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Autor: Moosmann, Yves / Brossard, Michel / Raeber, Pierre-Alain
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ESTIMATED LYME BORRELIOSIS INCIDENCE IN THE CANTON OF NEUCHÂTEL (SWITZERLAND)

MOOSMANN YVES¹, BROSSARD MICHEL¹,
PIERRE-ALAIN RAEBER² AND GERN LISE¹

¹Institute of Biology, University of Neuchâtel, Switzerland

²Swiss Federal Office of Public Health, Switzerland

Corresponding author : Gern Lise, Faculté des Sciences, Institut de Biologie, Emile Argand
11, Case postale 2, 2007 Neuchâtel (Switzerland). Tel. +41 32 718 3000. Fax +41 32 718 3001
E-mail: lise.gern@unine.ch

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Mots-clés: Borréliose de Lyme, Incidence, Manifestations cliniques, Canton de Neuchâtel (Suisse)

SUMMARY

In the present study, we assessed clinical aspects of Lyme borreliosis and estimated clinical incidence of this disease in the general population living in the canton of Neuchâtel (North-Western part of Switzerland), in three different regions characterized by their endemicity. The study period lasted from 1996 to 2001. Data on Lyme borreliosis cases were collected on the basis of positive serological tests and the diagnosis retrospectively evaluated by the attending physician. Results showed that dermatological problems were the most frequent diagnostic of Lyme borreliosis representing 78.1% of cases, mainly EM (68.7%), followed by manifestations affecting the nervous system (12%) and the joints, which has been diagnosed only occasionally (6.5%). The highest endemicity was observed in region 1 (lowest altitude 430 meters) with an incidence varying from 49.0/100 000 inhabitants to 72.7/100 000 according to years, followed by region 2 (lowest altitude 720 meters) with an incidence ranging from 18.7/100 000 inhabitants to 48.5/100 000 inhabitants, whereas region 3, (lowest altitude 940 meters) the mountain area, showed the lowest incidence which varied from 7.1/100 000 to 12.6/100 000 inhabitants. These clinical incidences are largely underestimated due to various reasons discussed thereafter.

RÉSUMÉ

La borréliose de Lyme est une maladie dont l'agent pathogène, *Borrelia burgdorferi* sensu lato, est transmis en Europe par la tique *Ixodes ricinus*. Cette maladie provoque parti-

culièrement des atteintes dermatologiques et neurologiques, et atteint également les articulations. Au cours de cette étude, nous avons estimé l'incidence annuelle de la Borréliose de Lyme dans la population du Canton de Neuchâtel (nord-ouest de la Suisse) dans 3 régions géographiques différentes, caractérisées par leur endémicité. L'étude s'est étendue de 1996 à 2001. Les données concernant les cas de borréliose de Lyme ont été récoltées à partir de résultats sérologiques positifs et le diagnostic a ensuite été rétrospectivement posé par les médecins ayant demandé l'examen sérologique. L'étude a montré que les problèmes dermatologiques représentaient 78.1% des cas et que l'érythème migrant prédominait (68.7%), suivaient ensuite les manifestations touchant le système nerveux (12%). Les problèmes liés aux articulations n'ont été observés qu'occasionnellement et ne représentaient que 6.5% des cas. La plus forte endémicité a été observée dans le bas du Canton, c'est-à-dire dans la région de plus faible altitude (limite inférieure d'altitude : 430m) avec une incidence annuelle variant, selon l'année, entre 49.0 et 72.7 cas pour 100 000 habitants. Dans la région de moyenne altitude (limite inférieure d'altitude : 720m), l'incidence variait entre 18.7 et 48.5 cas pour 100 000 habitants. Dans la dernière région, constituée de montagnes (limite inférieure d'altitude: 940 m) nous avons observé l'incidence la plus faible avec 7.1 à 12.6 cas pour 100 000 habitants. Ces incidences cliniques sont sous-estimées en raison principalement de la faible sensibilité des tests sérologiques en début de maladie. Ainsi plusieurs patients présentant un érythème migrant (manifestation apparaissant rapidement après la piqûre), mais n'ayant pas de tests sérologiques positifs, n'ont pas été inclus dans cette étude. D'autre part, nombre de médecins connaissant bien cette manifestation clinique la diagnostique sans avoir recours à la sérologie.

INTRODUCTION

Lyme borreliosis, the most prevalent tick-borne disease in the Northern hemisphere, is a multisystemic disorder caused by the spirochete *Borrelia burgdorferi* sensu lato (sl) which comprises 12 genospecies and several unnamed genomic groups. The tick *Ixodes ricinus* is the main vector of *B. burgdorferi* sl in Europe. Six different *Borrelia* genospecies have been found associated with *I. ricinus*: *B. burgdorferi* sensu stricto (ss) (JOHNSON *et al.* 1984), *B. garinii* (BARANTON *et al.* 1992), *B. afzelii* (CANICA *et al.* 1993), *B. valaisiana* (WANG *et al.* 1997), *B. lusitaniae* (LA FLÈCHE *et al.* 1997) and *B. spielmanii* (Richter *et al.*, 2006). All these *Borrelia* species, except *B. spielmanii*, have been described in Switzerland (PÉTER *et al.* 1995, JOUDA *et al.* 2003, 2004a,b, MORÁN CADENAS *et al.* 2007). The pathogenic role for humans of two of them, *B. valaisiana* and *B. lusitaniae*, remains unclear.

In Switzerland, the infection rate of *I. ricinus* ticks collected in various areas varies

between 5% and 47.5% (AESCHLIMANN *et al.* 1986, PÉTER *et al.*, 1995, JOUDA *et al.* 2003, 2004a,b, MORÁN CADENAS *et al.* 2007). As human infection with *B. burgdorferi* is the result of an infectious tick bite, the probability of acquiring Lyme borreliosis primarily depends upon tick-human encounters. Studies on the incidence of Lyme borreliosis in defined populations in Europe are scarce. In Switzerland, a few studies reported incidence of Lyme borreliosis in populations at risk, like orienteers (FAHRER *et al.* 1991, 1998) and a more recent study assessed clinical aspects and incidence of Lyme borreliosis over a period of 19 months in populations living in the Western part of Switzerland (NAHIMANA *et al.* 2000).

The goal of the present study was to assess clinical aspects of Lyme borreliosis and to estimate the incidence of this disease in a more restricted and defined population over a period of 6 years (1996-2001). We collected information on the clinical manifestations of Lyme borreliosis in a popula-

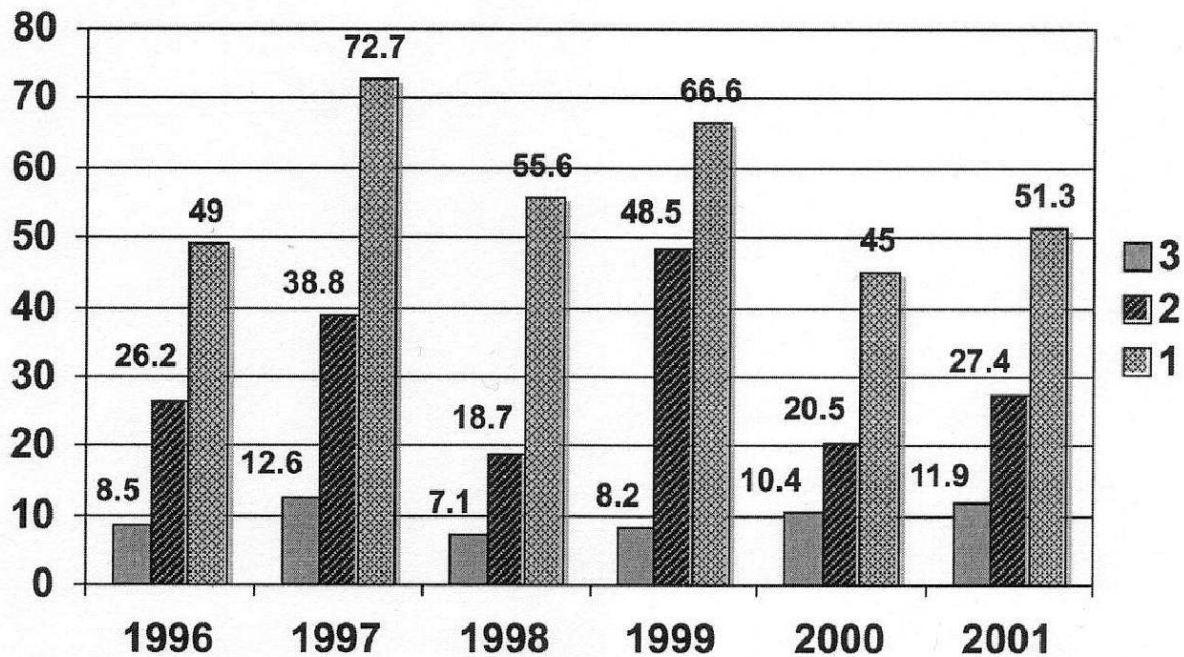


Figure 1 : Incidence of Lyme borreliosis cases according to the various geographic areas (number of cases for 100'000 inhabitants and year)
 Legend: 1: Region 1(Lakeside); 2: Region 2 (Valleys); 3: Region 3 (Mountain area)

tion living in an endemic area (Neuchâtel, Switzerland) where a high density of *I. ricinus* ticks has been reported (more than 300 ticks/100m²) (PERRET *et al.* 2000, JOUDA *et al.* 2004a, MORÀN CADENAS *et al.* 2007) and where *Borrelia* infection rates in nymph and adult ticks reach 33% and 43%, respectively (Jouda *et al.* 2004a, MORÀN CADENAS *et al.* 2007).

MATERIALS AND METHODS

Study area

The study area, Canton of Neuchâtel, is located in the North-Western part of Switzerland. To obtain large units of population for more stable estimates of incidence, the studied geographical area was divided into 3 parts from South to North. Region 1 is at the lowest altitude with the highest population density. This area, also called littoral zone, is constrained between the lake of Neuchâ-

tel and a mountain (Chaumont) reaching 1000 m above sea level. A high tick density was reported in this region (PERRET *et al.* 2000, 2004, JOUDA *et al.* 2004a, MORÀN CADENAS *et al.* 2007). Region 2 is delimited at the southern margin by a chain of mountains (including Chaumont) and at the Northern margin by mountains reaching 1200 m above sea level and is formed mainly by 2 valleys (Val-de-Ruz and Val de Travers). Region 3 is situated at the highest altitude and surrounded by mountains reaching 1200 m. The three regions are characterized by their lowest altitudes: 430, 720 and 940 meters for regions 1, 2 and 3, respectively.

All hospitals and private laboratories, except one, and most of the private physicians located in the study areas sent probes for confirmatory serological analysis to the Laboratory for Parasitological Diagnosis at the University of Neuchâtel (Switzerland). During the study, half of general practitioners, internists, dermatologists and paedia-

tricians with private practice and located in the study area received at least one questionnaire (see below) and were therefore concerned by the study.

Serologic assays:

The Dako IgM-capture assay (IDEIA™ *Borrelia burgdorferi* IgM) was performed according to the manufacturer's instructions, in order to evaluate the presence of IgM in the serum of patient. For IgG, a two-tests approach was used. Specimens were first tested by home-brew screening EIA and Immunofluorescence (IF) assays, as described by Fahrner *et al.* (1991). Positive serum specimens were then tested with a more specific IgG immunoblot. When available, paired sera and CSF were tested by the DAKO Lyme Neuroborreliosis ELISA kit (IDEIA™ Lyme Neuroborreliosis) in order to determine intrathecal specific antibody production (IP). For immunoblot, strains of *B. burgdorferi* B31, *B. garinii* NE83 and *B. afzelii* NE17 were used as antigens. When present, bands of 90, 31 (OspA) and 22 kD (OspC) counted for 10 points each; bands of 72, 65, 58-56, 43, 39, 34 (OspB), 30, 29 and 17 Kd counted for 5 points each. Each other band counted for one point. The immunoblot was considered as positive when the total of points was equal or superior to 31.

Incidence of Lyme borreliosis

The incidence of Lyme borreliosis in the human population was proceeded from positive serological results and clinical diagnosis made by physicians. Physicians who sent probes to the "Laboratory for Parasitological Diagnosis" for a Lyme borreliosis serology were retrospectively asked to fill up a questionnaire for each patient presenting a positive serological result (IgM and/or Immunoblot IgG and/or positive intrathecally-produced Ig index). The questionnaire was used to collect information on motivation for asking the serology, on tick bite

and clinical manifestations. Physicians were asked to retrospectively evaluate as "certain", "probable", "possible" or "excluded" the post-test probability of Lyme Borreliosis for each patient. This evaluation was left to the physician hands and was therefore based on their own knowledge of the disease and their own diagnostic criteria. The study started April 22 1996 and ended December 31 2001.

Incidence of Lyme borreliosis was estimated by dividing the number of patients with confirmed and probable Lyme borreliosis by the number of inhabitants living in each region. Annual variations of total residential population (data obtained from the Swiss Federal Statistical Office, Neuchâtel, Switzerland) in the study areas were less than 0.4% between 1996-2000, the residential population was therefore considered as stable and data for year 2000 were considered for the whole study period. Regions 1, 2 and 3 include 86'871, 26'492 and 52'368 inhabitants, respectively.

RESULTS

During the study period, a total of 984 questionnaires were sent to physicians accompanying positive serological test results. Altogether, 730 questionnaires (74.2%) were returned and could be included in the study. Half (374/730, 51.2%) of the questionnaires concerned patients clinically diagnosed by the physicians as confirmed or probable Lyme borreliosis. For 238 patients (32.6%) Lyme borreliosis was diagnosed as possible, and unlikely or excluded for 118 patients (16.2%).

For the group of patients with confirmed or probable Lyme borreliosis, clinical manifestations were retrospectively classified using clinical case definitions according to Stanek *et al.* (1996). When a patient presented more than one disorder, only the latest disease stage reached was considered (Table 1). Dermatological problems were the most frequent diagnostic of Lyme borre-

		N=	% of patients	
CUTANEOUS	Erythema migrans (EM)	257	68.7%	78.1%
	Lymphadenosis benigna cutis (LBC)	8	2.1%	
	Acrodermatitis chronica atrophicans (ACA)	16	4.3%	
	Subacute or chronic dermatological manifestations (= dermatological manifestations except EM, LBC or ACA)	11	3.0%	
NERVOUS	Early neuroborreliosis (meningitis, radiculoneuritis, facial palsy or other cranial neuritis, with/without positive IP)	23	6.1%	12.0%
	Chronic neuroborreliosis (with positive IP)	1	0.3%	
	Subacute or chronic neurological manifestations (= neurological manifestations except early or chronic neuroborreliosis)	21	5.6%	
MUSCULO-SKELETAL	Lyme arthritis	7	1.9%	6.5%
	Subacute or chronic articular manifestations (= articular manifestations except Lyme arthritis)	17	4.6%	
OTHER	Lyme carditis	2	0.5%	3.4%
	Non typical subacute Lyme borreliosis manifestations	11	2.9%	
Total:		374	100%	

N= Number of clinical manifestations

IP= Intrathecal production

Table 1 : Main clinical manifestations (n= 374) presented by 374 patients considered of having a confirmed or probable Lyme borreliosis between April 1996 and December 2001 in Canton of Neuchâtel.

		N=	% of patients	
CUTANEOUS	Erythema migrans (EM)	31	13.0%	28.9%
	Lymphadenosis benigna cutis (LBC)	1	0.4%	
	Acrodermatitis chronica atrophicans (ACA)	2	0.8%	
	Subacute or chronic dermatological manifestations (= dermatological manifestations except EM, LBC or ACA)	35	14.7%	
NERVOUS	Early neuroborreliosis (meningitis, radiculoneuritis, facial palsy or other cranial neuritis, with/without positive IP)	8	3.4%	41.2%
	Chronic neuroborreliosis (with positive IP)	0	0.0%	
	Subacute or chronic neurological manifestations (= neurological manifestations except early or chronic neuroborreliosis)	90	37.8%	
MUSCULO-SKELETAL	Lyme arthritis	0	0.0%	23.1%
	Subacute or chronic articular manifestations (= articular manifestations except Lyme arthritis)	55	23.1%	
OTHER	Lyme carditis	5	2.1%	13.5%
	Non typical subacute Lyme borreliosis manifestations, or no clinical data available	27	11.4%	
Total:		254		

N= Number of clinical manifestations
 IP= Intrathecal production

Table 2 : Clinical manifestations (n=254) presented by 238 patients considered of having a possible Lyme borreliosis.

liosis representing 78.1% of all confirmed/probable cases (Table 1). Among these cutaneous manifestations, erythema migrans (EM) was the most common (68.7% of all cases), followed by acrodermatitis chronica atrophicans (ACA) (4.3%) and lymphadenosis benigna cutis (LBC) (2.1%). Clinical manifestations affecting the nervous system were the second most frequent diagnostic

representing 12.0% of all cases, followed by joint manifestations (6.5%). For the group of patients with possible Lyme borreliosis, clinical pictures presented a shift to less specific manifestations of Lyme borreliosis (Table 2).

The distribution of the estimated incidences of confirmed/probable clinical cases of Lyme borreliosis in the studied popu-

lation varied according to region and year (Fig. 1). Region 1 was the region with the highest incidence, varying from 45.0/100 000 inhabitants to 72.7/100 000 according to years. Region 2 was the second most exposed region with an incidence ranging from 18.7/100 000 inhabitants to 48.5/100 000 inhabitants. Region 3, the mountain area, was the region showing the lowest incidence which varied from 7.1/100 000 to 12.6/100 000 inhabitants. The highest incidence was observed in 1997 in region 1.

DISCUSSION

Lyme borreliosis occurs throughout Europe and appears to be the most common vector-borne disease in humans as it is in North America, particularly in the USA. In the USA, national surveillance is in place through the Center for Disease Control (CDC). In Europe, surveillance methods vary considerably among countries (WHO, 1995). Moreover, in most European countries, notification of Lyme borreliosis is not compulsory and therefore very few is known on clinical incidence of this disease. Lyme borreliosis is notifiable to authorities in only two countries, Slovenia and Scotland (O'CONNELL *et al.*, 1998). In Slovenia an incidence of approximately 120/100'000, mainly EM, has been reported (STRLE & STANTIC-PAVLINIC, 1996) and in Scotland, only 10 cases per year are reported (O'CONNELL *et al.* 1998). In a study in Norway an incidence rate of 3.4/100'000 has been reported for 1999 (FOLKEHELSA, 1999).

In Switzerland, notification was previously conducted through diagnostic laboratories reporting serological positive tests. This has been abandoned in 1998, and from 1999 to 2003, EM was notified. An incidence between 2.4/100'000 (2000) and 3.9/100'000 (2001) was reported for EM, but these results do not take into account demographic, geographic and climatic disparities in Switzerland. In a previous study

undertaken in the population living in the whole French speaking part of Switzerland, incidences of Lyme borreliosis were reported to vary according to geographic areas (ranges: 9.0/100'000 inhabitants to 95.0/100'000 inhabitants, NAHIMANA *et al.* 2000). The highest incidence (95.0/100'000) was reported in our studied area. However, the incidence reported by NAHIMANA *et al.* (2000) was adjusted on the return rate of questionnaires and therefore cannot be compared with our results.

In the present study, we used an indirect method to estimate incidence of clinical Lyme borreliosis in the general population living in the North-Western part of Switzerland in three different areas characterized by their endemicity. Data on clinical Lyme borreliosis cases were collected on the basis of positive serological tests, and the diagnosis of Lyme borreliosis retrospectively evaluated by the participating physicians. Results showed that when the diagnosis was certain or probable, dermatological problems were the most frequent diagnostic of Lyme borreliosis representing 78.1% of cases, mainly EM (68.7%, accompanied or not by non-specific symptoms), followed by manifestations affecting the nervous system (12%) and the joints, which have been diagnosed only occasionally (6.5%). Interestingly, *B. afzelii* and *B. garinii* are the most frequent genospecies isolated from ticks in the lakeside area, followed by *B. burgdorferi* ss (JOU DA *et al.* 2004a, MORAN CADENAS *et al.* 2007). In NAHIMANA *et al.* study (2000) undertaken in a larger population living in the whole French speaking part of Switzerland and based on a protocol comparable with our protocol, EM was recorded in only 53.4% of confirmed or probable Lyme borreliosis. The difference between both studies might be due for example to different risks of Lyme borreliosis in the studied areas. In fact, these risks have been shown to considerably vary from one area to another in Switzerland (JOU DA *et al.* 2004b).

When the diagnosis of Lyme borreliosis was considered as possible (Table 2), the terms used by the physicians to describe the various clinical manifestations shifted from precise definitions (i.e. “erythema migrans”) to less specific description (i.e. “erythema” or “redness”). This reflects the difficulties for physicians to link laboratory data (positive serology) and clinical diagnosis of Lyme borreliosis. Moreover, in the lake-side area, 10% of inhabitants can present a positive IgG Western blot despite absence of any symptoms or tick bites in the last 6 months (unpublished data). Thus, to exclude a diagnostic of Lyme borreliosis in case of positive serology and chronic or acute non-typical manifestations compatible with the disease may be difficult. Therefore, we did not take into account possible cases in order to maximize the specificity of the epidemiological results.

Highest incidences were observed in the human population living in a recognized endemic area (region 1) where tick density is very high (PERRET *et al.* 2000, PERRET *et al.* 2004, JOUDA *et al.* 2004a, MORAN CADENAS *et al.* 2007) and where infected tick density can reach 68 infected nymphs per 100 m² (JOU DA *et al.* 2004a). The highest incidence was observed in 1997 with 72.7 cases for 100'000 inhabitants, 1997 was a year with a very high tick density (PERRET *et al.* 2000), the highest observed during the period 1996-2008 (unpublished data).

The procedure used here to estimate the incidence of Lyme borreliosis, i.e. the retrospective use of positive Lyme borreliosis serology, may have biased the obtained incidences. In fact, these incidences are underestimated. First, all positive serological tests were not considered since 25% questionnaires were not returned. Secondly, serological tests have relatively low sensitivity at the early stage meaning that many patients presenting EM, but having no positive serological tests, were not included in the study. Thirdly, EM is easily identified without serological tests and moreover,

awareness of this infection is very high among physicians in Neuchâtel, many of them diagnose EM without serological testing. Fourthly, only half of physicians with private practice and considered as first aid doctors participated to this study.

Reported annual incidence rates throughout Europe range from 3.4/100 000 inhabitants (JENKINS *et al.* 2001) in Southern Norway to 80 cases for 100'000 in Southern Sweden (BERGLUND *et al.* 1995) and 120 cases for 100'000 in Slovenia (STRLE & STANTIC-PAVLINIC, 1996). So the incidence in Neuchâtel, particularly in the high endemic region 1, appears to be among the highest reported in Europe considering that the estimated incidence in Neuchâtel is largely underestimated.

To sum up, if we compare epidemiological and ecological data from the Neuchâtel area with data from Europe, we can conclude that Neuchâtel offers one of the highest tick density in Europe (PERRET *et al.* 2000, 2004, JOUDA *et al.* 2004a), with high density of infected ticks (JOU DA *et al.* 2004a,b) and one of the highest incidence of Lyme borreliosis. These results allowed us to identify a population who should benefit from further studies on risk to be infected by *B. burgdorferi* s.l after a tick bite.

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