WHO WORKSHOP ON LYME BORRELIOSIS DIAGNOSIS AND SURVEILLANCE

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List of abbreviations

Ab        Antibodies
ACT       Acrodermatitis chronica atrophicans
B         Borrelia
BB        Borrelia burgdorferi
BL        Borrelia lymphocytoma
CDC       Centers for Disease Control, Atlanta, USA
CNS       Central nervous system
CSF       Cerebrospinal fluid
ECM       Erythema chronica migrans
EIA       Enzyme Immunoassay
ELISA     Enzyme-Linked Immunosorbent Assay
EM        Erythema migrans
I.         Ixodes
IFA       Immuno Fluorescence Antibody Test
IgG       Immunoglobulin G
IgM       Immunoglobulin M
LACB      Lymphadenosis cutis benigna
LB        Lyme Borreliosis
LD        Lyme Disease
Osp C     Outer surface protein C
Osp A     Outer surface protein A
PCR       Polymerase Chain Reaction
WB        Western-Blot-Test
1. INTRODUCTION

The WHO Regional Office for Europe and the Veterinary Public Health unit (VPH) at Headquarters have fostered research on the diagnosis and control of Lyme Borreliosis (LB) in humans and animals for many years (WHO/CDS/VPH/93.132, WHO/CDS/VPH/95.141, EUR/ICP/CDS 011, EUR/ICP/CDS 039, and EUR/ICP/OCD 010).

In order for efficient national and regional control strategies to be set up, development and implementation of a standardized diagnosis and surveillance of the disease is of utmost importance. To this end VPH and the Bacterial, Viral Diseases and Immunology unit (BVI) of WHO, jointly with the Ministry of Health, Poland, organized a workshop, the results of which are the subject of this report. The workshop had the following objectives:

- to promote the exchange of technical information and knowledge on strategies for the diagnosis and surveillance of LB in humans;
- to update/review knowledge on the prevalence of the disease in different parts of the world;
- to facilitate standardization of serological techniques for diagnosis of the disease in humans, and
- to identify future research needs in the field of:
  - laboratory diagnosis with emphasis on standardization of serological techniques;
  - national/international surveillance strategies of humans and animals, including clinical case definition.

Specialists (Annex 1: List of participants) on LB diagnosis and control in humans and animals were invited and they presented country reports on the four main subjects (Annex 2: List of papers presented).

- national reporting and surveillance system
- diagnostic procedures
- trends in prevalence in humans and animals
- research activities.

The country reports are available on request from the participants. An additional paper reported on the current activities within the European Lyme Borreliosis concerted action (Dr Gray).

After the presentation of the country reports the participants discussed in two groups:

- Standardization of serological tests for LB in humans
- National Surveillance and Diagnosis of LB
- Case Definition.

Recommendations on the above subjects were adopted during the closing plenary session of the workshop.

The participants wished to express their thanks and gratitude to WHO and the Ministry of Health, Poland, for the organization and conduct of the workshop.

2. COUNTRY REPORTS
(Abstracts)

Austria

There is no national reporting and surveillance system for LB in Austria. However, there is a very high standard of information about hazards of tick exposure in Austria due to the endemic existence of TBE.

The clinical manifestation rate after tick bites was found to be between 2-4%. The estimated annual average incidence of Lyme borreliosis is 300/100 000 inhabitants in Austria. The estimated average number of cases of Lyme borreliosis per year is 24 000 (total number of inhabitants: 8 000 000). Incidence of Lyme borreliosis varies in the nine states of Austria. In the eastern and southern states, Wien, Niederösterreich, Steiermark and Kärnten the estimated incidence reaches 350 cases /100 000 inhabitants. Burgenland, the most eastern state of the country, shows an incidence rate of 300/100 000. The same figure was found for Oberösterreich. Towards the western part of Austria, the incidence rate declines. However, Salzburg has a lower incidence (100/100 000) than Tyrol (250/100 000). Incidence is lowest in the most western state of Austria, Vorarlberg (50/100 000).
Erythema migrans (77.5%) and neurological disorders (15.7%) are the most frequent clinical manifestations of LB. However, it is speculated that Erythema migrans, counts for more than 90% of all cases in Austria.

Diagnostic procedures at the Institute for Hygiene, University of Vienna, comprise techniques including culturing, ELISA, immunoblot, and PCR. Both Borrelia afzelii and B. garinii are used for seroimmunological tests. Internal standardization is constantly performed; interpretation of test results is based on many clinical studies and experience accrued over more than a decade. However, various commercially available test systems are in use in the country.

Research on LB in Austria is done at the Medical Faculties of the Universities of Vienna, Graz, and Innsbruck and at the Institute of Biomedical Research, Immuno company. Work comprises studies of the clinical courses, therapeutic efficacy of various antimicrobials, pathophysiology, pathohistology, immunodiagnosis, molecular biology and diagnostic procedures. Work is partly done in collaboration with hospitals outside the universities and on international grounds. Additionally, field studies are being performed on the natural reservoirs of tick-borne pathogens including borreliae.

Czech Republic

Surveillance of LB cases in the Czech Republic (CR) started in 1990 with the establishment of 12 regional immunological laboratories, and LB working groups within the Ministry of Health in cooperation with the National Reference Laboratory at the National Institute of Public Health.

Standardization of the ELISA test based on comparison of 5 commercial kits, on provision of a reference serum panel, a reference antigen panel (16 strains typed by monoclonal antibodies and PCR). Eight laboratories are currently conducting Lyme borreliosis diagnosis. They use a combination of two tests (e.g., μ-capture and an indirect IgG ELISA).

Immunoblotting is used in all cases of prolonged IgM response and/or in IgG response without specific clinical findings. The PEG-ELISA technique utilizes with selected seronegative patients with specific clinical symptoms.

Lyme borreliosis is subject to obligatory notification in the Czech Republic.

- The follow-up of the incidence of the disease began in 1986. Routine serological diagnostics of LB was introduced in 1988. In the following years the number of cases reported ranged from 14 to 20 per 100 000 inhabitants. In 1993 and 1994 the morbidity rate of LB rose up to 36.6 and 39.4/100 000 respectively.
- LB affects all age groups with an increased incidence beyond the 25th years of life. The highest morbidity rate occur in the age group 45–54 years. Incidence in children peaks at the 5–9 years.
- Male and female ratio in patients with LB is 1:1.6. The exception is in patients suffering ACT with male/female ratio 1:10.
- Mode of transmission is tick-borne (54%), insect-borne (19%), in 27% of the cases the mode of transmission is unknown.
- Clinical manifestations of reported cases of LB: 66% dermatologic (82% EM), 14% rheumatologic, 12%, neurologic and 8% other manifestations. Cardiologic complications are observed in 1.1% of

Bulgaria

In Bulgaria approximately 3 500 case of LB are registered every year. Serological diagnosis of Lyme is performed by the IFA test and ELISA. Epidemiological and epizootological studies are continuing. Fourteen endemic areas have been established.

The major vector of B. burgdorferi is the tick ixodes ricinus. Nearly 26-90% of the adult ticks were found to be infected with B. burgdorferi in the 14 endemic areas.

Deer and roe-deer are the most important hosts for ticks Ix. ricinus among wildlife whereas goats and dogs are the hosts of predilection for Ix. ricinus in domestic animals.
patients.
• Incubation period: early localized stages - from 1 to 29 days, disseminated stage - average about 50 days, late stage- 3 months - 6 years.
• LB cases are reported during the whole year with peak in July and August.
• 92% of reported cases were confirmed serologically by ELISA, IFA and WB kits.
• The National Reference Laboratory on LB tests the proficiency of 53 diagnostic laboratories twice a year.

Denmark

Diagnostic tests still need to be improved. According to our view ELISAs based on B. burgdorferi flagellum and outer surface protein C (OspC) seem presently to be the most promising concept.

Standardization of serological tests is urgently needed, but this would demand a more convergent attitude and agreement regarding choice of test antigen and adjustment of assay specificity level (= cut off).

A widely, internationally accessible supply of standard sera from healthy persons and patients should be available.

At the Borrelia Laboratory, Statens Serum-Institut, it was planned to prepare three standard sera (one high and one low IgG reactive serum, and one IgM reactive serum) to be used by interested laboratories for standardization purposes. Due to the complexity of the field the project needs more consideration, and it has therefore been postponed.

Many previous attempts at proficiency testing and of standardization have been too ambitious and unsuccessful because too many laboratories are still not ready for a harmonization extending beyond their "in-house" or personal routine.

LB case definitions are necessary but are, according to our experience, incomplete and problematic regarding the entity of Lyme arthritis, which is difficult to define by rather specific diagnostic rules.

Surveillance of LB

Considering the rather low morbidity (and lack of mortality!) due to B. burgdorferi infections the value and need of an obligatory nationwide surveillance of LB is in our opinion questionable. If at all, only cases of Neuroborreliosis should be reported in order to obtain a reliable epidemiological index of this infection.

Attempts to monitor the occurrence of erythema migrans or even the quite unspecific condition of Lyme arthritis would yield data that are inaccurate due to misdiagnosis.

Considering the very low incidence of serious disease due to B. burgdorferi infections, a vaccination programme is, in our opinion, not justifiable. Prevention should be based on thorough information of professionals and nonprofessionals about the symptoms of this disease and its transmission, and should consequently focus mainly on clinical recognition and appropriate treatment.

Former Socialist Republic of Yugoslavia

• In the Former Socialist Republic of Yugoslavia (F.S.R.Y.), 1 724 patients were registered with tick bite and/or signs of disease from 1987 to 1994.
• Latent infection was discovered in 93 (5.4%) of persons who reported previous tick bites. Various clinical manifestations of the disease were found in 1 631 (94.6%) persons, of which 76% had confirmed tick bites. 85.6% with EM and 26.7% with serologic confirmation of LB.
• The mean year rate of morbidity in F.S.R.Y. was 2.3/100 000. Within the last eight years peaks were registered every three years.
• Male to female ratio in patients with LB was 1:1.4 with an average age of 39 years.
• Cases are being registered all year long with a peak in June (30.1%).
• LB was found in all parts of the F.S.R.Y. with an accumulation in the Belgrade area.
• Skin changes were diagnosed in 1 061 (61.5%), Lyme Neuro-borreliosis in 179 (11%), articular changes in 191 (11.7%)
and cardiological complications in 34 (2.1%) patients between 1987-1994.

- Specific LB antibodies were found in 30.6% of the patients (IFA, ELISA).
- Acarological studies revealed that 30.6% of the ticks collected from the Belgrade area were infected with BB.
- The natural reservoir of BB is found to be Apodemus flavicollis.

France

There is no national reporting in France. Only few data have been published on the prevalence and human incidence of the disease. One study concerns the entire territory of France and concludes with an average incidence of 16.5/100 000. Three other studies are of local scope: 18 departments in the west of the country, a small part in the centre of France (Berry-sud) established an LB incidence of 40/100 000. Another study investigated the correspondence of spatial occurrence of the disease and the presence of I. ricinus.

The study done in the commuter belt of Paris in 1991-93, showed 13.4% IgG and 2.52% IgM presence in the risk population (IFA), and from 19% to 24% IgG in large wild animals (ELGA = modified ELISA).

Investigation of ticks in the above area revealed the presence of Borrelia in ticks at: (IFA) 6% (larvae), 10% (nymphs) and 8.6% (adults).

Hungary

Epidemiological analysis of 1 175 cases

From the first recognized Hungarian LB cases in July 1984 to the end of 1989, the management of the disease remained centralized. During this period, a total of 3 304 patients were examined at the Lyme Disease Centre, Budapest. 1 175 of them fulfilled the CDC criteria of LB. Of these, 44% showed the typical sign of EM. Multiple EM rings were observed in 12.2% of the patients. In children, the monosymptomatic appearance was characteristic. Complications develop more often in adults. A strikingly large proportion of our EM patients (80%) noticed tick bites. Skin lesions were seen at the ear lobe in all of our eight borrelial lymphocytoma patients. Eighteen cases of Lyme carditis (1.5%) were diagnosed. Rhythm disturbances were the principal clinical signs in all of them. Three required temporary pacemaker treatment. Three hundred and fifty-eight cases (33%) showed neurological symptoms of LB. The protein level was elevated in almost every Lyme meningitis case. 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. Bilateral facial palsy was more frequently found among LB patients (p<0.005). Relapsing facial palsy patients often proved to be seronegative (p<0.05). In the idiopathic group multiple relapses were regularly seen, but never among LB patients. In our facial palsy material that consisted of 654 cases, 25.4% proved to have borrelia infection. Cranial nerves other than the facial nerve were seldom involved. The eye movements were restricted in 17 cases. Paraesthesias were rarely accompanied by severe paresis. Complete paraplegia developed in three cases only. Peripheral neuritis is a typical manifestation of neuro-borreliosis. Both mild paraesthesia and serious lancinating neuralgia can occur. This is a clear evidence that polyneuritis can be both an early and a late manifestation of LB. One hundred and ninety-three (18%) arthritis cases were recognized. In five patients, acute arthritis developed while ECM could still be seen. In one case, gonarthritis preceded ECM. When arthritis developed after a short incubation time, mainly large joints were swollen. In cases of a longer incubation period small joint inflammation predominated. Acrodermatitis chronica atrophicans (ACT) was diagnosed in 16 patients.

Ireland

The vector tick, I. ricinus, is abundant and widespread in Ireland, but few cases of LB are diagnosed in Ireland (approximately 0.6 cases per 100 000). Preliminary ELISA screening is carried out at the Virus Reference Laboratory, Dublin, and also some hospitals in the west and south of the country. Confirmatory Western blots are performed in 2 UK laboratories.
among forestry workers is high (20%), and recently, a survey of all general practitioners identified several high risk areas for tick bite and erythema migrans. Currently 23 laboratories are performing serodiagnosis of LB, mainly with ELISA, and at the National Institute of Public Health and the Environment, the ELISA result is verified on request. For this purpose IgG immunoblotting, IgM immunoblotting and inhibition ELISA are used. The inhibition ELISA measures antibodies to a *Borrelia* specific flagellin epitope, by competition of human antibodies and monoclonal antibody H9724 for this epitope. Criteria for a positive IgG immunoblot are: at least four bands including the flagellin. The IgM immunoblot is considered positive when Osp C reactivity is present.

**Netherlands**

In the Netherlands, a national surveillance system does not exist, and data on prevalence of LB are lacking. Approximately 25% of Ixodes ricinus ticks are infected with *B. burgdorferi sensu lato*. Four genomic groups are present: *B. burgdorferi sensu stricto*, *B. afzelii*, *B. garinii* and group VS116. Seroprevalence

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flagellin, recombinant antigens, and monoclonal antibodies for reaction development are used. Therefore, comparison of results is difficult.

Sera obtained from patients with clinical symptoms of Lyme borreliosis have been tested in parallel with different commercial kits using as the antigen: cell sonicate, isolated flagellin and recombinant pC, P41 (inner part of flagellin), and p100 proteins. The highest sensitivity was reached with an ELISA and a mixture of three recombinant proteins as antigen. It has been possible to detect elevated levels of BB specific IgM Ab in patients at the tenth day of EM and with LB symptoms lasting for more than three months. The ELISA based on isolated flagellin only (P41) has shown lower sensitivity, however 85.5% of the results were consistent with those obtained using recombinant ELISA.

The remaining tests, employing whole cell sonicate and conjugate of human monoclonal antibodies, were found to be less sensitive. Seventy two point five per cent of all sera seropositive as determined with recombinant ELISA turned out to be positive in both tests. The obtained results show the necessity for further standardization of serodiagnosis of Lyme borreliosis. It is imperative to define both sensitivity as well as specificity levels of available tests to achieve better recognition of the disease.

**Russia**

A substantial proportion of the global Lyme borreliosis endemic area is located in Russia. LD cases have been registered in 46 out of 50 administrative regions of Russia. *I. persulcatus* and *I. ricinus* are the main vectors for *B. garinii* and *B. afzelii* which are the only Borrelia genospecies found in Russia so far.

LD is reported in Russia according to the national system of sanitary - epidemiologic reporting and surveillance of infectious diseases. In 1993-1994, average morbidity was about 3.1/100 000 of population, the greatest number of cases being reported in the Urals and West Siberian regions. Thus in 1994, 14.7 cases/ 100 000 of population were registered in the Sverdlovsk region (the Urals). The prognosis is that at least 10,000 to 12,000 new cases may be discovered in Russia each year. City dwellers comprise 84 to 94% of LD patients. Taking into account the current economical situation in Russia, IFA, which is performed using the standard antigen prepared from the local *B. afzelii* strain Ip-21, will remain the routine laboratory test for LB in Russia for several years.

**Sweden**

Lyme disease incidence is not monitored on a national level in Sweden. The available data on infection prevalence in ticks and humans are based on studies. In a study of 3 141 *Ixodes ricinus* ticks, the prevalence of BB senso labo infection in northern Sweden (0-5%) was significantly lower (p<0.001) than for central and southern Sweden (7-29%). Sero-epidemiological studies on a national scale have not been conducted but in high-endemic areas on the Baltic coast, the rate of seropositives varied between 7 and 29%. In the area with 29% seroprevalence, disease incidence was on an average 4% during a two-year survey (Gustafson, R., 1994, Scand. J. Infect. Dis., suppl. 92, 1-63). The clinical manifestations of LB in Sweden are the same as described in other European countries. Laboratory confirmation by serology is offered by some 15 laboratories and a national quality assurance programme was started in 1991. Most laboratories used ELISA with sonicate antigen and/or the flagellar antigen kit from Dako. The results showed a high degree of congruence in evaluations (97%) for the six samples (four of LD and two of false-positives). In conclusion, quality assurance schemes are needed on a national basis and should be conducted on a European basis. A common, European standard for evaluation of WB would be of high priority for the reference laboratories using the assay. Seroprevalence studies in the general population on a national and European basis should be attempted.

**Switzerland**

Until now, no *Ixodes ricinus* population devoid of BB were observed in Switzerland. The infection rate of nymphs and adults ranges from 5-50% (larvae 3%).

_Lyme Borreliosis Diagnosis and Surveillance_
Ab prevalence among the general population may reach levels as high as 26.6%. Seroprevalence among populations at risk such as forestry workers and orienteers ranges from 25.6% to 34%.

A great heterogeneity among BB isolates is observed from different geographic areas. Since 1988, laboratories have to report serological positive Lyme tests to the Swiss Public Health Office, their number varies from 1 452 to 2 183 per year.

**United Kingdom**

Within the UK, Lyme borreliosis (LB) is notifiable only in Scotland and so incidence dates are incomplete. Lyme-endemic areas have been identified throughout the UK and in some of these IgG antibody prevalence rates are 10% or more. The most common symptoms associated with LB in the UK are EM and/or ‘flu’, and neuroborreliosis. Lyme carditis and arthritis are rare. The Public Health Laboratory Service’s Lyme disease reference unit (LDRU) at Southampton employs a two-step protocol for serological testing. All samples are tested by EIA followed, where appropriate, by immunoblot. Serological results are routinely reported with an accompanying interpretive comment. The LDRU has a strong clinical and scientific input. Staff are readily available by telephone to advise on appropriate clinical management on individual cases, to provide epidemiological information and advice on prevention of infection.

**USA**

LB is a nationally reportable disease in the United States and a uniform national case definition is used for surveillance.

LB is focally endemic in the United States. In 1994, more than 13,000 cases were reported, for a national reported disease rate of 5.2/100,000. The eight states with the highest rates (Connecticut, Rhode Island, New York, Maryland, New Jersey, Pennsylvania, Wisconsin, and Minnesota) had rates ranging from 10 to 70/100,000. A number of counties in the most endemic northeastern and north central states had rates exceeding 100/100,000;

Nantucket County, Massachusetts had a rate of approximately 1.000/100,000.

A collaborative effort is underway to establish standardized serodiagnostic testing that utilizes a two-test approach of EIA/IFA first test, followed by Western immunoblot testing of serum specimen yielding equivocal or positive first test results.

There is a diversity of enzootic cycles of BB in the US, involving various reservoir hosts, ixodid ticks and BB strains; only those cycles involving *I. scapularis*, *I. pacificus*, and BB, *sensu stricto* are of significant public health importance.

There is an effort to map the national distribution of tick vectors, and to develop an entomologic risk index that takes into account vector density and infection rate of vectors with BB.

Proficiency testing and quality control is required in all laboratories testing serum specimens for clinical diagnosis for patient management.

**The European Union Concerted Action on Lyme Borreliosis**

The EU has provided funds for arranging workshops and exchanging materials and personnel in a multidisciplinary programme for the assessment of risk in Lyme borreliosis. Approximately 20 research groups in 14 different countries are taking part and are involved in 10 distinct but related topics. These include biological topics such as vector transmission, reservoir hosts and spirochaete and tick strains, medical ones such as serodiagnosis, case definition and clinical risk and also epidemiological topics and mathematical modelling. Considerable interaction with other clinicians and scientists in Europe has taken place. It is intended that at its conclusion in 1996 it will be possible to make recommendations concerning the criteria and procedures for risk assessment of LB.
3. CONCLUSIONS AND RECOMMENDATIONS

3. 1 National Surveillance and Diagnosis of Lyme Borreliosis (LB) including Case Definitions

3.1.1 Necessity for LB surveillance

Disease surveillance has the objective of determining the incidence and distribution of a particular disease and, for human diseases, surveillance systems are usually operated for those that cause high morbidity and/or mortality. Lyme borreliosis does not qualify regarding the latter aspect and only in some countries regarding the former. Nevertheless, surveillance systems for Lyme borreliosis are necessary because of the complex nature of this disease together with the public disquiet often associated with it. Such systems are required to determine resource allocation and to provide information for counselling by physicians and health authorities to improve public knowledge. Surveillance data are major components of LB risk assessment and the production of risk maps, which should be important objectives for all affected countries.

3.1.2 Model of a surveillance system

The United States LB surveillance system has been thoroughly developed. Such a system does not exist in Europe though at present each country evaluates LB prevalence in its own way. Reporting of cases showing particular symptoms occurs in some countries but depends on a notification system and a high level of compliance. Most countries rely on serological testing for incidence estimation but this approach requires efficient linkage to genuine cases and also national quality control systems to assure laboratory performance.

As a first step towards harmonising surveillance in Europe and other regions it is recommended that each country should establish a central reference unit with responsibility for collation and reporting of data. In some countries such reference centres may also be involved in diagnosis and treatment. In others they may function mainly as medical statistics offices and would be linked to regional centres. However, it is also recommended that national reference centres should have a counselling function and would be responsible for data dissemination, particularly in relation to physician awareness and knowledge.

3.1.3 Indicators for the prevalence/ incidence of LB

Neuroborreliosis

This clinical manifestation of LB has been made notifiable in, for example, Denmark but the usefulness of this indicator will diminish in the future because of the increasing number of cases that are treated by general physicians without recourse to lumbar puncture, which requires hospitalisation. It is expected that this will result in a considerable decrease in reporting. It was also pointed out that where those genospecies not associated with neuroborreliosis, such as Borrelia afzelii, are prevalent, many cases of LB would be missed with this approach. Neuroborreliosis cannot therefore be generally recommended as an indicator for LB surveillance.

Erythema Migrans (EM)

EM is notifiable in, for example, the Former Socialist Republic of Yugoslavia, but since in most countries many patients with this symptom are treated by general physicians it is considered that the level of reporting would not be sufficient for surveillance purposes. It would be possible, however, to use the diagnosis of EM for periodic sentinel studies. This has been carried out in the USA and a survey on tick-bites and EMs is planned for The Netherlands. In France some physicians are linked by computer for similar sentinel surveys.

Serology

The detection of anti-borrelia antibodies is used by many countries to estimate the prevalence and incidence of LB. Reliable serodiagnosis is still problematic and as mentioned above quality assurance schemes are required. It is stressed, however, that even good
serodiagnosis alone is inadequate for surveillance, as it would simply be a measure of seropositivity, which in countries with high prevalence would be much greater than the disease level. It is strongly recommended, therefore, that clinical details should be made available where feasible for the evaluation of seropositive cases. It is, however, recognised that the practical problems involved in this may be considerable in some countries, particularly due to the increase in private testing and the often indirect links between the treating physicians and diagnostic laboratories. Even where efficient follow-up can be carried out, as in Denmark, 50% of the submitted clinical details may be irrelevant, indicating a significant lack of knowledge amongst non-specialist physicians.

Ecological indicators

Infected vector prevalence

The abundance and degree of infection with *B. burgdorferi*, of the principal vector ticks (members of the *Ixodes ricinus* species complex) provides a good indication of risk in a particular area. Other important data required are the degree of utilisation of the relevant area by the public and the relationship between intensity of *spirochaete* infection in ticks and tick infectivity. This may depend on both strains of *spirochaetes* and of ticks. These topics are the subject of study in the EU Concerted Action.

Tick-borne encephalitis (TBE)

Tick-borne encephalitis is a more serious disease than LB and many cases are hospitalised, which facilitates efficient reporting. The fact that TBE is transmitted by the same tick species that transmit LB in parts of Europe and Asia suggests that its prevalence may be used as an indicator for LB. However, TBE is a much more focal disease than LB and it also occurs in fewer countries. Furthermore, vaccination is often carried out against TBE, which further weakens any conclusions on relationships between the prevalence of TBE and LB. The presence of TBE in an area, may however, be useful as a crude indicator for the presence of ticks.

Reservoir hosts

The use of competent reservoirs of the *spirochaete* (able to infect ticks) or of tick hosts, as indicators for the measurement of risk is a worthwhile long-term objective. Although several species of animals have been identified as significant reservoirs, their individual roles in the maintenance of the *spirochaete* in a particular habitat are still difficult to determine. Deer have been used as indicators for LB in the USA and Europe because of their role as significant hosts of ticks in woodland. Their role as reservoir remains a subject of controversy. More data on the role of *spirochaete* and tick hosts are required before they can be used as surveillance indicators.

3.1.4 Responsibility for advice on clinical management and physician awareness/knowledge

Clinical management is the responsibility of the treating physician. The responsibility for advising the physicians should be that of the national reference centres. These centres should be able to respond to requests for information and also to disseminate information via the professional associations of medical specialists and general physicians. This will assist physicians in their clinical management of cases and will also facilitate the counselling of patients in relation to prevention.

3.1.5 Costs of surveillance systems and impact of LB on public health economics

The economic impact of LB in a particular country should determine the scale of any surveillance system. In countries with low prevalence and therefore little LB economic impact, the costs of a surveillance system could be minimised by linking it directly to a centralised diagnostic service, so that all serodiagnostic data may be used for surveillance. Considerable LB economic impact will occur in countries with high prevalence, resulting from medical consultations, laboratory diagnosis, drugs, hospitalisation costs, lost work days and invalidity compensations. Where these costs are high, more elaborate and expensive surveillance systems would be justified, which, in addition to serodiagnosis, may include sentinel and ecological surveys for the efficient
focusing of control measures. So far no attempt has been made to estimate the overall economics of LB in any country and it is recommended that this should receive attention as a specific objective, possibly through future WHO meetings or EU Concerted Actions.

3.1.6 Coordination of national reference centres

As recommended above, each country should have a reference centre with responsibilities for data collation, data dissemination and reporting. It is further recommended that one or more of these centres should be designated as a Lyme Borreliosis Surveillance Centre, with the responsibility for networking with the other national centres and reporting to WHO. It is further suggested that the WHO centre(s) should produce an annual Lyme Borreliosis Bulletin. Additional functions for any WHO centres may be appropriate as suggested for a proposed centre in the USA and it was agreed that the preparation of a list of possible terms of reference would be considered at the next meeting of the EU Concerted Action steering committee.

3.1.7 Case definitions

Good case definitions are essential for clinicians in relation to diagnosis and treatment and are also a vital component of any surveillance system. The Centers for Disease Control and Prevention (CDC), USA has produced case definitions for North America and, as part of the EU Concerted Action, European clinicians are in the final phase of determining case definitions for Europe. In general, they are similar to those produced by CDC, but there are some important differences. For example in the CDC case definitions, EM forms a large round lesion, but in the view of the European clinicians this round shape is not obligatory. Furthermore, the size condition of at least 5 cm is not appropriate in Europe for clinical diagnosis, though it may be acceptable for surveillance purposes. Other European symptoms, not included in the CDC case definitions, because of their apparent absence in North America, are borrelia lymphocytoma and ACT.

Final definitions of these and other clinical manifestations of Lyme borreliosis in Europe will be published in the near future.

3.2 Standardization of Serological Tests

Objectives of serological testing

Two key objectives of serological testing are:

- Support of clinical management;
- Provide data for epidemiological studies

3.2.1 Recommended specifications for serodiagnostic tests

Specificity

High specificity is required to minimize false-positive results. A minimum level of specificity of 98% is recommended.

Whether appropriate levels of specificity are obtained by use of a single test or combination of tests may depend on local circumstances. For example, in regions with low prevalence of infection where even a 98% specificity will result in a significant number of false positives, a second test aimed at raising specificity further may be helpful.

3.2.2 Requirements for determination of appropriate test cut-off

- Sample size: at least 100 sera

- Population: Healthy adults (e.g. blood donors) from the same locality as test population or from an area of comparable seroprevalence. If cut-off has been established previously in a control population elsewhere this cut-off should be confirmed using local control sera.

- Calculation of cut-off for quantitative tests: Using quantitative tests, antibody levels in the normal population are skewed. Thus, parametric methods for calculation of cut-off are inappropriate. A non-parametric method (e.g. 98-percentile) is recommended.
- Specificity of qualitative tests: For qualitative assays such as immunoblot, specificity should be determined according to the protocol described above for quantitative tests, i.e. a minimum of one hundred sera from an appropriate control population.

3.2.3 Requirement for international standard sera

At Statens Serum Institut, the WHO International Standardization Centre had, in collaboration with the Borrelia Laboratory, planned to collect and evaluate international standard sera for laboratory standardization purposes, including immunoblot assays. It was thought that three such sera should be identified (strongly IgG positive, low IgG positive, and IgM positive) and be quantified in terms of international units per ml. However, this project needs further consideration due to the complexity of the field. The project has subsequently been postponed.

3.2.4 External quality assurance schemes and proficiency testing

Serological testing for Lyme borreliosis is difficult due both to the low levels of specific antibody associated with early infection (giving rise to false negative results) and relatively high prevalence of cross-reacting antibody in the healthy population (giving rise to false positive results). Further, any positive results must be interpreted in a clinical context due to the prevalence of specific antibodies, i.e. true asymptomatic seropositives, particularly in high endemic areas.

Several national quality assurance schemes have been organized or are planned to address these problems. In view of the difficulties in serological testing for Lyme borreliosis and the increasing number of laboratories offering testing, such national programmes should be encouraged.

As a complement to such national schemes we recommend an international quality assurance scheme with the following aims:

- to measure precision of testing;
- to assess appropriateness of advice given by the laboratory with regard to interpretation of serological data in a clinical context;
- to serve as a teaching tool to educate physicians.

This quality assurance scheme should comprise regular distributions of 6-12 sera from cases of clinically confirmed LB or negative controls, both of confirmed serological status. Sera should not be pooled or diluted. Results will be returned to each laboratory with a teaching sheet indicating appropriate interpretation in a clinical context.

Such an international quality assurance scheme is currently being organized under the auspices of the EU Concerted Action on Lyme Borreliosis. The organisers of this scheme invite interested laboratories to contact them for more information (contact: Dr Edward Guy, Public Health Laboratory, Singleton Hospital, Swansea SA2 8QA, United Kingdom).

3.2.5 Immunoblot proficiency testing

No consensus presently exists for immunoblot criteria for seropositivity. This may be due to differences in immunoblot methodology, geographical and strain variations, different interpretations for band significance (total number of bands vs specific bands), and subjective interpretation (weak vs strong bands).

In order to achieve a consensus we recommend the following:

Key laboratories (in 5 or 6 countries) with extensive experience of the use of immunoblot for laboratory diagnosis should compare methodologies and receive for testing LB sera collected from geographically distinct regions.

3.2.6 Role of national licensing institutions

Few countries, e.g. Germany and USA, presently have national licensing arrangements and others, e.g. Poland, are considering these.

We recommend that any new LB serological test should conform to the following criteria:
Specificity: At least 98% based on testing of sera from more than one hundred healthy adults. Sera of patients with diseases that result in potentially cross-reacting immune responses, e.g. syphilis, should be tested in order to establish the levels of cross-reactivity.

Sensitivity: The stated sensitivity of particular tests should be determined using clinically confirmed LB sera.

Before any new test can be recommended for diagnostic testing, specificity, sensitivity, and precision should be equal to, or better than, presently available methods.

Information included in any kit should stress that positive results must be interpreted in a clinical context.
Annex 1 List of participants

Dr Elisabeth Aberer, Department of Dermatology, University of Graz, Auenbrugger Platz 8, A-8036 Graz, Austria
Tel: +43 316 385 37 82, Fax: +43 316 3852371

Dr L. Angelov, Department of Epidemiology and Infectious Diseases, Medical University, 15A V. Aprilov Av, 4000 Plovdiv, Bulgaria
Tel: +359 32 44 61 63, Fax: +359 32 44 32 28

Dr N. Axelsen, Director, Department of Clinical Biochemistry, Statens Serum Institut, 5 Artilerivej, 2300 Copenhagen S, Denmark
Tel: +45 3268 3268, Fax: +45 3268 3868

Dr D. T. Dennis, Bacterial Zoonoses Branch, CDC, Vector-borne Infectious Diseases, PO Box 20 87, Foothills Campus, Fort Collins, CO 80 522, USA
Tel: +1 303 221 6453, Fax: +1 303 221 6476

Dr Radmila Dmitrovic, Gradski zavod za zastitu zdravlja, 29 November 54a, Belgrade, the Federal Republic of Yugoslavia (Serbia/Montenegro)
Tel: +381 11 337 122, Fax: +381 11 3227 828

Dr Lise Germ, Institut de Zoologie, Emile Argand 11, 2000 Neuchâtel, Switzerland
Tel: +41 38 233 052, Fax: +41 38 233 001, E-mail: Lise.Germ@zoool.unine.ch

Dr Marta Granström, Department of Microbiology, Karolinska Hospital, S-174 76 Stockholm, Sweden
Tel: +46 8 729 35 64, Fax: +46 8 30 80 99, E-mail: marta@amb.ks.se

Dr J. Gray, University College Dublin, Department of Environmental Resource Management, Faculty of Agriculture, Belfield, Dublin 4, Ireland
Tel: +359 1 706 7777, Fax: +353 1 283 7328

Dr E. Guy, Public Health Laboratory Service, Singleton Hospital, Sgeti, GB-SA2 8QA Swansea, United Kingdom
Tel: +44 1792 205 666, Fax: +44 1792 202 320

Dr K. Hansen, Borrelia Laboratory, Department of Treponematoses, Statens Serum Institut, Amager Boulevard 890, DK-2300 Copenhagen S, Denmark

Dr Dagmar Hulinská, Director, WHO Collaborating Centre for Research on Borreliosis, National Institute of Public Health, Šrobárova 48, CZ-100 42 Prague 10, Czech Republic
Tel: +42 2 673 105 78-84, Fax: +42 2 673 11 188

Dr K. Korenberg, Gamaleya Institute of Epidemiology and Microbiology Academy of Medical Sciences, Gamaleya Str. 18, Moscow, Russian Federation
Tel: +7 095 193 4395, Fax: +7 095 115 1255

Dr A. Lakos, Ptak s. 156, Nagykovácsi, H-2094, Hungary
Tel/Fax: +36 1 138 99 03, E-mail: H189RAD@ella.hu

Dr Olga Lesnyak, Lyme Disease Centre, Regional Hospital No. 1, 185 Volgogradskaya Str., Yekaterinburg 620102, Russian Federation
Tel: +7 3432 431 950,
WHO/CDS/VPH/95.141

Dr D. Pavlovic, Institute of Neurology, University of Belgrade, Dr Subotica 6, 11000 Belgrade, the Federal Republic of Yugoslavia (Serbia/Montenegro) Fax: +38 111 322 7828 (Dr Radmila Dmitrovic)

Dr Claudine Perez-Eid, Unité d’Ecologie des Systèmes Vectoriels, Institut Pasteur, 75724 Paris Cedex 15, France Tel: +33 1 45 68 8728, Fax: +33 1 40 61

Dr S. Rijpkema, Laboratory for Bacteriology and Antimicrobial Agents, National Institute of Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, PO Box 1, NL-3720 BA Bilthoven, The Netherlands Tel: +31 30 742 889, Fax: +31 30 292 957, E-mail: Sjoerd.Rijpkema@rivm.nl

Dr Janet Robertson, Lyme Disease Reference Unit, Public Health Laboratory, Southampton General Hospital, Southampton SO16 6YD, United Kingdom Tel: +44 1703 794 810, Fax: +44 1703 774 316

Dr G. Stanek, Hygiene-Institut, Universität Wien, Kinderpitalgasse 15, A-1095 Wien, Austria Tel: +43 1 404 900, Fax: +43 1 404 90295

Dr T. Talaska, Arztlisches Labor Frankfurt (Oder), Am Kleistpark 1, D-15230 Frankfurt (Oder), Germany Tel: +49 335 558 1100, Fax: +49 335 528 1160

Dr Y. Yanagihra, Department of Microbiology, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka-shi, 422 Japan Tel: +81 54 264 5716, Fax: +81 54 264 5715

Participants from the host institute and other Polish colleagues

Dr A. Buczek, Katowice Medical School, Clinic of Biology and Parasitology, Katowice, Poland

Dr S. Tylewska-Wierzbanska, National Institute of Hygiene, Chołmska 24, 00791 Warsaw, Poland Tel: +48 22 494 051 (ext. 250), Fax: +48 22 497 484

Dr J. Zabicka, National Institute of Hygiene, Chołmska 24, 00791 Warsaw, Poland

Observer

Dr M. Schlumberger, médecin-épidémiologiste, Association pour l’aide à médecin Préventive (AMP), 3 avenue Pasteur, 92430 Marnes-la-Coquette, France Tel: +33 1 47 95 80 30, Fax: +33 1 47 95 80 35

Secretariat

Dr K. Stöhr, Veterinary Public Health, Division of Communicable Diseases, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (Secretary) Tel: +41 22 791 2529, Fax: +41 22 791 0746, E-mail: STOHRK@WHO.CH

Dr E. D. Tikhomirov, Programme on Bacterial, Viral Diseases and Immunology, Division of Communicable Diseases, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (Co-Secretary) Tel: +41 22 791 2688, Fax: +41 22 791 0746

18 Lyme Borreliosis Diagnosis and Surveillance
Annex 2  Lyme Borreliosis case definition and differential diagnoses
(prepared by Dr E. Aberer, under discussion)

In October 1994 and in May 1995 G. Stanek (Vienna) invited experts to discuss clinical case
definitions for Lyme Borreliosis on behalf of the EU Concerted Action on Lyme Disease,
Vienna, 14-16 October 1994, and at the Symposium on the Therapy and Prophylaxis for

1. Erythema (chronicum)
migrans (EM)

The definitions coined at the EU Concerted Action meeting in Vienna were further
discussed at the Portoroz Symposium. Currently, the many suggestions submitted by
experts from Europe and USA for a clinical case definition of erythema (chronicum)
migrans are processed and await a final review.

Suggestion for definition

At the Department of Dermatology of the University of Vienna Medical School
approximately 100 patients/year with EM were investigated and treated. From our experience
erythema migrans (EM) (it was suggested that the term erythema chronicum migrans is not
appropriate, since the erythema does not become chronic because of antibiotic treatment)
can be seen in 11 different shapes as reported by Aberer [1].

<table>
<thead>
<tr>
<th>Erythematous patch</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 1. homogenous red with peripheral expansion</td>
<td>• unspecific arthropod bite reaction, erysipeloid</td>
</tr>
<tr>
<td>• 2. with fading centre</td>
<td>• erythema anulare centrifugum</td>
</tr>
<tr>
<td>• 3. with elevated border</td>
<td>• urticaria</td>
</tr>
<tr>
<td>• 4. with central nodule</td>
<td>• unspecific tick bite reaction, lymphocytoma</td>
</tr>
<tr>
<td>• 5. with central vesicles</td>
<td>• contact dermatitis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oval or round erythematous patch with peripheral accentuation</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 6. erythematous ringlike patch</td>
<td>• erythema anulare centrifugum</td>
</tr>
<tr>
<td>• 7. with palpable purpura</td>
<td>• folliculitis, vasculitis</td>
</tr>
<tr>
<td>• 8. with induration</td>
<td>• granuloma anulare</td>
</tr>
<tr>
<td>• 9. with scaling</td>
<td>• tinea corporis, nummular eczema</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bluish patch</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 10. apparently persistent round to oval with discreet peripheral erythematous hue</td>
<td>• unspecific arthropod bite reaction in regression</td>
</tr>
<tr>
<td>• 11. geographically confined bluish patch with erythematous border</td>
<td>• fixed drug eruption</td>
</tr>
</tbody>
</table>

Based on the above clinical manifestation our definition is:

EM is an enlarging erythematous to bluish-red patch with advancing border; central
clearing and a history of arthropod bite is common.

Justification:
- Since the advancing border is typical for all clinically different lesions EM can also
be diagnosed under the size of 5 cm.
- A ring- or oval shape of EM does not occur in all patients. Therefore, annular or ring-
like forms should not be mentioned in the definition.
- The question of the duration of EM can often not be answered since patients do not
notice skin changes for several days or weeks.
On the other hand EM can already be diagnosed 4 days after a tick bite.

- Antibodies against BB can be determined in only 30-50% of EM patients. Therefore a positive serology is not obligatory for diagnosis and case definition.
- EM can be accompanied by constitutional symptoms in 25% of patients which we suggest as a major variant of EM in contrary to the minor variant (in analogy to other dermatoses such as erythema exsudativum multiforme minor, major). It has been suggested that EM minor is only a localized infection. Recent data about Borrelia-DNA shedding in these patients, however, have shown, that Borrelia or fragments of them must pass the kidney, so that "localized EM" can no longer be considered as a localized disease.

2. Borrelia-lymphocytoma (BL)

Definition:

BL is a painless bluish-red nodule or swelling at the earlobe, helix, mamilla or scrotum; antibody titres against B. burgdorferi or culture of B. burgdorferi from a skin biopsy should be positive; histologically, a B-cell pseudolymphoma is present."

Lymphadenosis cutis benigna (LACB) arises typically on the earlobe, helix, on the nipple or on the scrotum as a soft red or bluish-red, well circumscribed nodule. On the other hand a general enlargement or swelling of the nipple, areola mammae or helix can be observed due to the dense mononuclear infiltration in the dermis.

Differential diagnoses:
- ear erysipelas relapsing polychondritis insect bite reaction pemiones
- breast malignant lymphoma breast carcinoma

BL can also arise in the centre of erythema migrans (differential diagnosis: persistent arthropod reaction), in an EM area, at the border of EM or on other body sites as single or multiple papular, plaque or nodular eruptions with histological signs of a B-cell pseudolymphoma.

The histological type of B-cell pseudolymphoma (LACB) can be seen in other conditions such as persistent nodular arthropod bite reactions, lymphomatoid drug eruptions, or for unknown reasons. Therefore, a positive serology is necessary for the diagnosis of BL as well as the histological examination for the diagnosis of LACB on all body sites but the ear lobe.

3. Acrodermatitis chronica atrophicans (ACA)

Description of the morphology of lesions is indispensable for a reliable dermatological diagnosis. Therefore, the suggestion for definition of ACA is:

ACA is a red or bluish-red, not sharply demarcated patch or swelling of parts of the extremities with possible skin induration over bony prominences leading to skin atrophy. Serum IgG antibodies against B. burgdorferi are positive. The diagnosis has to be confirmed histologically.

ACA develops in weeks, months to years with no subjective symptoms, sometimes from an erythema migrans. A history of tick bite is not always reported by the patients. ACA can be accompanied by regional or sock-like neuropathies which persist for months after treatment together with erythematous skin lesions. Fibroid nodules at elbows or interarticular areas can precede ACA or go hand in hand with the development of ACA. Anetoderma - like skin lesions can be seen concomitant with ACA.

In the literature a few reports concerning laboratory diagnoses of ACA have been published so far by Wilske et al., Asbrink et al., Hansen et al., Aberer et al., Olsson et al. [2,5,6,7,8]. Hansen et al. found 100% IgG seropositivity in ACA patients. In addition, 12% of patients showed IgM antibodies [6]. From our own experiences the clinical diagnosis of ACA was doubtful in seronegative cases. No cases of seronegative ACA patients have yet been published.
The clinical differential diagnoses of ACA are:

- Erysipelas: This acute infectious disease with fever and erythema has a short history contrary to ACA which develops in weeks to months.
- Erysipeloid caused by an infection with Erysipelothrix rhusiopathiae which also presents as an acute dermatosis.
- Stasis dermatitis and stasis oedema can show bluish-red swellings. In stasis oedema, concomitant stasis dermatitis is present with papules, scaling, crusts and superficial varicosity of the legs or a post-thrombotic syndrome.
- Perniones show a non-specific histology.
- Acrocyanosis: symmetrical distribution, iris-phenomenon, no inflammation histologically.
- In the case of secondary sclerotic changes circumscribed scleroderma has to be delineated. The later disease is sharply demarcated and shows peripheral spreading contrary to ACA [3]. In the systemized type of circumscribed scleroderma (livid discolouration and induration of a whole extremity the delineation to ACA is difficult since the histological findings are very similar to ACA [4].

References
3. A berer E, Klade H. Reply to: Localised scleroderma is not a B. burgdorferi infection in France. Dermatology 1992; 184:287-288