Review

LYME BORRELIOSIS; A GENERAL SURVEY

G. Trevisan

SUMMARY

In this article a general review on Lyme borreliosis is given. The most important topics as ecology, clinical aspects, congenital borreliosis, risks in blood transfusions, diagnosis, differential diagnosis, course, prognosis and therapy are shortly discussed.

KEY WORDS
Lyme borreliosis, general survey

INTRODUCTION

Lyme disease was reported beginning in 1975 in the city of Lyme (Connecticut) by authors from Yale University who had studied a group of children affected by an unusual epidemic of arthritis (l); Willy Burgdorfer identified the etiologic agent in 1982 (2). Since then, many microbiological and clinical data have been collected and the Authors maintain that the disease has to be reconsidered and evaluated again. In Europe, two previously unknown Borrelia species, i.e. *B. garini* and *B. afzelii* causative of Lyme disease were recently identified (3, 4). They are responsible for late manifestations of the disease which differ in terms of frequency and severity from the typical manifestations caused by *Borrelia burgdorferi* (5).

Hence, Lyme disease is not caused by a single agent and therefore clinical and laboratory findings which were so far considered as the common denominator of the disease have to be reviewed. Erythema (chronicum) migrans (EM) is the first clinical manifestation: the lesions are found in the infections caused by the three Borrelia varieties under consideration. Is EM specific to Lyme disease or, conversely, is it to be found in other diseases? Generally speaking, in the areas endemic for Lyme disease EM is correlated with Borrelias infection; however, EM-like manifestations could not be correlated with Lyme borreliosis elsewhere. There might possibly be other varieties of Borrelia which cannot develop in culture mediums favourable to *Borrelia burgdorferi* and other viral agents.

ECOLOGY

Lyme disease is a zoonosis caused by Borrelia burgdorferi, a Spirochete borne by hematogenic Artropoda, i.e. Ixodidae ticks: the following species have been identified: *I. dammini* and *Dermacentor variabilis*, and *I. pacificus* in the U.S.A. on the West and East coast respectively (6, 7); *I. persulcatus*, in Asia and *I. ovatus* in Japan, *I. hololcyclus* in Australia and *I. ricinus* in Europe (6, 8, 9). There are other hematophagous insects which sometimes act as causative agents of the disease: fleas and mosquitos in the U.S.A., tabanids of the *Crisops caecutiens* variety in Austria and the ticks of the rock-pigeons (*Argas reflexus*) in the Trentino-Alto Adige region in Italy (8, 10, 11).

Numerous pathogens other than *Borrelia burgdorferi* are tick-borne: *Ixodes dammini* is implicated as vector of Babesia microti, i.e. protozoa causing babesiosis, and *Ixodes ricinus* has a role in transmitting tick-borne encephalitis virus.

Ixodidae ticks infest domestic mammals such as sheep, dogs and cats, as well as medium and small size wild mammals, birds and reptiles, having a remarkable ability to adjust to different climates, so that they are widely distributed (7). For humans to get contaminated there have to be carrier or reservoir animals: they are primarily large mammals such as deers, *Odocoileus virginianus* in the U.S.A., as well as fallow and roe deers which, being largely infested by ticks, can carry them to considerable distances (7).

According to most recent theories, the reservoir animals are small rodents as *Peromyscus leucopus* in the U.S.A. and *Clethrionomys glareolus* and *Apodemus flavicollis* in Europe (12). Although they are mainly found on mice and deers, ticks get nourishment from the blood of other animals as well, such as large size ungulata, as Tamias striatus, and birds or reptiles (7).

Humans are exposed to contamination in woods where Lyme disease is endemic: the ecological niches of Ixodidae are found in the warm-humid areas where underwood plants are abundant, at a height ranging from 600 to 1,200 meters, reaching as far as 1,500 m in cold climate countries such as Sweden (13).

Ticks, which are influenced by humidity, are highly active during spring and autumn when they can lay as much as 500-3,000 eggs. Until a few years ago humans rarely got infected: on the contrary, a progressive increase in the number of Lyme disease cases is reported nowadays as residential areas are built in the vicinity of woods and more recreational activities are carried out in the open air.

CLINICAL ASPECTS

Lyme borreliosis (LB) is classically described as having three clinical stages (14a) (Table I). Similarly to syphilis, the natural course of LB can be divided into early and late infection (14b, 14). The early infection correspond to the first stage; the late infection correspond to the second and third stage. According to skin manifestations the second stage may be further divided into two phases, the first and second efflorescences (14c). Recently, according to general and neurological symptoms a new classification has been proposed (14d). The disease spreads to different organs and systems: skin, joints, nervous system, heart and eyes are most frequently affected (13, 15, 16, 17).

The typical manifestation of the primary stage (Table II) is erythema chronicum migrans (ECM), which is sometimes associated with general symptoms typical of other infectious diseases: oligomyoarthralgias of short duration, headache and neck stiffness, fever, conjunctivitis, non productive cough, swelling of the testes, changes in some laboratory parameters such as elevated liver transaminases, proteinuria and haematuria. In the past, annular lesions where considered a typical manifestation of the first stage: on the contrary, at present such lesions are known to be a sign of the second stage. The second stage generally starts between the third and eighth month, namely after patients recovered from ECM; it lasts for 5-6 months and sometimes even longer.

The most significant manifestations are found in skin, nervous, myoarticular and heart systems (Table III).

Similarly to syphilis, two different stages, also called "efflorescences", are evident in the skin: the first is of early onset and multiple annular erythemas and recurrent urticariod rashes are present, which are often associated with disseminated micro-lymphoadenopathy. After 3-4 months the second efflorescence appears and the most typical skin manifestation is lymphadenosis benigna cutis (LABC).

The third stage does not start earlier than seven months from the advent of the infection: skin lesions are mainly of atrophic and sclerodermal nature and the extra-dermal involvement, severely affecting both joints and nerves is chronicized: there is no longer

Table I. Lyme borreliosis classifications

CLASSIC CLASSIFICATION:		
FIRST STAGE SECOND STAGE THIRD STAGE		Steere AC et al. (14a)
MODERN CLASSIFICATION:		
EARLY LYME BORRELIOSIS	First stage	
LATE LYME BORRELIOSIS	Second stage Third stage	Steere AC (14b)
CUTANEOUS CLASSIFICATION:		
FIRST STAGE SECOND STAGE THIRD STAGE	First efflorescence Second efflorescence	Trevisan G & Cinco M (14c)
NEUROLOGICAL CLASSIFICATION:		
- EARLY LB WITHOUT DISSEMINATION - EARLY LB WITH DISSEMINATION, INCLUDING GENERAL AND NEUROLOGICAL MANIFESTATIONS - LATE LB - ASYMPTOMATIC LB - LATE LYME ENCEPHALOPATHY		Schmutzhard E (14d)

Table II. Clinical manifestations of Lyme Borreliosis during the first stage

SKIN	Erythema (chronicum) migrans
JOINTS	Oligo-myoarthralgia
OTHER SYMPTOMS	Headache, stiff neck, regional lymphoadenopathy, fever, conjunctivitis

a clear-cut distinction between the features typical of this period and the manifestations of diseases of a different etiology (Table IV).

Late skin manifestations vary significantly from one country to the next. In the U.S.A., acrodermatitis chronica atrophicans (ACA) is particularly rare and imported from outside whereas in Europe it is frequently found in Sweden and Germany (18). In Italy, ACA is most frequently reported in areas endemic for the disease (Friuli, Liguria and Trentino) (11, 19). Further research is needed to shed light on the relationships with other sclerodermal and arthropodermal forms of the disease such as lichen

Table III: Clinical manifestations of Lyme Borreliosis during the second stage

FIRST EFFLORESCENCE:	
SKIN	multiple annular erythemas urticarioid rashes
JOINTS	myoarthralgias
OTHER SYMPTOMS	disseminated microlymphoadenopathy
SECOND EFFLORESCENCE:	
SKIN	lympadenosis benigna cutis, roseolar symptoms, urticaria
JOINTS	myoarthralgias, tendonitis, osteomyelitis
NERVOUS SYSTEM	meningitis, encephalitis, facial palsy, Garin-Bujardoux-Bannwarth syndrome, myelitis
LYMPHATIC SYSTEM	regional or general lymphoadenopathy, lymphomas, lymphoproliferative syndromes, megalosplenias
LIVER	hepatitis
HEART	A-V heart block, myocarditis, pancarditis
EYES	iritis, iridocyclitis, choroiditis, panophthalmitis, haemorrhage and retinal detachment
AIRWAYS	nonexudative angina, non productive cough
KIDNEYS	proteinuria, microhaematuria
GENITALIA	orchitis, softening of the testes
GENERAL SYMPTOMS	fever, malaise, prostration

Table IV: Clinical manifestations of Lyme Borreliosis during the third stage.

SKIN	acrodermatitis chronica atrophicans, lichen sclerosus et atrophicus, morphea, atrophoderma, Parry-Romberg facial hemiatrophy
JOINTS	chronic arthritis, bone calcifications and cysts
NERVOUS SYSTEM	chronic progressive encephalomyelitis, encephalopathy, multiple sclerosis-like syndrome, peripheral neuropathy, optic nerve atrophy, ataxia, pseudo tumor cerebri, psychiatric syndromes
EYES	keratitis
GENERAL SYMPTOMS	megalosplenia, lassitude

sclerosus et atrophicus (LSA) (20, 21), morphea (20, 21, 22, 23), scleroderma (24) artrophoderma of Pierini and Pasini (25) and Schulman's fasciitis (26, 27).

In the U.S.A., these affections and Lyme disease are apparently not correlated (28). Conversely, *Borrelia burgdorferi* was cultured from skin affected by circumscribed scleroderma in Austria and Germany (22, 29, 30). In Europe, sclerodermal manifestations are more frequently found correlated with Lyme disease than in America, most likely because Borrelia strains have a different distribution in the two continents (31). Etiology of scleroderma varies and Borrelia is apparently more frequently responsible

for it in localities where the disease is endemic, particularly if strains other than Borrelia burgdorferi sensu stricto are present.

BORRELIOSIS DURING PREGNANCY AND CONGENITAL BORRELIOSIS

Borreliae can cross the placental barrier and contaminate the conceptus during the first months of pregnancy (32, 33); the risk of contamination during the first three months of pregnancy is higher (34, 35). Schlesinger reported the first case of mother-fetus transmission in 1985 (35); in 1986, Mac Donald isolated Borrelias in the liver of aborted fetuses of infected mothers (32). In the past the Authors (36) carried out a retrospective study on the incidence of idiopathic miscarriages in a group of 91 women and reported positive serology; in some cases, patients previous history was positive for ECM during gestation or shortly before pregnancy. Malformations, fetal death in uterus, pre-term deliveries and rashes in the newborn are possible consequences of the infection during pregnancy (33, 34). Congenital Lyme disease is possible, though rare, and reports are scarce (33).

RISKS IN BLOOD TRANSFUSIONS

In theory, contamination secondary to transfusion of infected blood is possible, though extremely rare; some authors observed that *Borrelia burgdorferi* can survive for approximately 40 days in frozen plasma, 45 days in blood red cells and 6 days in the platelets whenever the common methods to preserve blood products are used in blood banks. In 1983, Benach et al. isolated spirochetes from blood specimens taken from two patients affected by LB (37); in 1989, Badon et al. reported that *B. burgdorferi* survives in blood products (38); in 1990, Johnson et al. reported that Borrelia burgdorferi can survive in blood treated for transfusions (39); in 1992, Mc Guire detected Borrelias in human blood using PCR and Bohme et al. reported that patients who received blood transfusions from patients with positive serology for LB but who had no clinical symptoms of the disease were not affected (40). Hence, even though the infection might be transmitted through blood transfusions, the risk of contamination is extremely low and contamination could only occur in case blood of recently infected patients is used.

DIAGNOSIS

Owing to the protean nature of Borreliosis, the diagnosis of clinically suspected Lyme disease must be substantiated by laboratory tests to differentiate it from other diseases.

Both direct and indirect methods can be used.

Direct methods are extremely useful during the early stages of the disease when the antibodies are not yet circulating or in case serum assays are negative. Direct methods are more expensive, less practical and take more time than indirect ones: however, the diagnosis is made in case Spirochetes are isolated from tissue cultures. False negatives are numerous.

Direct diagnosis rests upon the findings of histochemical, immunohistochemical, cultural and genetic hybridization assays on body tissues and fluids (41, 42, 43). Polymerase chain-reaction (PCR) using genetic amplification of Borrelia genome in vitro offers new opportunities (44, 45, 46).

Indirect diagnosis rests upon evidence of anti-borrelia antibodies in the skin, sera, CSF and sinovial fluid in affected subjects using indirect immunosorbent assay, immunofluorescence, enzyme-linked immunosorbent assay, immunoperoxidase on slides, microscopic agglutination, hemagglutination and Western blot tests (43, 47, 48).

Establishing the diagnosis on the findings of serum assays is particularly difficult: only the flagella antigen is found in the three Borrelia varieties, whereas other antigens vary.

When flagella antigens are looked for, false positives are more numerous.

DIFFERENTIAL DIAGNOSIS

Differentiating the clinical pictures from other diseases is necessary as LB is characterized by a polymorphous pathology. While differentiation of ECM from other lesions is rarely difficult, manifestations in the joints have to be carefully evaluated: all the more so as they have been mistaken for Still's disease for many years (49). Skin lesions have to be differentiated from collagenosis, reticulohystiocytosis, lymphoma, sarcoidosis, anetoderma and atrophoderma secondary to inflammatory processes or endocrinous disorders. Clinical pictures characterized by joint and/or neurological involvement, associated with fever and myalgias have to be differentiated

from fibromyalgic syndrome, dermatomyositis and other diseases where the cause of infection is unknown.

COURSE, PROGNOSIS AND THERAPY

Lyme disease course depends on a prompt diagnosis and antibiotic treatment: if treatment is promptly started at the advent of ECM, patients can often, even if not always, recover. The evolution of the disease is influenced by constitutional factors such as resistance to bacterial agents and adequate response of the immune system.

The quod vitam prognosis is generally good, with the exception of cardiac and neurological late complications which are lethal.

The treatment of Lyme Borreliosis is based on antibiotics and drugs to abate symptoms (24, 50): the choice must rest upon the clinical picture, stage of the disease, symptoms, age of the patient, sex and other concomitant factors, such as pregnancy. Lyme disease involves all systems and districts of the body: the evaluation of the action of drugs on Spirochetes and their therapeutic efficacy is mandatory.

Antibiotic treatment, even if correctly administered, does not always sterilize germs, which can be cultured from biopsies of skin which has apparently healed (51). Probably Borrelia can locate itself inside the cells and therefore minimum bactericidal dose (MBC) is to be preferred to minimum inhibiting concentration (MIC). When tetracyclines and some macrolide antibiotics with more than 14 atoms of carbon are used satisfactory results can be obtained (52, 53, 54).

Erythromycin is active in vitro at MIC 50 of 0.03 microg/ml and MIC 90 of 0.06 microg/ml; however, the in vivo activity is extremely scarce as the 14 C atoms structure does not allow a stable binding to be formed with the ribosomes of Borrelia (55). Macrolide antibiotics 15 and 16 membered allow a more stable ribosomal binding and are therefore much more active and effective in vivo.

Some patients affected by Lyme arthritis who do not respond to treatment as they have a flare-up of the disease, whose picture is sometimes very severe, have evidence of a particular type of HLA, and present DR4 or DR2 (56).

VACCINATION

A vaccine is being developed which has been used in animal experiments only (57). The vaccine may use the OspA surface antigens, as the antibodies against such proteins are important in the control infection of *Borrelia burgdorferi*. However, such a vaccine would only be used as a protection against *Borrelia burgdorferi sensu stricto* and not against all Borreliae. Unfortunately, mutations were reported so that preparing the vaccine is extremely difficult. At any rate, in the future vaccines will offer good opportunities in the prophylaxis in subjects exposed to risk.

REFERENCES

1. Steere AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, Ross MR, Steele FM: Lyme arthritis: An epidermic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 1977; 20: 7-17.

2. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP: Lyme disease: -a tick-borne Spirochetosis. Science 1982; 216: 1317-1319.

3. Baranton G, Postic D, Saint Girons I, Boerlin P, Piffaretti JC, Assous M, Grimont PA: Delineation of Borrelia burgdorferi sensu stricto, Borrelia garini sp. nov. and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol 1992; 42: 378-83.

4. Davidson B, MacDougall JH, Saint Girons I: Physical map of the linear chromosome of the bacterium Borrelia burgdorferi 212, the causative agent of Lyme disease. J Bacteriol 1992;174: 3766-3774.

5. Saint Girons L Old IG, Ojaimi C, MacDougall J, Davidson BE: Physical and genetic maps of the Borrelia burgdorferi sensu lato chromosomes. Ann Rheum Dis 1993; 52: 397.

6. Arthur DR. The Ecology of ticks with reference to the transmission of protozoa. In Soulsby EJL, Editor. Biology of parasites. New York: Academic Press 1966; 61-84.

7. Anderson JF. Epizootiology of Lyme Borreliosis. Scand J Infect Dis Suppl 1991; 77: 23-24.

8. Masuzawa T, Okada Y, Yanagihara Y, Sato N. Antigenic properties of Borrelia burgdorferi isolated

from Ixodes ovatus and Ixodes persulcatus in Hokkaido, Japan. J Clin Microbiol 1991; 29: 1568-1573.

9. Manilla G: Distribuzione delle zecche presenti in Italia (Acari ixodoidea). Fragmenta Entomologica 1986; 19: 57-81.

10. Magnarelli LA, Anderson JF. Ticks and biting insects infected with the etiologic agent of Lyme disease Borrelia burgdorferi. J Clin Microbiol 1988; 26: 1482-1486.

11. Trevisan G, Crovato F, Marcuccio C, Fumarola D, Scarpa C. Lyme disease in Italy. Zbl Bakt Hyg 1986; 263: 459-463.

12. Bosler E, Coleman JL, Benach JL, Massey DA, Rahan JP, Burgdorfer W, Barbour AG. Natural distribution of the Ixodes dammini spirochete. Science 1983; 220: 321-322.

13. Trevisan G, Cinco M. Lyme disease: a general survey. Int J Dermatol 1990; 29: 1-8.

14. Abele DC, Anders KH. The many faces and phases of borreliosis II. J Am Acad Dermatol 1990; 23: 401-410.

14a. Steere AC, Bartenhagen NH, Craft JE, et al. Clinical manifestations of Lyme disease. Zbl Bakt Hyg A 1986; 263: 201-206.

14b. Steere AC. Lyme disease. N Engl J Med 1989; 321: 586-596.

14c. Trevisan G, Cinco M. Lyme borreliosis in childhood. Eur J Pediat Dermatol 1992; 2: 81-112.

14d. Schmutzhard E. Manifestazioni neurologiche ed internistiche della Malattia di Lyme. (Abstract) 3° Convegno Internazionale "Malattie infettive nell'Arco Alpino", Siusi allo Sciliar, 24-26 marzo 1994, 27-28.

15. Cox J, Krajden M. Cardiovascular manifestations of Lyme disease. Am Heart J 1991;122:1449-1455.

16. Smith JL. Neuro-ocular Lyme borreliosis. Neurol Clin 1991; 9: 35-53.

17. Linde van der MR, Crijns HJGH. Aspects of Lyme carditis. Ann Rheum Dis 1993; 52:389.

18. Åsbrink E, Hovmark A, Olsson I. Clinical manifestations of acrodermatitis chronica atrophicans in 50 swedish patients. Zbl Bakt Hyg 1986; AT263: 253-261.

19. Trevisan G. Epidemiologia della borreliosi di Lyme nel Friuli-Venezia Giulia. Ann It Derm Clin Sperim 1990; 44:127-137.

20. Aberer E, Stanek G. Histological evidence for spirochetal origin of morphea and lichen sclerosus et atrophicans. Am J Dermopathol 1987; 9: 374-379.

21. Ross SA, Sanchez JL, Taboas JO. Spirochetal forms in the dermal lesions of morphea and lichen sclerosus et atrophicus. Am J Dermatopathol 1990; 12: 357-362.

22. Aberer E, Stanek G, Ertl M, Neumann R. Evidence for spirochetal origin of circumscribed scleroderma (morphea). Acta Derm Venereol (Stockh.) 1987; 67: 225-231.

23. Aberer E, Neumann R, Stanek G. Is localized scleroderma a Borrelia infection, Lancet 1985; ii: 278.

24. Berger BW, Johnson RC, Kodner C., Coleman L. Failure of Borrelia burgdorferi to survive in the skin of patients with antibiotic-treated Lyme disease. J Am Acad Dermatol 1992; 27: 34-37.

25. Berman A, Berman GD, Winkelmann RK. Atrophoderma (Pasini-Pierini). Findings on direct immunofluorescent, monoclonal antibody, and ultrastructural studies. Int J Dermatol 1988; 27: 487-490.

26. Sepp N, Schmutzhard E, Fritsch P. Shulman syndrome associated with Borrelia burgdorferi and complicated by carpal tunnel syndrome. J Am Acad Dermatol 1988; 18: 1361-1362.

27. Stanek G, Konrad K, Jung M, Ehringer H. Shulman Syndrome, a scleroderma subtype caused by Borrelia burgdorferi. Lancet 1987; i: 1490.

28. Tuffanelli DL, Tuffanelli DR, Hoke A. False positive Lyme antibody test in morphea. J Am Acad Dermatol 1993; 1: 112-113.

29. Åsbrink E, Hovmark A. Successful cultivation of spirochetes from skin lesions of patients with Erythema chronicum migrans Afzelius and Acrodermatitis chronica atrophicans. Acta Path Microbiol Immunol Scand Sect 1985; 93: 161-163.

30. Weber K, Preac-Mursic V, Reimers CD. Spirochetes isolated from two patients with morphea. Infection 1988:16: 29-30.

31. Stanek G, Wewalka G, Groh V, Neumann R, Kristoferitsch W. Differences between Lyme disease and European arthropod-borne Borrelia infections. Lancet 1985; i: 401.

32. Mc Donald AB: Human fetal borreliosis, toxemia of pregnancy and fetal death. Zbl Bakt Hyg 1986; 263: 189-200.

33. Edly SJ. Lyme disease during pregnancy. NJ Med 1990; 87: 557- 560.

34. Schutzer SE, Janninger CK, Schwartz RA. Lyme disease during pregnancy. Cutis 1991; 47: 267-268.

35. Schlesinger PA, Duray PH, Burke BA, Steere AC, Stillman MT, Stillman MT. Maternal-fetal transmission of the Lyme disease spirochete, Borrelia burgdorferi. Ann Int Med 1985; 103: 67-68.

36. Candussi G, Carlomagno G, Luksa V, Magaton Rizzi G, Trevisan G. Lyme Borrelia positive serology associated with spontaneous abortion in an endemic Italian area. Acta Europaea Fertilitatis 1988; 19: 279-281.

37. Benach JL Bosler EM, Hanrahan JP, Coleman JL, Habicht GS, Bast TF, Cameron DJ, Ziegler JL, Barbour AG, Burgdorfer W, Edelman R, Kaslow RA. Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med 1983, 308: 740-742.

38. Badon SJ, Fister RD, Cable RG. Survival of Borrelia burgdorferi in blood products. Transfusion 1989; 29: 581-583.

39. Johnson SE. Borrelia burgdorferi: survival in experimentally infected human blood processed for transfusion. J Infect Dis 1990; 162: 557-559.

40. Bohme M, Schwenecke S, Fuchs E, Wiebecke D, Karch H. Screening of blood donors and recipients for Borrelia burgdorferi antibodies: no evidence of B. burgdorferi infection transmitted by transfusion. Infusionsther Transfusionsmed 1992; 19: 204-207.

41. De Koning J, Bosma RB, Hoogkamp-Korstanje JAA. Demonstration of spirochaetes in patients with Lyme Disease with a modified silver stain. J Med Microbiol 1987; 33: 261-267.

42. Park HK, Jones BE, Barbour AG. Erythema Chronicum Migrans of Lyme Disease:diagnosis by monoclonal antibodies. J Am Acad Dermatol 1986; 15: 406-410.

43. Berardi VP, Weeks KE, Steere AC. Serodiagnosis of early Lyme disease: analysis of IgM and IgG responses using an antibody-capture enzyme immunoassay. J Infect Dis 1988;158:754-760.

44. Debue M. Detection of Borrelia burgdorferi in biological samples using the polymerase chain reaction assay. Res Microbiol 1991; 142: 565-572.

45. Guy EC, Stanek G. Detection of Borrelia burgdorferi in patients with Lyme disease by the polymerase chain reaction. J Clin Pathol 1991; 44: 610-611.

46. Rosa PA, Schwan T. A specific and sensitive assay for the Lyme disease spirochete, Borrelia burgdorferi using the Polymerase Chain Reaction. J Infect Dis 1989; 160: 1018-1029

47. Hansen K, Hindersson P, Strandberg Pedersen N. Measurement of antibodies to the Borrelia burgdorferi flagellum, improves serodiagnosis in Lyme disease. J Clin Microbiol 1988; 26: 338-346.

48. Hausen K. Lyme neuroborreliosis. Ann Rheum Dis 1993; 52: 389.

49. Cimmino M, Trevisan G. Lyme arthritis presenting as adult onset Still's disease. Clin Exper Rheumatol 1989; 7: 305-308.

50. Stiernstedt G. Therapeutic options on Lyme borreliosis. Ann Rheum Dis 1993; 52: 393.

51. Preac-Mursic V, Weber K, Pfister HW, et al. Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis. Infection 1989;17: 355-359.

52. Preac-Mursic V, Wilske B, Schierz G, Sa E, Groa B. Comparative antimicrobial activity of the new macrolides against Borrelia burgdorferi. Eur Jr J Clin Microbiol Infect Dis 1989; 8: 651- 653.

53. Harf R, Panterix G, Gaspar A, Leclerq M. Etude in vitro de la penetration de la josamycine dans les cellules phagocytaries humaines. Medicine et Maladies Infectieuses 1985; suppl: 46- 48.

54. Trevisan G, Cinco M. Efficacy of Josamycin in Lyme borreliosis treatment. J Chemother 1991; 4 (suppl.): 451-452.

55. van Rensburg CEJ, Anderson R. Intracellular antimicrobial activity of Josamycin and Etythromycin. Chemioterapia 1985; 4 suppl 2: 973-975

56. Steere AC, Dwyer E, Winchester R. Association of chronic Lyme disease with HLA-DR4 and HLA-DR2 alleles. N Engl J Med 1990; 323: 219-223.

57. Fikrig E, Barthold SW, Kantor FS, Flavell RA. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. Science 1991; 250: 553-556.

AUTHOR'S ADDRESS

Giusto Trevisan, M. D., professor of dermatology, Dept. of Dermatology, University of Trieste Ospedale di Cattinara, Strada per Fiume, 34149 Trieste-Italy