were tested for Bb. Both of them contained specific IgG antibody (1:64 and 1:128) but neither of the newborns showed clinical signs of infection.

Screening tests

The cut-off level was based on testing sera from 200 blood donors. Moreover, 516 inhabitants from Budapest and 224 residents from Veszprém county, as well as 125 infants with enteritis were tested. Of them 6%, 4.5% and 2.3% proved to be positive. Among the infants, no antibody titer above 1:128 was found.

Epidemiology

In Figure 7, the number of tested patients can be seen in yearly distribution. As the disease became well known in Hungary, more requests for borrelia antibody testing arrived. The number of tested patients increased exponentially, but the proportion of Lb cases decreased year by year.

Frequency of symptoms

The three main symptoms are shown in Figure 8. Only frank arthritis is included here. It is important to underline that 20% of our patients had more than one major symptom consecutively or at a time (Fig. 9). In many cases, this complex clinical picture led to the suspicion of Lb. There was a group of patients which could not be enrolled into any of the nine major symptoms. This group was not processed in the further statistical calculations. The importance of ECM is outstanding in diagnosing Lb. In Figure 10, only cases with complicated ECM and their accompanying symptoms are shown. The frequency of joint manifestations is impressive.

Tick bite and its occurrence

Hungary is divided into 64 equal squares along the latitudes and longitudes in Figure 11. Those patients are represented here who had information about the geographical place of tick bite. The bars are proportional to the number of tick bites in each square and they roughly draw the relief map of Hungary. Half of those patients who had noticed a tick bite were proved to have borrelia infection. Our data suggest that Lb is prevalent in every area of Hungary where ixodid ticks can be found. Many patients were bitten by a tick in their own garden in Budapest. In our material altogether 886 patients noticed tick bite, and 932 did not. Information about tick bite has not been registered in one-third of our patients (Fig. 12). One-fifth of Lb cases did not notice tick bite. The frequency of tick bite recognized by symptoms is shown in Figure 13. It was striking that those patients remembered the bite, whose dermatological involvement (ECM and LBC) appeared at the site of it. The tick bite was less frequently observed or remembered in the facial palsy group. This is probably due to the screening characteristics of this manifestation. (Neither the physician nor

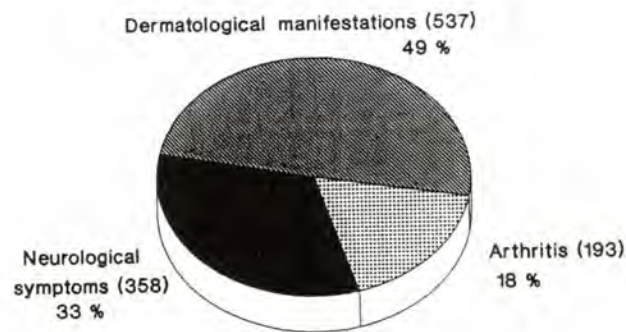


Fig. 8. Distribution of the main symptoms of Lyme borreliosis in Hungary, 1984-1989 (n=1175)

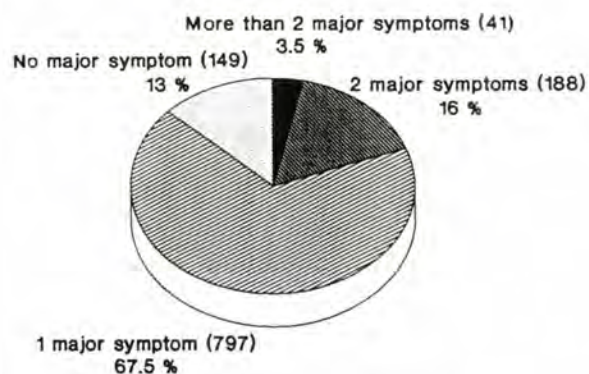


Fig. 9. Multiple symptoms in Lyme borreliosis (n=1175)

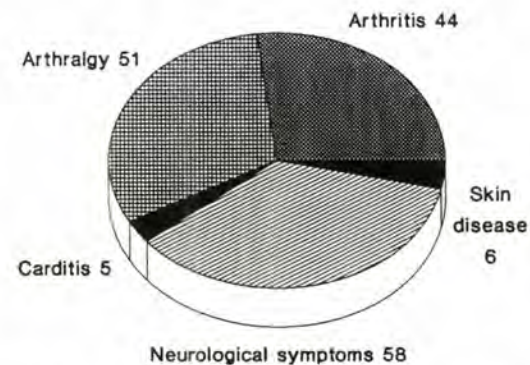


Fig. 10. Erythema chronicum migrans (n=519) distributed by complications (n=148)

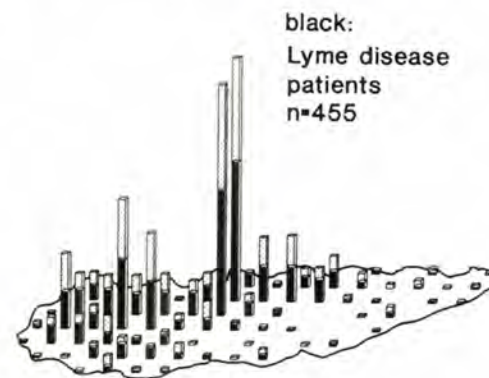


Fig. 11. Geographical distribution of tick bites in Hungary based on 807 patients

the patient considered borrelia infection as a real possibility, when the questionnaire was filled in.) The risk of neuroborreliosis was 2.42 times higher in cases of multiple tick bites (Table 4).

Seasonality

The total number of Lb cases and the number of seropositive patients are shown in Figure 14, in monthly distribution, according to the first clinical symptom. Taking into consideration that much more patients' material was sent to our lab for serological testing in the summer months, a better picture can be drawn if Lb cases are represented in the proportion of the patients tested (Fig. 15). An accumulation became visible in the early spring and late autumn.

ECM showed a midsummer seasonality while ACA had no seasonality at all. The analysis of the few LBC cases suggests a more balanced seasonal distribution than that found for ECM. Dividing the rheumatological manifestations into arthralgia and frank arthritis seems to be justified by differences in the seasonal distribution of the two forms (Fig. 16). Neuroborreliosis has a seasonal pattern too. It is most pronounced in the CNS manifestations in the midsummer months, while in the case of peripheral neuritis an autumnal peak can be seen. Facial palsy has a relatively balanced distribution. Because of the screening characteristic of the latter symptom, it is worth to show the seropositive cases as a percentage of all cases examined (Fig. 17). A weak accumulation of the seropositive cases in the early spring and a stronger one in the autumn can be seen. The seasonal pattern can be better understood by showing the incubation time from the tick bite to the onset of different symptoms. It is the shortest in ECM: from a few days to 4 months (median: 10 days). The longest incubation time (1-3 years) was found in ACA. In most of the neurological manifestations an incubation time of 10-50 days (median: 32 days) was measured. Most of the frank arthritis developed from two months to two years, but 15% of them appeared in one month after the tick bite (Fig. 18). Arthralgia may develop at the onset of infection or several months later. Similar data can be derived by analysing the time that elapsed from the onset of ECM to the appearance of complications (Fig. 19). Neurological symptoms usually appear within a short time. Three peaks can be seen in the arthritis cases: an early form, almost at the time of development of ECM, the second with an incubation time of 3-8 months, and a late form appearing after 3 years. Arthralgia may develop at any time during the long disease process.

Age

The average age of the total examined population was 33.3 years, and that of the Lb cases was 34.3 years. The youngest seropositive was a 14-month-old patient suffering from ECM and facial palsy, the oldest an 83-year-old man with arthritis. Figure 20 shows that most of the patients examined were in the 10 to 15-year-old age group and in young adulthood. In contrast, the ratio of Lb cases in the examined population was highest in the youngest and in the oldest age groups. The high

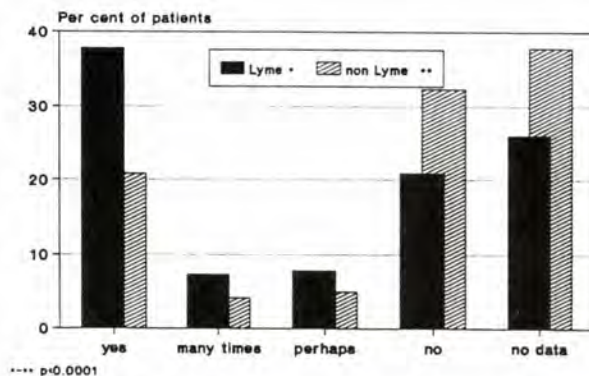


Fig. 12. Tick bite in the case history of Lyme and non-Lyme patients (n=3304)

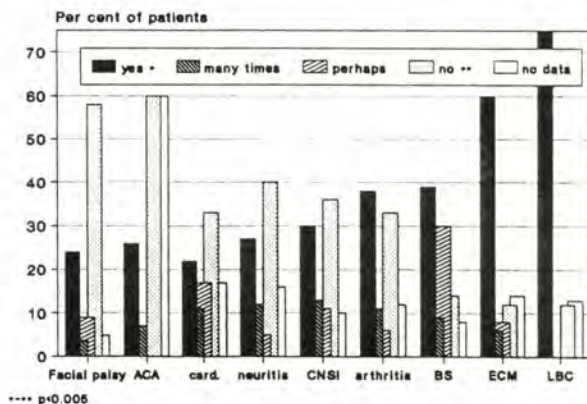


Fig. 13. Tick bite in the case history of different symptoms (n=1175)

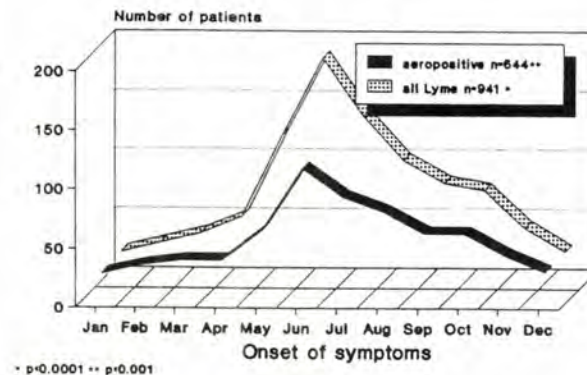


Fig. 14. Distribution of the patients as a function of the onset of disease (n=2400)

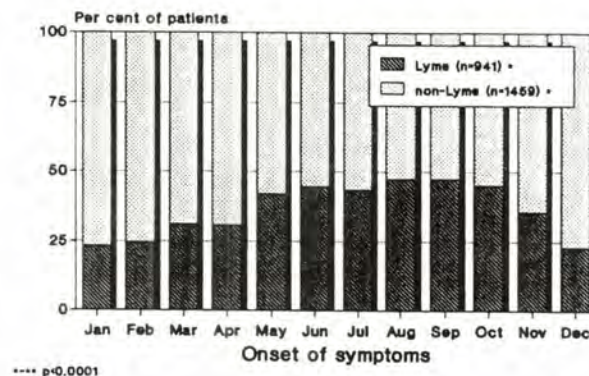


Fig. 15. Seasonal distribution of Lyme borreliosis in the proportion of all tested patients (n=2400)

Table 4
Frequency of multiple tick bite in neuroborreliosis

Etiology	tick bite		sum
	single	multiple	
non-Lyme	188	25 (11.7%)	213
Lyme	121	39 (24.4)	160
Sum	209	64	373

$p < 0.001$

Table 5
Relative risk of different symptoms of *Borrelia burgdorferi* infection in children

	Risk factor	p
Lyme (all)	0.7	NS*
ACA	0.0	0.004
Arthritis	0.78	NS
BS	0.8	NS
Carditis	0.6	NS
ECM	1.32	0.01
Facial palsy	0.9	NS
CNSI	0.9	NS
LBC	23.12	0.0002
Periph. neuritis	0.27	0.03

* not significant

Table 6
Frequency of arthritis among symptoms caused by *B. burgdorferi*

	Arthritis		sum
	No	Yes	
adult	617	244 (28.3%)	861
child	212	45 (18.6%)	257
sum	829	289	1118

$p < 0.001$

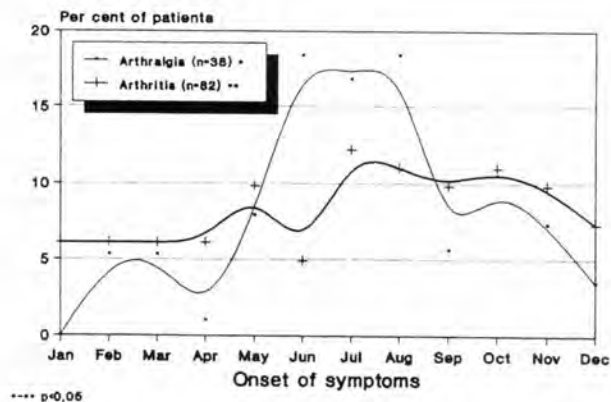


Fig. 16. Rheumatological involvement in Lyme borreliosis (n=120)

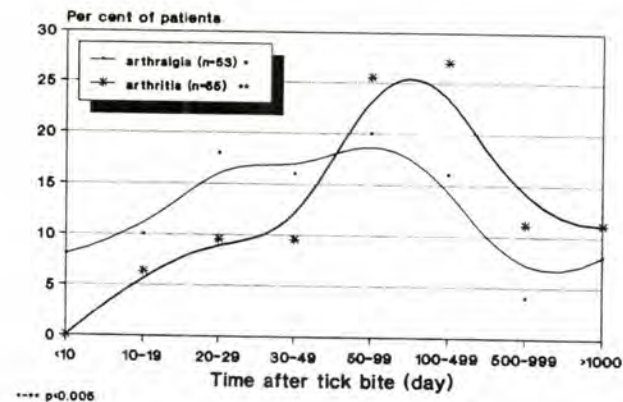


Fig. 18. Incubation period from tick-bite to arthritis (n=113)

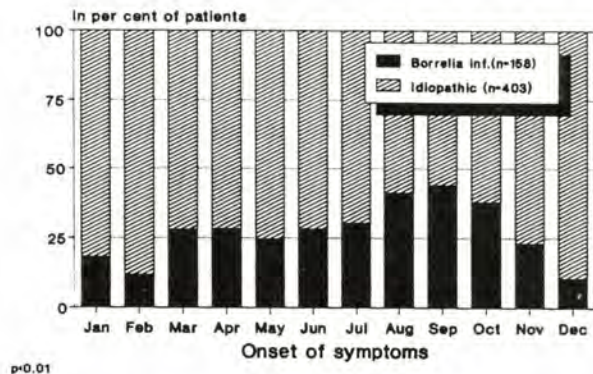


Fig. 17. Seasonal distribution of idiopathic Bell's palsy and facial palsy caused by *B. burgdorferi* (n=561)

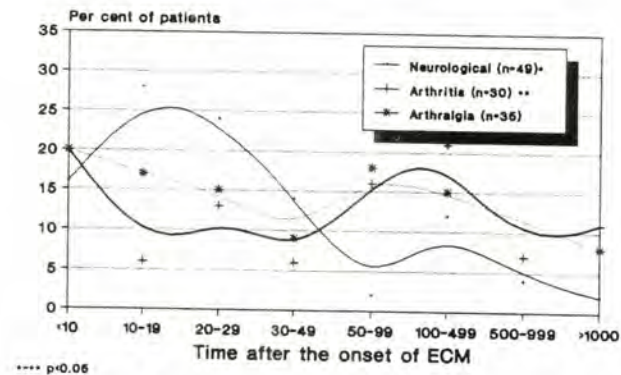


Fig. 19. Time elapsing between the onset of ECM and neurological or rheumatological symptoms

incidence of monosymptomatic ECM among young children is apparent. The ratio of monosymptomatic and total ECM cases decreased with age (Fig. 21). The relative risk of the development of Lb symptoms in children is shown in Table 5. Lyme arthritis is rare in childhood (Table 6). Among Lb patients, facial palsy was twice as common in children (Table 7).

Sex

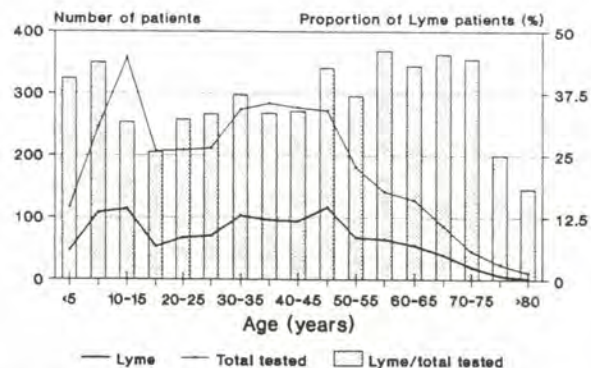
A total of 1563 males and 1741 females were examined. Among Lb patients 589 males and 586 females were found. The relative risk of Lb was higher in men (relative risk: 1.12; $p < 0.05$). Without ECM, the odds ratio of Lb was 1.5 times higher in men ($p < 0.001$). Males were more often seropositive ($p < 0.001$) and infrequently suffered from ECM ($p < 0.05$). Neuritis and carditis caused by Bb were found more often among men ($p < 0.05$).

Serology

The time elapsing from the tick bite till serology is very important in the judgement of a serological test. Each interval of Figure 22 contains ten per cent of the patients. A relatively big portion of patients was found to be seropositive shortly after the tick bite. The mean antibody titers are shown in Table 8. Only the typical Lb symptoms are presented here.

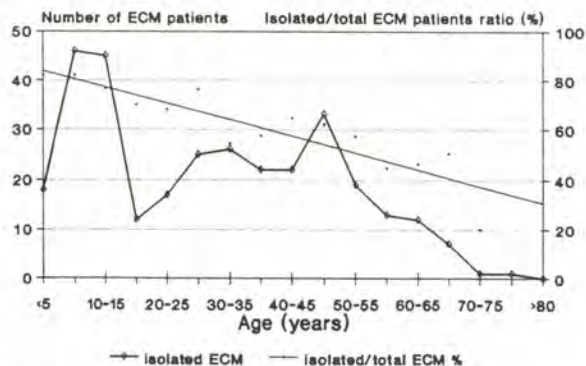
Which parameters determine the seropositivity? It can be assumed that systemic infection has a bigger chance to result in seropositivity than a localized one. Time is another factor that significantly affects seropositivity. Figure 23 shows the seropositivity ratio as a function of time elapsing from tick bite to the onset of ECM. "Complicated ECM" contains cases not just with major symptoms (e.g. facial palsy or meningitis) but with all the general symptoms (e.g. headache, fever, fatigue) as well. It is not surprising that more seropositives were found among patients with complicated ECM, but it is astonishing that incubation time has an effect on the serological results: the longer time elapsed from tick bite the more patients became seropositive in the complicated ECM group. In monosymptomatic ECM, the opposite was found. Taking all ECM cases, the time that elapsed from the onset of ECM to serology had no effect on the seropositivity ratio. The surprising result was due to two opposite tendencies: complicated ECM cases became seropositive more often as a function of the elapsed time, while monosymptomatic cases were more often found to be seronegative (Fig. 24). In Fig. 25, the incidence of complication is shown as a function of the time that elapsed from the onset of ECM to the serology (start of therapy). There is a significant increase in each group, but seropositive ECM became more frequently complicated with the elapsed time than did the seronegative one.

Because most of the patients had become seropositive by the time of the serological test, seroconversion was registered in 109 patients only. A significant difference was found in seroconversion rate between the different clinical symptoms of Lb (Fig. 26). It can be assumed that the early manifestations of Lb give a possibility



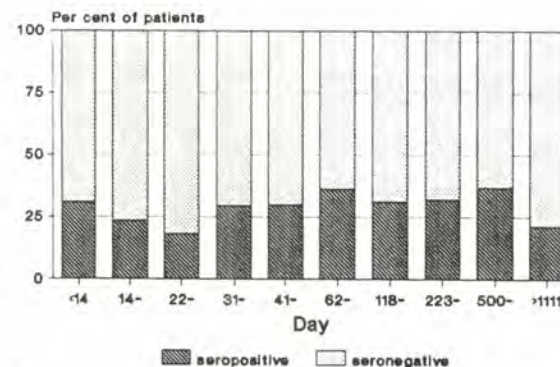
$p < 0.0001$

Fig. 20. Age distribution of all patients tested (n=3067)



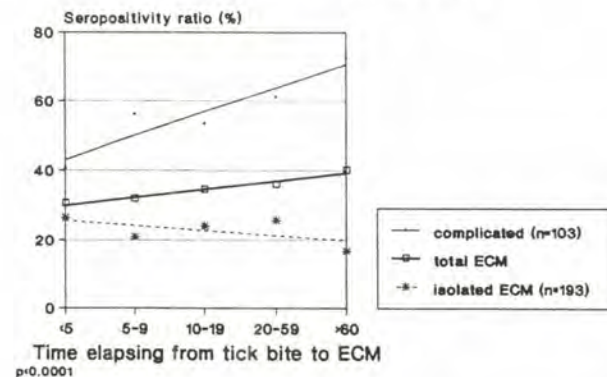
$p < 0.005$

Fig. 21. Age distribution of uncomplicated ECM cases (n=319)



$p < 0.05$

Fig. 22. Time elapsing from tick bite to the first serological test (n=983)



$p < 0.0001$

Fig. 23. Seropositivity ratio as a function of time that elapsed from tick bite to the onset of ECM (n=296)

Table 7
Frequency of facial palsy among manifestations caused by *Borrelia burgdorferi*

	other symptoms	facial palsy	sum
adult	755	106 (12.3%)	861
child	199	58 (22.5%)	257
sum	954	164	1118

$p < 0.001$

to demonstrate the seroconversion. ECM is an exception because the diagnosis usually coincided with the prompt start of therapy, and it usually prevented the further elevation of antibody titer. BS usually had become seropositive by the time it was diagnosed. The highest per cent of seroconversion was found among Bell's palsy patients. This was probably due to the serological screening of this illness. Six patients proved to be false positive: three had syphilis, two leptospirosis and one case of systemic lupus erythematosus.

Therapy

Our patients had been treated with several antibiotics, but only the penicillin- and doxycycline-treated groups were big enough for statistical analysis. Patients were not selected randomly for the different treatments: the treatment depended on the physicians' choice. The original treatment was altered only if progression was noted. Only patients receiving monotherapy were selected for further analysis. "Low" and "high" doses of antibiotics were distinguished. In adults, for the treatment of ECM, 6 megaunits/day phenoxymethylpenicillin or panemecillinum for nine days and at least 200 mg/day doxycycline for 30 days were considered a high dose. Every oral or intramuscular treatment for other concomitant symptoms was considered a low dose. In the latter cases, at least 20 megaunits/day penicillin G for 10 days was accepted as a high dose.

ECM cases are the best for the judging the efficacy of a treatment, because in this manifestation it is easy to demonstrate both the start and the end of the disease.

Table 8
Antibody titers in different manifestations

	average titer (reciprocal)	average of exponents (2^x)
ECM	76.1	6.25
LBC	137.2	7.1
BS	427.5	8.74
ACA	739.2	9.53

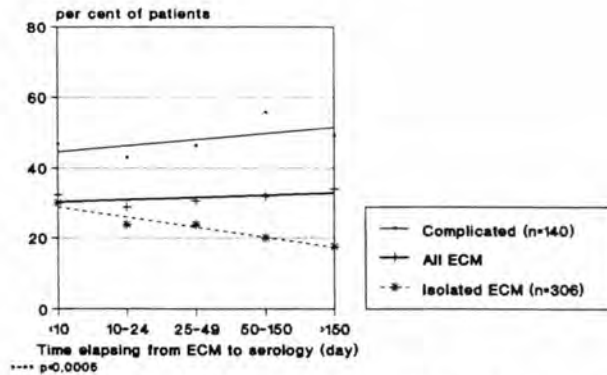


Fig. 24. Seropositivity ratio as a function of time that elapsed from ECM to the serology (n=446)

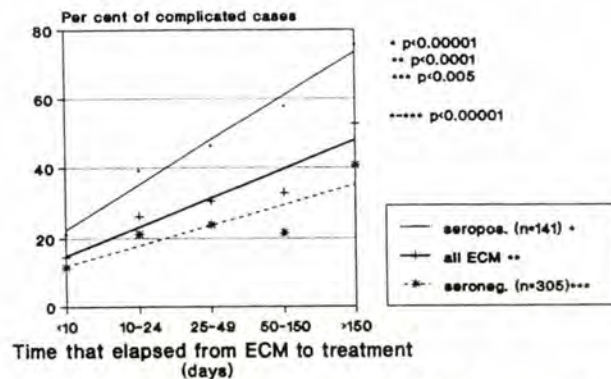


Fig. 25. Frequency of complications in ECM patients as a function of time that elapsed from the onset of symptoms to treatment (n=446)

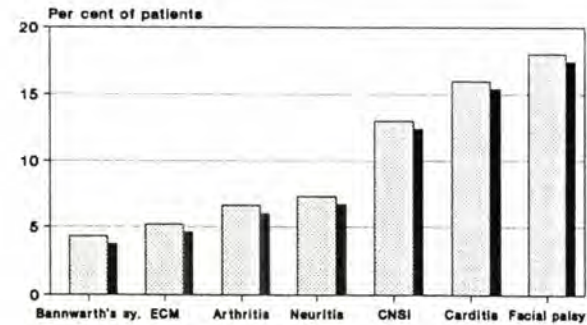


Fig. 26. Seroconversion rate in different symptoms of Lyme borreliosis (n=109)

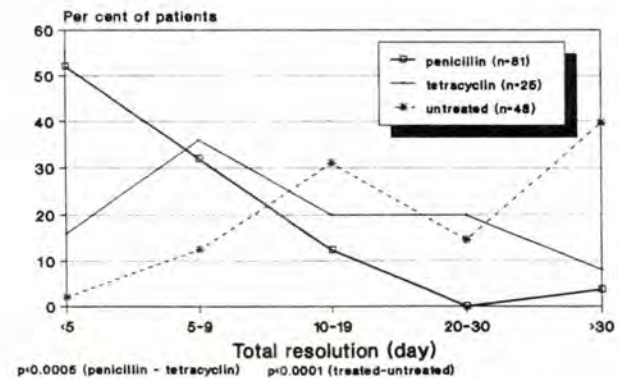


Fig. 27. Influence of the antibiotic choice on the improvement of erythema chronicum migrans (n=154)

Table 9
Borrelia isolation from ticks by different techniques

methods	dark- field	direct IF	indirect IF	cultivation
Tick-isolates				
MK5	—	—	N*	+
MK6	—	—	N	+
GyK3	+	N	+	—
GyK4	+	N	+	+
GyK6	—	N	—	+

* N=not done

All the other symptoms may have an obscure onset or finish. A significantly faster improvement was seen in the penicillin- than in the doxycycline-treated group (Fig. 27). In Figure 28, the data of patients followed up for a long term can be seen. The seronegative ECM cases only exceptionally became seropositive after treatment. Antibody titer decreased significantly in most of the antibiotic treated patients, but only 25% of them became seronegative. Not just the antibiotic itself, but the dosage also had a strong influence on the rapidity of improvement. Only a slight difference was found between the low-dose treated and the untreated groups (Fig. 29). On the contrary, high-dose treatment resulted in a rapid disappearance of ECM.

Treatment failure

Twelve (5.1%) of the 234 ECM patients followed up for a long-term developed new symptoms of Lb despite having been treated with adequate antibiotics. In five of them, the second symptom of Lb appeared during the treatment, so they may not be considered as treatment failures. Major symptoms developed in four of the remaining seven cases: meningitis, facial palsy and recurrence of ECM were observed, each after the low-dose oral penicillin treatment. The fourth patient was treated with 250 mg tetracycline q.i.d.; ECM disappeared on the 14th day. A year later, arthritis of the knee and the elbow developed, then improved spontaneously. A fluctuating, second degree atrioventricular block developed 18 months after the onset of ECM, and it was cured after ceftriaxone treatment. Slight, spontaneously improving symptoms such as headache and arthralgia developed in the further three cases. In cases without ECM, a second illness was registered in five more cases.

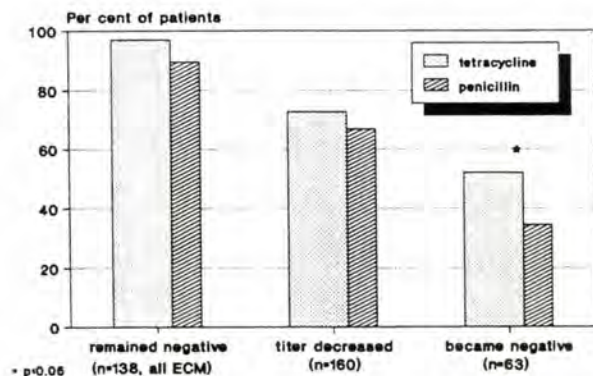


Fig. 28. Effect of antibiotic treatment on the result of the serological test (n=298)

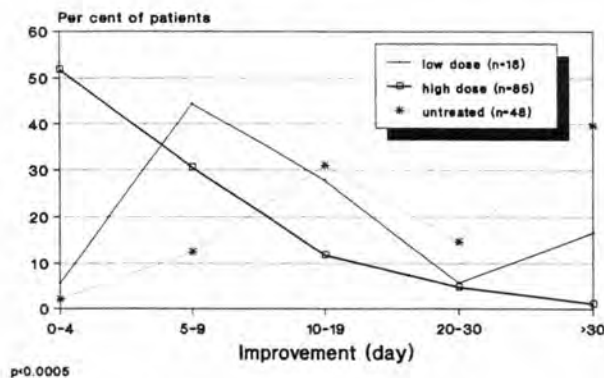


Fig. 29. Comparison of low- and high-dose antibiotic treatment of erythema chronicum migrans (n=151)

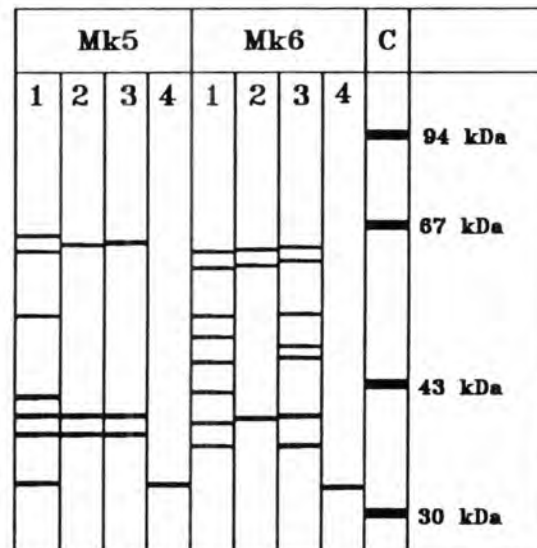


Fig. 30. Antigen pattern (Western blot analysis) of two *Borrelia burgdorferi* strains isolated from ticks. Strain Mk5 is shown in the first series of lane 1-4, Mk6 is in the second series of lane 1-4. Sera used as antibody were originated from: American case of arthritis (1); Hungarian farmer with arthritis (2); Bannwarth's syndrome (3); blood donor (4). Migration of the molecular weight standards is shown on the right.

Isolation of *Borrelia burgdorferi* from ticks

Thirty-one *Ixodes ricinus* ticks were dissected (78). Five of them proved to be infected with Bb (Table 9). In two ticks, direct dark-field microscope examination revealed 10-12 μ long spirochetes indistinguishable from our standard borrelia strains cultivated under artificial conditions. These two tick isolates could also be detected by indirect immunofluorescence test, but gave weaker reaction than the standard strain. One of the spirochete strains which could be visualized by dark-field microscope, had also been seen in the culture medium but it was then lost during the serial passages. Cultivation was successful from three other ixodes females, but only two of them (Mk5 and Mk6) grew well enough for further analysis. The third one (GyK4) was heavily contaminated. All the cultivated strains gave a strong reaction with IFA. The antigenic pattern was analysed by Western blot (Fig. 30). Both of them produced strong reaction with all the patients' sera in the characteristic 60 and 41 kDa molecular weight range. A slight difference of the antigenic pattern could be detected between the two strains. Strain Mk6 showed more bands in the 41-60 kDa region. The specific band of 32 kDa OspA gave a slight reaction only, and the 35 kDa molecular weight OspB could not be visualized.

Spirochetes could not be detected in the few collected haemaphysalis ticks.

DISCUSSION

Epidemiology

The prevalence of Lb in Hungary is impossible to determine yet. The fact that most of the patients had been seen by 4-6 doctors till the correct diagnosis was established, suggests that Lb is still underestimated in Hungary. On the other hand, twice as much patients were tested in 1989 than in the previous year yet the number of Lb cases showed only a small increase. On the whole, one-third of the 3304 patients was proved to have Lb. This proportion corresponds to the findings of the Centers for Disease Control (CDC) in U.S.A. (22). Our data are comparable with those, because we followed the case definition of the CDC (23).

Lb occurs in all regions of Hungary where the vectors are abundant. The morbidity data of some areas of the U.S.A., where 1-10% of the population becomes infected in every year (45, 80, 145), are probably not approached in Hungary. Ticks – especially the immature forms – may hardly be noticed. It is important to note that the tick bite remains unrecognized in many cases (17, 149, 166). In spite of this, a strikingly big portion of our ECM patients (80%) noticed tick bite. Of the 231 ECM cases reported by Ásbrink (6) 38 per cent recall tick bite.

It is a widespread opinion that the American Lb differs from the European one. Lyme-arthritis has not been recognized in Europe for a long time, while only a few cases of neuroborreliosis were diagnosed in the U.S.A. (22, 130). There is a significant difference in the distribution of Lb symptoms between the European countries. Half of the patients has neurological involvement in the northern part of Europe

(Sweden, Norway and the Netherlands), while in the southern countries most of the patients have ECM (160). It has been proved that there is some difference between the Bb strains isolated in the U.S.A. and in Europe (162), and this may account for the difference in the clinical picture between the two continents.

The state of medical knowledge may be a major factor in the epidemiological data. Steere was a rheumatologist who described the disease while he was looking for the origin of an arthritis epidemic. So the attention of the American physicians was drawn to the arthritis. The first cases in Europe were described by neurologists (1, 2, 167), who had realized that chronic meningitis responded to penicillin treatment. ECM as well as ACA have also been known in Europe, since the early descriptions of Afzelius and Herxheimer. Very probably, neurological and dermatological manifestations overshadowed arthritis in Europe.

The first recognized Hungarian Lb cases represented the wide range of Lb symptoms (71). So Hungarian physicians were informed of the main manifestations: ECM, neuroborreliosis and arthritis. Since our laboratory was the only one in Hungary where serological test was available in 1986-1989, our position was exceptional from the epidemiological point of view. Patients representing the whole spectrum of Lb have been sent to our laboratory from every region of Hungary (75). It is unique that the clinical and laboratory work has remained in one hand for a long time.

The distribution of the different symptoms of Lb in Hungary was found to be halfway between the two extremes described in Europe and in the U.S.A. (11, 22, 129). Half of our patients had ECM, one-third had neuroborreliosis, but arthritis was the leading symptom in 20%. The frequency of Lyme-arthritis is especially remarkable when the complications of ECM are analysed separately: the number of arthritis cases is almost equal to the number of patients with neurological involvement.

Epidemiological studies based on big populations described an age distribution similar to ours: an accumulation of cases was found in the age ranges of 5-15 and 30-50 years (21, 22, 44, 107, 129, 138). Strikingly different data can be obtained when the ratio of Lb cases within the total number of tested patients is calculated. Predominance of the youngest and oldest age groups was found in this way. This phenomenon can be explained by the fact that many of the characteristic symptoms of Lb (e.g. headache, paraesthesia, arthralgia) are rarely recognized in the youngest age groups, or are thought to be a consequence of degenerative illnesses in the older ages (156).

Clinical picture

Erythema chronicum migrans

It starts shortly after tick bite. The longest incubation time was four months in our cases, the shortest was 48 hours: this corresponds to data of the literature (6, 158). Because of the usually short incubation period, ECM is unambiguously a seasonal disease but it may occur in the winter months, too (158). A female and child

predominance was remarkable in our material. A female predominance was also described by others (6, 138). In children, the monosymptomatic appearance is characteristic, while complications more often develop in adults. This phenomenon can be explained by that women may rather visit a doctor with a simple "cosmetic" problem. Children are examined more thoroughly and their illness may be recognized at the early stage. The clinical picture is characteristic and may be problematic to judge in only few cases. In most of our patients, ECM was not accompanied by other symptoms, apart from a mild local pain or disaesthesia. Multiple ECM was observed in eight per cent by Åsbrink (6), and with an even greater frequency in the U.S.A. (11). In an early Hungarian study (151), multiple ECM was found in 23% of all cases. This figure decreased to half in the present study. The diagnosis of multiple ECM may sometimes be difficult, but in Lb only a few rings appear. It is important to emphasize that it is not worth to wait for serological test results in a doubtful case: the prompt response to antibiotic treatment supports the diagnosis.

Two-thirds of our ECM cases were presented without any joint symptoms. General symptoms have been mentioned with varying frequency, 15-70% (6, 110, 158). High fever, rash, sore throat, cough, abdominal pain, vomiting, loss of weight have been observed (11). We did not find such symptoms. Fever was rarely observed and high fever never occurred in our cases.

Although the number of complications increases with time that elapses to the therapy, those American data (145) according to which all of the untreated ECM patients would later develop arthritis are certainly not true for the Hungarian cases. In our experience, complications develop only in a small portion of the untreated ECM patients. Significantly more complications appear in seropositives than in seronegatives during the time elapsing from ECM to treatment. The risk of complication in a case of a seronegative ECM which started five months ago is not higher than that in an acute but seropositive one. Our data suggest that things are determined at the moment of infection. A similar observation was described by Steere (147): he rarely observed arthritis in cases of untreated ECM but with low antibody titer. Spontaneous recovery may take several weeks or months. In one of our patients, ECM recurred over a year. Åsbrink (6) reported an average course of ten weeks. Sixty per cent of our untreated cases recovered within one month.

Lymphadenosis benigna cutis (LBC)

LBC is a rare form of Lb (130). Most of our LBC patients were children. Of the two possible main localizations (breasts, earlobes) we have seen the latter one only. Of the nine patients reported by Hovmark et al. (57), only two were children. Both of them had LBC on the ears. In the elderly, LBC developed on the breasts. Mainly children with LBC in the ears were reported by Weber and Neubert (158). Spontaneous recovery may take up to one year. LBC was preceded by ECM in one, and by other major complications in two children. ECM and facial palsy appeared on the same side as LBC developed.

Carditis

Carditis is a rare form of Lb (150) but the only manifestation which may cause death (97). The first European case of Lyme carditis was described in 1984 (56). Less than two per cent of our cases had heart involvement. In childhood, it is even less frequently observed (11), we found only three children with ECG disturbances. In a report summarizing 66 cases of European Lyme carditis (154), a strong male predominance was found, comparable with our results. Fluctuating AV block was the characteristic symptom in our cases. The usually supraventricular rhythm disturbances may change rapidly (141, 154, 168) and the AV block may lead to asystolia (127, 153). Temporary pacemaker treatment was needed in three of our cases. Pacemaker implantation may be needed in 20-30% of carditis patients (4, 141, 154). Pericarditis is rare, but a little pericardial fluid may be disclosed by echocardiography (46, 85, 141). The incubation time was about one month in our cases. Although chronic carditis has been reported in many publications (30, 48, 49, 137), the course was usually benign and spontaneous improvement was documented in many of our patients.

Neurological involvement

BS combines all signs of neuroborreliosis: CNS involvement, cranial and peripheral nerve neuritis. All manifestations which cannot be included in this group can be considered as a *forme frust* of BS. Facial palsy was discussed separately because of its predominance. The other cranial nerves were rarely involved.

The detailed discussion of BS is explained by the fact that this manifestation is so characteristic that it can be diagnosed on the basis of the case history, even without examining the patient. Migrating paraesthesia, sometimes severe radicular pains and fatigue are the typical signs (2, 117, 155, 167). The symptoms are often vague and the patients may be misdiagnosed of having a psychosomatic disease. The objective origin of the symptoms would be disclosed by neurophysiological tests (41, 43). In the other group of patients, symptoms resemble vertebral disc herniation (32, 104). Typical BS was seldom accompanied by profound neck stiffness or other meningeal signs.

The CSF findings of neuroborreliosis are usually considered to be uncharacteristic. Cell counts of 1-200 are generally reported (44, 50, 66). In the present study, we have noted a higher upper range, with values above 1000 cells/mm³ in 3.4% of the patients. The presence of plasmocytes in the CSF has been mentioned in few previous studies only (66, 149), while in the present study it was shown to be a regular finding in the carefully examined CSF samples. Unfortunately, in most cases of Lyme meningitis the results were given only as "lymphocytic", or "mononuclear cells in the CSF". Although only 14 CSF samples of Lyme meningitis were examined carefully, 9 out of the 14 contained at least 5% plasmocytes.

In Lyme meningitis, CSF protein is stated to be almost invariably elevated (2, 105, 114). Also in our material, the CSF protein level was significantly higher in

Lyme meningitis cases than in controls. Elevation of protein levels was even more striking in Bannwarth's syndrome: only one of our cases had normal CSF protein.

In Lyme meningitis, CSF glucose is generally either stated to be normal in a majority of previous studies or not mentioned at all (2, 39, 63, 112, 114, 125). Low CSF sugar has only been reported by a few investigators (12, 31, 149).

Our data show that in Lyme meningitis, especially in Bannwarth's syndrome, significantly more patients have low CSF glucose levels than meningitis controls. The selection of controls may influence the statistical analysis, especially since patients with tick-borne encephalitis are known to have elevated CSF sugar levels. In our control material there was, however, no difference in CSF sugar content between the tick-borne encephalitis cases and the other members of the control group.

The combination of chronic lymphocytic pleocytosis + elevated CSF protein + decreased glucose content is generally considered to be unique for tuberculous meningitis. Our results show that this rare CSF profile is also a regular finding in Lyme meningitis. Especially in a case of chronic lymphocytic meningitis accompanied by radiculoneuritis, the probability of borrelia infection is very high. In our material, four cases of typical Bannwarth's syndrome had been treated with antituberculous drugs for weeks until the correct diagnosis of borrelia infection was established. Although antituberculous treatment will have to be given if mycobacterial infection is suspected, the differential diagnosis of borrelia infection must be kept in mind in endemic areas.

It is important to emphasize that pleocytosis in the CSF is one of the diagnostic criteria of BS, but CNS involvement caused by Bb may occur with a mild CSF abnormality or even without a pathological CSF finding. A case of peripheral neuritis need not be accompanied by CSF disturbances (43, 103, 165).

Peripheral facial palsy is a frequent symptom of LB (106, 115, 149). We tried to find a dividing line between the idiopathic and borrelia induced Bell's palsy. In our facial palsy material which consisted of 654 cases, 25.4% proved to have borrelia infection. A lower positivity ratio was reported by Jonsson (62) and Olsson (111), 16 and 20%, respectively. We could prove statistically that the bilateral facial palsy is very probably caused by Bb. A high incidence of bilateral palsy among Lb cases has also been reported by others (24, 106, 114). The relapsing cases of facial palsy – especially if the relapse was multiple or occurred more than 6 months apart – were usually seronegative. The most typical hallmark of borrelia infection is a facial palsy accompanied by meningitis. The probability of borrelia origin is about 60% in that case.

Lyme meningitis differed from other lymphocytic meningitis cases by its longer course and by the lack of meningeal signs. Only a few cases showed sensory loss or convulsion. These "conventional" encephalitic symptoms seem to be infrequent in neuroborreliosis but a few similar case reports have been published by others (33, 36). Five per cent of our cases showed memory impairment that could be measured by neuropsychiatric or neurophysiological examinations (43, 128).

Peripheral neuritis is a typical manifestation of neuroborreliosis. Both mild paraesthesia and serious lancinating neuralgia can occur. One of our patients was

operated on (laminectomy was performed) because his neurological symptoms mimicked vertebral disc herniation. Similar cases have been reported (67, 104). Although the motoneuron damage rarely resulted in complete palsy, irreversible muscular atrophy developed in two of our patients. Mild and latent paresis was more frequently found. In many cases, neuritis appeared shortly after tick bite or by the appearance of ECM, but all the ACA patients complained of similar symptoms. This is a clear evidence that polyneuritis can be both an early and a late manifestation of Lb (55).

The first case of progressive borreliosis was described by Pachner and Steere in 1985 (114) and Ackermann reported on 8 patients (1). We found a similar case in the following year. Since then, a lot of patients and various symptoms have been reported as having late or progressive borreliosis. Although there is no connection between Lb and multiple sclerosis (25, 123, 131), the clinical symptoms sometimes may be similar (1, 31, 67, 73, 165). The MRI result can also be similar in both illnesses (39, 42, 126, 165). The diagnosis may be difficult because EEG and CSF findings can be normal in chronic neuroborreliosis (40).

Halperin classified the basic types of late neuroborreliosis (43). Memory loss and impairment of cognitive functions were consistently found in these cases, even in the absence of "organic" signs of neurologic disorder. Mild peripheral neuropathies were demonstrated by electrophysiologic methods. Severe dementia and MS-like symptoms (21, 92) are exceptional and extreme forms of late neuroborreliosis.

Arthritis

In a few cases, arthritis may appear in the early stage, at the same time as ECM. Knees are typically involved in Lyme arthritis (51, 53, 54, 144, 147). In our patients, the large joint manifestations appeared typically after a short incubation period. Small joint polyarthritis is frequently found after a longer latency. Arthritis can be the sole manifestation of Lb (34, 51) and can mimic septic arthritis (34, 59), juvenile rheumatoid arthritis (82) or Reiter's syndrome (81). Involvement of the temporomandibular joint is typical of Lyme arthritis (52, 144, 147). Beyond several clinical symptoms of LB, *B. burgdorferi* infections remains inapparent in many cases (3).

Acrodermatitis chronica atrophicans

ACA has the longest latency period. The pathogen can be cultivated from a biopsy specimen drawn from an area where ACA has existed for as long as 30 years (6). It is a manifestation typical of adulthood (7, 8, 138). There is a clear female predominance (55). ACA is the only form of Lb in which no spontaneous remission occurs. The bluish-red oedematous dermatitis is usually thought to be of vascular origin. Sometimes morphea-like morphology appears (8, 83).



Photo 1. *Borrelia burgdorferi* with different staining in histological sections
(J. De Koning)

Photo 2-6. Different manifestations of erythema chronicum migrans
(author's photos)

Stages of Lyme borreliosis

The course of Lb can be divided into stages. ECM is the first stage, the second one is characterized by neurological and cardiac symptoms, while the third stage is equal to arthritis (135, 152). Our data show that it is very difficult to fit the various manifestations into stages. ECM and ACA are exceptions. Although ECM may have signs indicative of systemic infection, our data suggest that ECM remains a localized infection in most patients even when it is left untreated. The other extreme is ACA. It is a chronic, progressive illness in each case. To fit organ manifestations into stages is questionable because all the other symptoms may have acute, subacute and chronic forms.

The incubation period can influence the appearance of symptoms. For example, in ACA the long latency period is coupled with a chronic progressive course. The same phenomenon can be observed during the early period of ECM. The longer the time that elapsed after tick bite, the higher the risk of complications. Similarly, acute BS has a tendency for spontaneous recovery, while symptoms appearing after a longer period – e.g. ACA-associated polyneuritis – have a chronic progressive course. Arthritis is also a good example: there seems to be a shift from the large joints to the small ones as the incubation period lengthens. Large joint arthritis has a fluctuating but basically benign outcome, while small joint arthritis is a progressive and always chronic manifestation of Lb.

Theoretically, this phenomenon may be explained by the fact that Bb must adapt to the host and this takes time. Adaptation can also be seen *in vitro*. For example, a strain which had grown well in our own culture medium could hardly be multiplied in the Statens Seruminstitut. The normal Bb concentration could only be achieved after the 4th-5th passage. Inversely, a Danish strain, growing well in the Danish culture medium, could be cultured effectively in Hungary only after several passages. The same phenomenon is likely to exist *in vivo*. Bb isolated from ACA grew slower than another strain cultured from ECM (6). There are three possibilities concerning the relationship between the pathogen and its host. If the adaptability of the spirochete is poor, Bb remains in the skin and will invariably be eliminated, even in untreated patients. In rapidly generalized infections with acute onset and a usually benign outcome the host's defense mechanisms overcome the pathogen. The third possibility is the case of the slowly adapting Bb. During the long adaptation period the microbe "learns" how to evade the host's defence mechanism.

Serology

Our data show that IFA is a reasonably good method for borrelia antibody testing (77). Especially in the early cases it can be false negative because of the low antibody titer, and in the treated cases no seroconversion can be detected. Sometimes it is obviously false positive, but high titers are seldom misleading in the everyday clinical practice. Of course, this statement holds true only of the well controlled methods. A technique which gives a false positive result in 20% of cerebrovascular diseases or in multiple sclerosis (100, 101) is unsuitable for diagnostic purposes,

similarly to that which indicated a 30% seropositivity ratio for healthy blood donors (108).

Borrelia antibody testing in the CSF has higher sensitivity and specificity in neuroborreliosis (64, 148, 149). Intrathecal production of *borrelia*-specific antibody can be detected in almost every case (9, 98, 107, 163). However, it must be remembered that intrathecally produced antibodies against irrelevant viruses are frequently found in multiple sclerosis (37). That is why intrathecal production of *borrelia* antibodies should not be accepted as a proof of neuroborreliosis without some reserve (113, 123).

All of the serological methods give false positive or false negative results in some cases (65, 94, 96, 98, 116, 122). Which factors exert any influence on seropositivity? One of them is time. The highest antibody titers can be measured in the late form of Lb, especially in ACA. On the other hand, only 35% of ECM patients had elevated antibody titers. Seroconversion usually occurred 4-6 weeks after the first symptoms of infection had appeared. Another major factor influencing seropositivity is the generalization of infection. The chance of generalization is increasing with time but generalization does not take place in every case.

The appearance of *borrelia* antibodies takes at least 6 weeks (95, 134). Three weeks after the onset of ECM only an IgM response can be detected even by the very sensitive capture ELISA (13). In spite of this fact, two weeks (!) after tick bite we found elevated IgG titers in a considerable portion of patients. Sixty per cent of this fast-responding group had ECM, so the probability of false positivity is low. We are convinced that fast antibody response seen in a certain part of the patients supports our opinion mentioned earlier, namely that the outcome of the infection is determined soon after the tick bite. Fast response is a sign of systemic infection even in cases when clinical symptoms are missing. This theory is supported by the results of an animal experiment in which *intraperitoneally* infected mice (generalized infection from the first minute) produced IgG antibodies on the 5th (!) day (10).

Antibody response has been shown to be prevented by antibiotic treatment. No one knows the reason why no antibody production is induced against Bb surviving the antibiotic treatment (29, 133).

Therapy

Several years before the first isolation of Bb, some investigators suspected that the causative agent of ECM and BS was a penicillin-sensitive spirochete. The first Lb cases were treated with antibiotics suitable for the treatment of syphilis. Steere administered 250,000 IU of penicillin G or 250 mg tetracycline or erythromycin q.i.d. against ECM. Although the last one proved to be ineffective, the others significantly shortened the illness and reduced the risk of complications. Arthritis developed in one-third of the penicillin treated patients, and the joint symptoms were less severe than in the untreated group (140). When the unstable penicillin-G was exchanged with phenoxymethylpenicillin, a compound of better absorptive properties, complications occurred in less than 10% of patients (142). Penicillin adminis-

tered intravenously at a dose of 20 million units proved to be effective both in neuroborreliosis and in arthritis (143, 146). None of the 132 ECM patients treated orally with 2x1 g phenoxymethylpenicillin developed any complications, while 20% of the placebo-treated group produced progressive symptoms (6). Similar results have been published by others (14, 110, 136), but therapeutic failures have also been observed both in penicillin- and in tetracycline-treated patients (26, 33, 68, 70, 120, 157).

Penicillin treatment was highly effective in our ECM patients as well. A significantly faster improvement was seen in the penicillin- than in the tetracycline- (usually doxycycline-) treated group. The rate of improvement, is even more influenced by the dosage of the antibiotic. Low-dose administration can hardly shorten the duration of erythema chronicum migrans, while high-dose treatment is significantly more effective. Residual symptoms were seen only in 10% of our cases. In contrast to the excellent clinical response seen by us, *in vitro* antibiotic susceptibility results show that Bb is almost resistant to penicillin (!) but sensitive to tetracycline and even more sensitive to erythromycin and ceftriaxone (60, 121). It seems to be absurd that an *in vitro* resistant microbe appears sensitive *in vivo*. How can that be?! Our preliminary results also support the opinion of others that traditional MIC and MBC values cannot be used in the case of very slowly multiplying microbes such as Bb (109). This is why erythromycin, a drug showing excellent efficacy *in vitro*, proves ineffective *in vivo* (58). Even in an animal model experiment in which erythromycin was administered at a dose 20 times the human dose, it was not able to kill Bb (61, 121). The *in vitro* results were virtually proved by Johnson's animal experiment. As much as 200 mg/kg penicillin was administered and 90% of the hamsters remained infected. However, the antibiotic was given only once a day and only for 5 days (60)! Hansen applied a treatment model similar to the human practice and treated infected gerbils with penicillin at a dose of 100 mg/kg t.i.d. for 10 days. All treated animals became free from borrelia (47)!

To achieve an appropriate CSF level, doxycycline should be given at a dose of 200 mg b.i.d. (5, 169), which is hardly tolerable. Because of its bacteriostatic effect, microbes are able to survive quite a long treatment and start to multiply again (91). Orally or intramuscularly administered penicillin does not get into the CSF but a high antibiotic level can be achieved in the serum (86). A minimum incubation time of 72 hours is needed to kill borrelia (91). For that reason, orally administered penicillin can be recommended only for the treatment of early ECM. In our experience, high doses (4x1 g *per os*) of penamercillinum are used. That is well tolerated and highly sufficient to prevent the development of further symptoms and to cause prompt improvement. At the later stages of Lb, only the intravenously administered antibiotics are effective. Intravenous administration of penicillin at a dose 3x6 g for 14 days seemed to be an effective treatment of systemic borrelia infection. Our opinion is also supported by the results of some other *in vitro* studies (15, 91). Laboratory and human clinical studies are agreed on that ceftriaxone is more effective than penicillin (27, 28, 60, 89, 90, 159). Rocephin treatment is convenient because of the once-a-day administration. This antibiotic is usually well tolerated and results in a

relatively high CSF level. As we have only few patients who receive ceftriaxone monotherapy, we could not collect enough data for statistical analysis. In the present circumstances we do not think of Rocephin as a first choice of treatment. A similar conclusion was drawn by Neu (109).

Ticks, borrelia isolation and prevention

At least 20 different diseases are transmitted to humans by ticks (38), the commonest being Lb (139). The primary vectors of the etiologic agent of Lb are hard-bodied ticks belonging to the genus *Ixodes*. *Ixodes dammini*, *I. scapularis*, *I. pacificus*, *I. dentatus*, *I. persulcatus* and, in Europe, *I. ricinus* are the main vectors. Although other blood sucking insects have also been incriminated in the transmission of Bb, there are no epidemiological data to support this theory. Lb is becoming more prevalent because of the increased exposure of humans to infected ticks. Humans may be attacked by ticks even indoors after the pets have carried the ticks inside.

During their two-year life cycle, ixodid ticks develop through four stages of growth, namely egg, larva, nymph and adult. Each stage (except egg) feeds only once upon blood from a host animal. In the U.S.A. the white footed mouse (*Peromyscus leucopus*) is a particularly important host. In Europe, several small rodents are considered as a natural host of Bb. As natural hosts of ticks, birds play an important role in spreading ticks in new areas. All stages feed on humans.

Sixty per cent of ticks collected in Shelter Island, a highly infected area, contained Bb (18). In the Swiss Plateau an infection rate of 36% was found (19). On well-kept lawns, near the homes of Lyme disease patients a tick prevalence of approximately 1 per m² was found. Thirty-three per cent of nymphs and 55% of the adult ticks contained spirochetes (35). Thirty-four per cent of *Ixodes persulcatus* ticks examined in the Leningrad area was infected by Bb (69). In the most severely infected region of Germany 40% of the ticks harboured spirochetes (164). Similar prevalence was found in other parts of Europe (138). In contrast, less than 1% of ticks contains the tick-borne encephalitis virus, even in the hyperendemic regions (124).

We have dissected only 31 *Ixodes ricinus* ticks: six (16%) of them contained spirochetes. Light and electron-microscopic examination, IFA and Western blot analysis proved that the isolated strains are the causative agents of Lb. Both strains that could be studied (Mk5 and Mk6) showed the characteristic 41 kDa flagellar and the 60 kDa common bacterial antigens. Only a faint band was demonstrated in the region of 32 kDa OspA, which is typical of the American isolates. The 35 kDa OspB was missing, typically of the European strains (162). Our data are not sufficient to judge the Hungarian infection rate but very probably it is similar to the European situation.

The direct fluorescent technique was reported to be better for detecting Bb in ticks (118). In our hands, dark field microscopy and cultivation were more successful than the former method. Haemaphysalis ticks are seldom infected (20, 79), so our failure to find specimens harbouring borrelia is not surprising.

In an experimental animal model, Piesman et al. found that when infected ticks were removed within 24 hours after attachment, the risk of borrelia transmission was very low, while 72 hours later it reached 100% (119). Although nymphs are seldom infected, because of their tiny size they play an important role in borrelia transmission (93).

Prevention

Wearing closed clothes is frequently recommended for personal prophylaxis. To take this advice is almost impossible in hot weather, and its benefit is also questionable. Ticks seldom if ever bite on the naked parts of the body. They do not like climbing up trees and never throw themselves on the victim but can easily be collected from grass and bushes. Ticks are encountered on well-maintained lawns as frequently as in forests.

Personal protection. Repellents applied to clothing including the insides of trouser cuffs and socks prevent tick bite. Permethrin has been shown to provide excellent protection from tick bites. There are also repellents that do not kill ticks when impregnated into clothing such as N,N-diethyl-m-toluidide ("SZUKU", "Protect B"). The taping of trouser cuffs and tucking them in the footwear prevent ticks from easily crawling onto the untreated skin inside the clothing. Ticks are more easily observed on light- than on dark-coloured clothing. Repellents applied to the skin may help prevent the attachment of ticks but their effectiveness is lost within a few hours after application.

Tick attachment and removal. Ticks attach to their hosts by piercing the skin and inserting the hypostome with its recurved denticles. A cement-like protein mixture, which is secreted by the tick, and the anchoring hypostome hold the tick firmly in position. Ticks may be removed by grasping them as close to the skin as possible with a pair of forceps or tweezers. A steady upward pressure is exerted to detach the tick from the skin. An antiseptic may be applied onto the skin. Humans visiting tick-infested areas should periodically and thoroughly inspect their body and promptly remove ticks. Nymphs feed on their hosts for 4-7 days and adults for 8-11 days. Ticks are most likely to transmit spirochetes after they have fed for more than 48 hours. Prompt removal of ticks clearly diminishes the risk of infection. Ticks to be removed should never be greased with petrol, paraffin, body milk or any other chemical. During removal one should keep in mind that the tick's gut may be filled with borrelia. Any technique that may cause regurgitation can be dangerous.

Eradication of ticks would be more effective than personal protection. Although drastic methods have been applied, their effect remained transient (132). A method of lasting effect and safe the environment has been developed by Mather et al. (99).

CONCLUSION

This analysis is based on data collected for five years. Together with our co-workers, we described the first Hungarian cases and all the important clinical manifestations during that period.

Some fairly new data have been derived from the statistical analysis. One is that the incubation period may influence the appearance of clinical symptoms. The longer the period between tick bite and the onset of ECM, the higher the probability of complications. In cases when incubation is longer, symptoms present themselves less rapidly but there is bigger chance of a more serious chronic progressive course. Peripheral neuritis, memory loss and mental disturbances, as well as small joint arthritis are more common after a longer latency period.

We are convinced that the outcome of infection is determined soon after the tick bite. Fast antibody response is a sign of systemic infection even when clinical symptoms are still absent.

Physicians should be familiar with the symptoms and signs of Lb and aware of the possibility of both false negative and false positive serological results. Flagellar antigen ELISA is more sensitive than IFA in the early cases.

Facial palsy, when it is bilateral or accompanied by meningitis, is very probably caused by Bb. The relapsing cases are usually idiopathic. Facial palsy caused by Bb has a more serious onset but a better outcome in comparison with the idiopathic forms.

In Bannwarth's syndrome, not only high CSF protein but also low CSF sugar is frequently found. The combination of chronic lymphocytic pleocytosis + elevated CSF protein + decreased glucose content is generally considered to be typical of tuberculous meningitis. Our results show that this rare CSF profile is a regular finding also in Lyme meningitis. Especially in cases of chronic lymphocytic meningitis accompanied by radiculoneuritis, the probability of borrelia infection is very high.

In spite of the generally accepted opinion, in our experience Lb cannot be assigned to stages on the basis of the organ manifestations. All the organ manifestations (e.g. dermatological, cardiological, neurological and rheumatological) have acute, subacute, chronic and chronic progressive forms.

The efficacy of penicillin and tetracycline was compared in ECM patients. A significantly faster improvement was seen in the penicillin- than in the tetracycline- (usually doxycycline-) treated group. The rate of improvement was even more influenced by the dosage regime of these antibiotics. Low-dose administration can hardly shorten the duration of erythema chronicum migrans, while high-dose treatment is significantly more effective.

Half of the Hungarian Lb cases presented with ECM, one third had neurological symptoms and 18% showed frank arthritis. All the tick-infested territories of Hungary are infected by Bb. The largest number of Lb cases were bitten by ticks in Western Hungary, along the northern coastline of Lake Balaton and in the mountains of Buda.

ECM – particularly the monosymptomatic form – occurs more frequently in childhood and in females. Lb may occur in all age groups. Bannwarth's syndrome is more frequent in men than in women. Acrodermatitis occurs only in adulthood.

Lb is one of the most important clinical and bacteriological discoveries of the last decades. Many fields of medicine are involved in the protean disease. Lb is prevalent in Hungary. Its outcome depends mainly on the physician's knowledge: to cure a long-lasting, chronic illness only the correct diagnosis should be established and the appropriate treatment administered.

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Lakos A.: Lyme borreliosis Magyarországon, 1984-1989

A Lyme borreliosis (Lb) az utóbbi évtizedek egyik legfontosabb klinikai és bakteriológiai felfedezése. A kórokozó, a *Borrelia burgdorferi* 1982-es izolálása számos kórkép eredetének tisztázásához vezetett. Európában és Észak-Amerikában az Lb a leggyakoribb kullancs által terjesztett betegség. A jelen feldolgozás célja a klinikum és a hazai epidemiológia leírása. A szerző 1984-től 1989-ig 1175 beteg diagnosztizálásában vett részt. A betegek 44%-ának volt az Lb-re jellemző erythema chronicum migrans, 30%-uknak idegrendszeri, 25%-nak reumatológiai tünetei voltak. A közlemény ezen betegek klinikai, terápiás és epidemiológiai sajátosságait tárgyalja.

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