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- Lyme borreliosis
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Abstract

Lyme *borreliosis* is an infectious disease caused by bacteria belonging to the genus *Borrelia*, transmitted to humans by the bite of infected hard ticks of the genus *Ixodes*. National surveillance data are very scarce across Europe. Contribution of biology proves essential for diagnosis in the late manifestations. Our aim was to investigate the retrospectively frequency of Lyme positive serologies obtained from two French laboratories, their geographical distribution and their evolution over time. Sera tested were those received at CERBA between January 2007 and December 2011 and Biomnis between 2010 and 2011. IgG and IgM serum isotypes were detected by EIA. Antibodies specificity was analysed by western blot. Between 2010 and 2011, 83 528 patient samples were analyzed and 5 800 patients had positive serology for Lyme disease in France. The standardized rate of positive Lyme serologies (PLS) observed in our study was 4.63 cases per 100,000 person-year in 2010-2011. The regions localized at the center, the East and the North-East of France had a high incidence of PLS whatever the year. These areas have a dense forest cover. They represent a favorable habitat for ticks as well as for human outdoor activities. Prevention should be strengthened in these regions particularly in the elderly population. Percentage of positive patients over year was correlated with the annual temperatures, suggesting that climate change may impact Lyme incidence. A strong increase of the incidence was observed between 2010 and 2011 in six regions requiring an enhanced monitoring in the future.

ABBREVIATIONS

PLS: Positive Lyme Serology; NLS: Negative Lyme Serology

INTRODUCTION

Lyme borreliosis is an infectious disease caused by bacteria belonging to the genus *Borrelia*. This genus includes several pathogenic species: *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia bavariensis* and *Borrelia spielmanii*; *Borrelia valaisiana* and *Borrelia lusitaniae* were also detected in human skin samples [1,2].

Borrelia is transmitted to humans by the bite of infected hard ticks belonging to a few species of the genus *Ixodes*. In Europe, *Ixodes ricinus* is the main vector of the disease. This tick does not harbor host specificity and feeds on more than 240 different species, however, it is found predominantly on birds, mammals and even reptiles [3]. In nature, small mammals, especially rodents are with birds, among the most important reservoir species of *Borrelia burgdorferi* providing both hosting and prolonged survival of the pathogen.

Lyme disease predominates in temperate climates and is the most common vector-borne infection in North America and Europe [4]. In contrast to the United States, where Lyme borreliosis is a notifiable disease, national surveillance data are very scarce across Europe [5]. Variations have been reported across European countries with less than 5 cases per 100,000 people in Ireland to 300-350 cases per 100,000 people in Austria, where the highest incidence is currently reported. Variation has also been observed within countries at the regional level. For example, in France, the incidence varies greatly depending on the region, with variation in regional incidence from 0/100,000 in the Mediterranean coastal areas and 86/100,000 in the Alsace north-eastern region [6]. The global average being of 9.4 cases per 100,000 people in a prospective work in 1999-2000 by 875 general practitioners participating in the "Sentinel" network [7]. Recent data based on the clinical and biological observations of a network of French general practitioners report an incidence of 42 cases per 100,000 people, with differences according to regions [8]. Local incidence of Lyme disease is directly correlated with the density of tick-vector populations [9].

Lyme borreliosis is a multi-system disorder affecting a wide range of tissues including skin, nervous system, joints, heart, and less frequently other organs [10]. The most common presenting symptom is the characteristic skin lesion: erythema migrans

(EM) that usually appears within 3-30 days after the infectious tick bite.

However, neurological manifestations and arthritis start within weeks or months after the initial infection or skin lesion. The clinical manifestations of neuroborreliosis observed in Europe are due to the geographic spread of *B. garinii* [10,11]. Arthritis is a late manifestation targeting large joints. It is mostly reported in North America dominated by *B. burgdorferi* sensu stricto species. Cardiac involvement has been reported during both the acute phase and the chronic stage, but is rare (<5%). Symptoms are improved with appropriate Lyme disease treatment [10].

Early Lyme disease is best diagnosed by recognizing an EM lesion, which is present in 70-90% of cases. Serology is often negative early in the disease course as it may take 4-6 weeks after contamination for IgM antibodies to *Borrelia* to appear and 6-9 weeks after contamination for IgG to be present [5,10]. Serological testing is used to confirm a clinician's suspicion of Lyme disease presenting with evidence of disseminated disease, such as arthritis, carditis or neurological involvement [5].

If the contribution of biology is low stage in early manifestations of the disease, it proves essential for diagnosis in the late manifestations. The difficulty of implementation and the disappointing results of culture and molecular diagnosis, explain instead why serology is used in the diagnosis of Lyme disease in current practice. The aim of our study was to investigate the retrospectively frequency of Lyme positive serologies obtained from two French laboratories CERBA and Biomnis, their geographical distribution and their evolution over time. Results showed that the regions localized at the center, the East and the North-East of France had a high incidence of PLS whatever the year. Percentage of positive patients over year was correlated with the annual temperatures, suggesting that climate change may impact Lyme incidence.

MATERIALS AND METHODS

Laboratory diagnosis

From 2007 to 2011, the French territory is composed of 95 departments and 22 regions (Table 1). CERBA and Biomnis laboratories cover most of the Lyme blood and CSF analyses performed in the French territory during the period 2010-2011. Biomnis laboratory was omnipresent in 6 regions (Bretagne, Bourgogne, Auvergne, Pays de la Loire, Rhône-Alpes and Franche-Comté) where as CERBA laboratory was omni present in 14 regions (Corse, Aquitaine, Picardie, Nord-Pas-de-Calais, Ile-de-France, Centre, Lorraine, Limousin, Haute-Normandie, Basse-Normandie, Champagne-Ardenne, Midi-Pyrénées, Poitou-Charente, Alsace) (Table 2).

Sera tested were those received at CERBA between January 2007 and December 2011 and Biomnis between 2010 and 2011 for biological diagnosis. The distribution of laboratories that sent sera to CERBA from Lyme disease suspected patients from the period 2007 to 2011 for diagnosis are presented in Figure (1).

Cerba

IgG and IgM serum isotypes were detected by EIA with the Enzygnost Lyme kits (Siemens, Germany) using micro titration plates coated with *B. afzelii* Pko strain antigens and in addition, for IgG, the recombinant VlsE protein. Dilution buffer contains an ultrasound sonicated antigen of *Treponema phagedenis* in order to limit interference with *T. pallidum*. IgG and IgM CSF isotypes were detected with the Vidas kit (bioMérieux, France). Antibodies specificity was analysed by using the Euroimmun western blot. The strips included separated proteins of a *B. afzelii* strain plus the recombinant VlsE protein. Thresholds in IgG and in IgM by EIA were, respectively, of 7.0 U/ml and of 0.7 U/ml. Results were interpreted at a weak, non-significant level, between 7.0 and 14.0 U/ml for IgG and between 0.7 and 1.3 U/ml for IgM. Serology was interpreted as positive if IgG was greater than 14.0 U/ml or IgM was greater 1.3 U/ml. Weak positives were not included as positive Lyme serology. Western blot interpretation is described in Table (3). This test was fully automated: depositing, reading by camera, score calculation and interpretation.An Internal Quality Control, made from a pool of positive serology sera, was used in each assay by EIA and in each assay by western blot. External Quality Controls were performed with the College of American Pathologists trials sent twice a year. From 2010 to 2011, another external quality control test was also performed. It was provided by the French organization "Prospective Biology" with 3 shipments per year. Screening serology and western blot for antibodies to *Borrelia* were accredited according to EN 15189 NF.

Biomnis

In 2010 and 2011, the laboratory Biomnis used Elisa kits Enzygnost Borreliosis (Lyme Enzygnost link VlsE / IgG. Enzygnost Borreliosis / IgM Siemens, Germany) for the detection of IgG and IgM in serum and CSF samples (*B.afzelii* Pko strain with VlsE recombinant protein in IgG kit). An intrathecal synthesis index was also calculated based on the comparison of the levels of antibodies with the IgG kit and assay of albumin and total IgG according to the recommendation of Siemens based on the formula of Reiber. The positive threshold was 10 U/mL of IgG and 1.0 for IgM index for serum (sera dilution to respective 1/231 and 1/42nd); confirmation of serology was determined above the significance level of 25 U/mL for IgG serum titer and 1.5 indexes for IgM. In CSF, the threshold for IgG was 10 U/mL and 1.2 for IgM index (the dosage of the two isotypes is performed on a ½ dilution of the CSF). Two quality controls close to the positive threshold (serum pools charged and tested in the laboratory) were analyzed in each Elisa series at random position. Screening for antibodies to *Borrelia* was accredited according to EN 15189 NF.

Confirmation serology was performed with the kit immunoblot Recom Line Mikrogen distributed by Diasorin. Only recombinant antigens were deposited on the immunoblots and *B.burgdorferi* sensu stricto, *B.afzelii*, *B.garinii*, *B.spielmanii* and *B.bavariensis* strains represented for antigens OspC, p18 and VlsE. Interpretative criteria are given in Table 4. These tests were fully automated: depositing, reading by camera, score calculation and interpretation. The external quality control was provided by the French organization "Prospective Biology" with 3 shipments per year.

Ninety four departments from twenty two regions of France participated in the study between 2010 and 2011. The

Table 1: List of French departments and corresponding regions.

Region	Department number	Department name	Region	Department number	Department name
			Languedoc-Roussillon	11	Aude
				30	Gard
Alsace	67	Bas-Rhin		34	Hérault
	68	Haut-Rhin		48	Lozère
Aquitaine	24	Dordogne		66	Pyrénées Orientales
	33	Gironde	Limousin	19	Corrèze
	40	Landes		23	Creuse
	47	Lot-et-Garonne		87	Haute Vienne
	64	Pyrénées Atlantiques	Lorraine	54	Meurthe-et-Moselle
Auvergne	3	Allier		55	Meuse
	15	Cantal		57	Moselle
	43	Haute-Loire		88	Vosges
	63	Puy-de-Dôme	Midi-Pyrénées	9	Ariège
Basse-Normandie	14	Calvados		12	Aveyron
	50	Manche		31	Haute-Garonne
	61	Orne		32	Gers
Bourgogne	21	Côte-d'Or		46	Lot
	58	Nièvre		65	Hautes Pyrénées
	71	Saône-et-Loire		81	Tarn
	89	Yonne		82	Tarn-et-Garonne
Bretagne	22	Côtes-d'Armor	Nord-Pas-de-Calais	59	Nord
	29	Finistère		62	Pas-de-Calais
	35	Ille-et-Vilaine	Pays-de-la-Loire	44	Loire Atlantique
	56	Morbihan		49	Maine et Lore
Centre	18	Cher		53	Mayenne
	28	Eure-et-Loir		72	Sarthe
	36	Indre		85	Vendée
	37	Indre-et-Loire	Picardie	2	Aisne
	41	Loir-et-Cher		60	Oise
	45	Loiret		80	Somme
Champagne-Ardennes	8	Ardennes	Poitou-Charente	16	Charentes
	10	Aube		17	Charente-Maritime
	51	Marne		78	Yvelines
	52	Haute Marne		79	Deux-Sèvres
Corse	20	Corse		86	Vienne
Franche-Comté	25	Doubs	Provence-Alpes-Côte d'azur	4	Alpes-de-Haute-Provence
	39	Jura		5	Hautes-Alpes
	70	Haute Saône		6	Alpes-Maritimes
	90	Territoire de Belfort		13	Bouche-du-Rhone
Haute-Normandie	27	Eure		83	Var
	76	Seine Maritime		84	Vaucluse
Ile-de-France	75	Paris	Rhone-Alpes	7	Ardèche
	77	Seine-et-Marne		1	Ain
	91	Essonne		26	Drôme
	93	Seine-Saint-Denis		38	Isère
	94	Val-de-Marne		42	Loire
	95	Val-d'Oise		69	Rhône
	92	Hauts-de-Seine		73	Savoie
				74	Haute-Savoie

Table 2: Laboratory coverage between 2010 and 2011.

Region	Biomnis	Cerba
Corse	1%	99%
Aquitaine	1%	99%
Picardie	3%	97%
Nord pas de Calais	5%	95%
Ile de France	4%	96%
Centre	6%	94%
Lorraine	9%	91%
Limousin	11%	89%
Haute Normandie	11%	89%
Basse Normandie	16%	84%
Champagne-Ardenne	28%	72%
Midi-Pyrénées	31%	69%
Poitou-Charentes	32%	68%
Alsace	39%	61%
Languedoc Roussillon	53%	47%
Provence-Alpes-Côte d'Azur	57%	43%
Bretagne	61%	39%
Bourgogne	72%	28%
Auvergne	76%	24%
Pays de la Loire	77%	23%
Franche Comté	91%	9%
Rhône Alpes	96%	4%

*(per 100,000 person-years)
** (per 100,000 person-years) [95%CI]

Table 3: Western blot interpretation criteria in IgM and in IgG (CERBA).

	Other specific positive proteins among: p83, p39, p21, p19 and p17	No other specific positive proteins
Positive OspC	Positivity in IgM	Positivity in IgM
Weak positive OspC	Positivity in IgM	Weak positivity in IgM
Negative OspC	Positivity in IgM	Negativity in IgM

	Two or more other specific positive proteins among: p83, p39, p21, p19 and p17	One other specific positive proteins among: p83, p39, p21, p19 and p17	No other specific positive proteins
Positive VlsE	Positivity in IgG	Positivity in IgG	Positivity in IgG
Weak positive VlsE	Positivity in IgG	Positivity in IgG	Weak positivity in IgG
Negative VlsE	Positivity in IgG	Weak positivity in IgG	Negativity in IgG

department of Mayenne (department 53, see Figure (1) for localization) was excluded from the analysis because of the low number of requests recorded (n=25). Analysis of the evolution of positive Lyme serology between 2007 and 2011 was performed in nine regions (Aquitaine, Centre, Corse, Haute-Normandie, Ile-de-France, Limousin, Lorraine, Nord Pas-de-Calais and Picardie) where an excellent coverage was provided by CERBA laboratory with more than 89% of requested patients' serology analysed.

DATA COLLECTION

The following data were collected: i) general characteristics (age, region, origin of requests), ii) biological data (IgG, IgM, Western Blot), iii) climate (monthly rainfall and maximum temperature) from the Météo France Register between 2007 and 2011, iv) data on the French population (population per region, per year and per age (≤ 19 years, between 20 and 39 years, between 40 and 59 years, between 60 and 74 years and ≥ 75 years)) from the INSEE Register between 2007 and 2011.

STATISTICAL ANALYSIS

Crude incidence rate of positive Lyme serology (PLS) was calculated as the number of positive serology cases divided by the number of residents for each department, region and year. Age-standardized PLS rates and 95% confidence interval (CI) were calculated for each region and year using the direct method based on the French population (INSEE). Regions of France

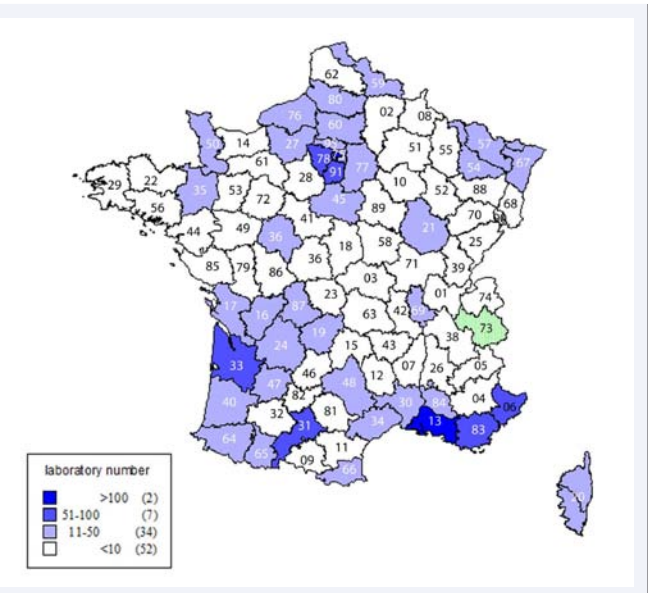

Figure 1 Distribution of laboratories that sent to CERBA sera from Lyme disease suspected patients from the period 2007 to 2011.

Table 4: Western blot interpretation criteria in IgM and in IgG (Biomnis).

First step: evaluation of antigen scores		
Antigen	**IgG scores**	**IgM scores**
p100	5	5
VlsE	5	5
p58	4	4
p41	1	1
p39	5	4
OspA	5	5
OspC	5	8
p18	5	5
Second step: total scores		
	IgG evaluation	IgM evaluation
≤ 5	negative	negative
6	equivocal	equivocal
≥ 7	positive	positive

were classified in 6 groups (< 2, between 2 and 3.99, between 4 and 6.99, between 7 and 9.99, between 10 and 19.99 and ≥ 20 cases per 100,000 person-years) corresponding to an incidence of PLS from 0 to more than 20 cases per 100,000 person-years. Temperature was expressed as mean and rainfall was expressed as median and range. Comparison of age was based on Mann & Withney test. Comparison of incidence rates was based on the Chi^2 test for incidence rate difference. Two-by-two comparisons were performed, followed by the Bonferroni correction. Rainfall between 2009 and 2011 was compared by the Wilcoxon matched-pairs signed-ranks test. A p value of ≤ 0.05 was considered to denote statistical significance. Data were analysed with STATA software version 12.0 (Stata Corporation, College Station, Texas).

IXODES RICINUS' SAMPLING

Tick sampling was achieved through the flag method that collects the questing ticks. This method uses a lure that mechanically simulates a host's passage. The collector drags a square of fabric measuring $1m^2$ over a distance of 10 meters at a rate of 50cm per second, in order to collect ticks in an area of $10m^2$. This method is repeated 16 times randomly within each plot. They were collected in Alsace (2003-2004), in Aquitaine (2010-2011), in Auvergne (2005-2006), in Franche-Comté (2010-2011), in Limousin (2005-2006) and in Lorraine (2003-2011).

Ixodes ricinus' density

Ticks' density, expressed in number of ticks per 100 m^2, is estimated from the total collected ticks' number according to Ferquel et al, [12].

Infection rates

The ticks' infection rate is calculated using the following formula: p = f / k

f: number of ticks infected with *B. burgdorferi* sensu lato in each plot

k: number of ticks analyzed in each plot

RESULTS

Incidence of positive Lyme serologies in France in the 2010-2011 periods

Between 2010 and 2011, 47, 428 requested patients' serologies were analysed by CERBA laboratory and 36,100 by Biomnis laboratory (Table 5). 23% of requests came from hospital. 8.8% of patients were under 15 years and the median age was 49 years [33-63] (Table 6).

5,800 (6.9%) patients were positive in the IgG and/or the IgM screening confirmed by western blot (4.5% of patients coming from hospital and 5.7% of patients coming from local laboratories; 8.9% of patients with unknown source). Patients with positive Lyme serology (PLS) were older than patients with negative Lyme serology (NLS) (57 [44-67] versus 49 [33-62], p<0.001). 5.2% of patients with an age less than 15 years were positive (Table 6). The age-standardized PLS rate was 4.63 cases per 100,000 person-years and varied from 0.60 to 24.70 according to the region. The incidence of PLS was particularly high in the region of Limousin (24.70 cases per 100,000 person-years) followed by the region of Auvergne, Rhône-Alpes and Alsace (14.60, 11.19 and 10.92 cases per 100,000 tiperson-years, respectively) (Table 7). In the region of Limousin and Auvergne, the incidence of PLS was particularly high and was consistent over departments (from 18.87 to 38.56 cases per 100,000 person-years in Limousin and from 13.26 to 25.29 cases per 100,000 person-years in Auvergne). However, in most cases, large differences could be observed in the same area according to the department. In Alsace, the incidence was three times higher in Bas-Rhin than in Haut-Rhin (14.80 versus 4.59 cases per 100,000 person-years, p<0.001). In Lorraine, the incidence was twice higher in Meuse (30.15 cases per 100,000 person-years) than the other departments (Vosges, Moselle and Meurthe-et-Moselle with 13.69, 7.70, and 5.53 cases per 100,000 person-years, respectively) (p<0.001). In Rhône-Alpes, a high incidence was observed in five of the eight departments (from 11.16 to 21.17 cases per 100,000 person-years in Savoie and Ain, respectively) whereas an average incidence was observed in Drôme, Rhône and Isère (5.44, 7.24 and 7.47 cases per 100,000 person-years, respectively) (Figure 2).

Table 5: Total request patients' serology.

Laboratory	2007	2008	2009	2010	2011	Total
Cerba	22 037 (100%)	23 253 (100%)	23 184 (100%)	22 513 (58%)	24 915 (56%)	115 902 (76%)
Biomnis				16 215 (42%)	19 885 (44%)	36 100 (24%)

Table 6: Age of patients for which a Lyme serology was requested.

	Lyme serology		Total	p-value
	Negative (n=77 728)	Positive (n=5 800)		
Age (years)				
Mean (SD)	47.1 (20.6)	53.8 (19.3)	47.5 (20.6)	<0.001
Median	49	57	49	
Q1-Q3	33-62	44-67	33-63	
≤ 15 years	6 945 (8.9%)	382 (6.6%)	7 327 (8.8%)	<0.001
> 15 years	70 622 (91.1%)	5 409 (93.4%)	76 031 (91.2%)	

Table 7: Distribution of the population and PLS cases between 2010 and 2011.

Region	Population	Number of PLS	Crude PLS rate[a]	Adjusted PLS rate[b]
Corse	309 693	4	0.64	0.60 [0.58-0.61]
Nord-Pas-de-Calais	4 038 157	53	0.66	0.68 [0.67-0.69]
Haute-Normandie	1 836 954	39	1.06	1.07 [1.06-1.08]
Ile de France	11 786 234	288	1.22	1.29 [1.29-1.30]
Provence-Alpes-Côte d'Azur	4 899 155	155	1.58	1.52 [1.52-1.53]
Languedoc Roussillon	2 636 350	111	2.09	2.02 [2.01-2.03]
Centre	2 548 065	136	2.66	2.61 [2.59-2.62]
Pays de la Loire	3 265 168	175	2.67	2.68 [2.67-2.70]
Picardie	1 914 844	114	2.97	3.04 [3.02-3.06]
Bretagne	3 199 066	204	3.17	3.11 [3.10-3.12]
Basse Normandie	1 473 494	105	3.56	3.47 [3.45-3.49]
Midi-Pyrénées	2 881 756	220	3.80	3.66 [3.65-3.68]
Poitou-Charentes	1 770 363	143	4.03	3.78 [3.76-3.80]
Champagne-Ardenne	1 335 923	119	4.46	4.42 [4.39-4.44]
Aquitaine	3 232 352	339	5.22	4.94 [4.92-4.96]
Bourgogne	1 642 115	293	8.91	8.46 [8.43-8.49]
Franche Comté	1 171 763	228	9.71	9.61 [9.57-9.64]
Lorraine	2 350 920	463	9.84	9.79 [9.76-9.81]
Alsace	1 845 687	394	10.66	10.92 [10.89-10.95]
Rhône Alpes	6 230 691	1381	11.03	11.19 [11.17-11.21]
Auvergne	1 347 387	422	15.64	14.60 [14.56-14.64]
Limousin	742 771	414	27.84	24.70 [24.64-24.76]

[a](per 100,000 person-years)
[b](per 100,000 person-years) [95%CI]

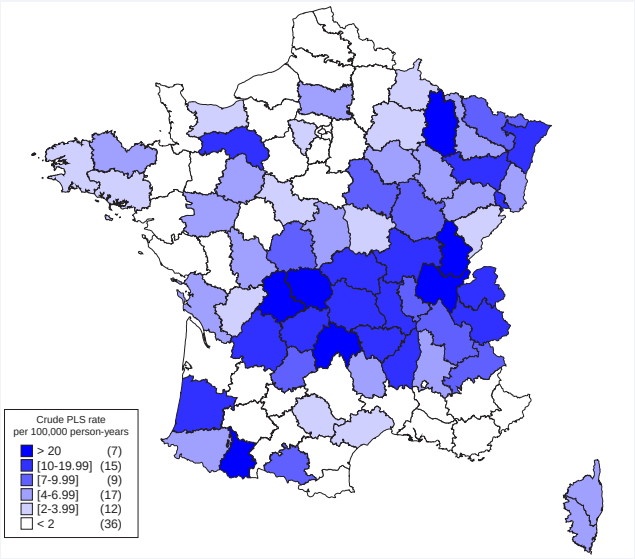

Figure 2 Crude PLS rate according to French departments for the period 2010-2011. The geographical origin of patients with positive Lyme serology was determined from the postal code of the patients. The geographical areas were classified into six categories corresponding to the crude PLS rate per 100,000 person-years as indicated in the figure.

The geographical origin of patients with positive Lyme serology was determined from the postal code of the applicant laboratory. The geographical areas were classified into six categories corresponding to the crude PLS rate per 100,000 person-years as indicated in the Figure (3).

Evolution of the incidence of positive Lyme serologies between 2010 and 2011

The incidence of PLS was significantly higher in 2011 (5.28 versus 3.98 cases per 100,000 person-years, p< 0.001). An increase of the incidence was observed in most of regions and was particularly high in six regions (Rhône-Alpes (+5.34),

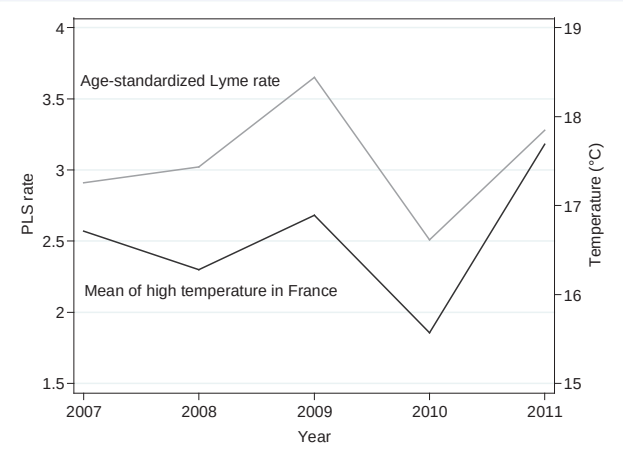

Figure 3 Evolution of the PLS rate and the mean of maximum temperature in France between 2007 and 2011. Evolution of positive Lyme serology was followed between 2007 and 2011 in nine regions (Aquitaine, Centre, Corse, Haute-Normandie, Ile-de-France, Limousin, Lorraine, Nord Pas-de-Calais and Picardie). Climate (monthly rainfall and maximum temperature) were obtained from the Météo France Register between 2007 and 2011.

Lorraine (+4.07), Auvergne (+3.97), Picardie (+3.65), Limousin (+3.55) and Champagne-Ardenne (+3.35)) (Table 8). The region of Limousin had the highest PLS rate in France followed by Auvergne whatever the year. The region of Rhône-Alpes became the third highest PLS rate in 2011. The incidence of PLS in Alsace remained stable whereas a strong increase was observed in Lorraine (+4.07 cases per 100,000 person-years) (Table 8).

While a significant increase was observed in two departments of Limousin, a sharp decline was observed in Corrèze (-11.53 cases per 100,000 person-years) (Table 9). In Auvergne, the incidence rose sharply in three departments (from + 4.93 to + 9.78 cases per 100,000 person-years) while it remained stable in Puy-de-Dome. The incidence of all departments from Rhône-Alpes increased and the two largest increases were recorded in Haute-Savoie and Ain with an increase of 11.33 and 9.81 cases per 100,000 person-years, respectively. The same scenario was observed in Champagne-Ardenne and the two largest increases were recorded in Haute-Marne (+ 5.44 cases per 100,000 person-years) and Aube (+ 4.95 cases per 100,000 person-years) (Table 9). In Lorraine, the incidence remained stable in Meuse whereas a strong increase was observed in the other departments, the highest being recorded in Vosges (+ 5.77 cases per 100,000 person-years). The strong increase observed in Picardie (+ 3.65 cases per 100,000 person-years) was mainly due to the increase observed in only one department (Oise with an incidence ranging from 1.24 to 9.43 cases per 100,000 person-years) (Table 9).

Evolution of the incidence of positive Lyme serologies between 2007 and 2011 in 9 regions

Between 2007 and 2011, 4 403 (6.7%) patients were positive in the IgG and/or the IgM screening confirmed by western blot. Patients with PLS were older than patients with NLS (57 [44-69] versus 48 [32-62], p<0.001). The age-standardized PLS rate was 3.07 cases per 100,000 person-years. The age-standardized PLS rate varied from 2.52 to 3.65 cases per 100,000 person-years according to the year. A significant increase was observed in 2009 to reach a peak of 3.65 (p< 0.001) followed by a significant decrease in 2010 where the lowest crude PLS rate was observed (p< 0.001).

Table 8: Adjusted PLS rate per year.

Region	Adjusted PLS rate per 100,000 person-years		Variation
	2010	2011	
Corse	0.38	0.81	+0.43
Nord pas de Calais	0.71	0.65	-0.06
Haute Normandie	0.93	1.20	+0.27
Ile de France	1.22	1.36	+0.14
Provence-Alpes-Côte d'Azur	1.33	1.71	+0.38
Languedoc Roussillon	1.79	2.25	+0.46
Centre	2.93	2.29	-0.64
Pays de la Loire	2.65	2.71	+0.06
Picardie	1.21	4.86	+3.65
Bretagne	2.40	3.81	+1.41
Basse Normandie	3.25	3.69	+0.44
Midi-Pyrénées	3.86	3.47	-0.39
Poitou-Charentes	2.73	4.83	+2.10
Champagne-Ardenne	2.74	6.09	+3.35
Aquitaine	4.39	5.49	+1.10
Bourgogne	7.19	9.73	+2.54
Franche Comté	10.05	9.17	-0.88
Lorraine	7.75	11.82	+4.07
Alsace	10.74	11.10	+0.36
Rhône Alpes	8.51	13.85	+5.34
Auvergne	12.62	16.59	+3.97
Limousin	22.93	26.48	+3.55

Table 9: Crude PLS rate per year and department.

Region and Department	Crude PLS rate[a]	
	2010	2011
Limousin		
- Corrèze	24.64	13.11
- Creuse	34.14	42.98
- Haute-Vienne	24.72	35.54
Auvergne		
- Allier	10.79	15.72
- Cantal	21.60	28.96
- Haute Loire	11.16	20.94
- Puy-de-Dome	14.08	14.99
Rhône Alpes		
- Ain	16.24	26.05
- Ardèche	12.06	19.85
- Drôme	4.54	6.34
- Haute Savoie	9.35	20.68
- Isère	5.31	9.61
- Loire	12.68	17.20
- Rhône	6.03	8.45
- Savoie	7.95	14.33
Lorraine		
- Meurthe-et-Moselle	3.96	7.09
- Meuse	29.39	30.90
- Moselle	5.36	10.03
- Vosges	10.80	16.57
Champagne-Ardenne		
- Haute-Marne	2.72	8.16
- Aube	3.63	8.58
- Marne	2.83	5.13
- Ardennes	1.77	4.24
Picardie		
- Oise	1.24	9.43
- Aisne	0.93	1.48
- Somme	1.40	1.22

[a](per 100,000 person-years)

Evolution of the temperature and the rainfall between 2007 and 2011

The mean of maximum temperature varied from 15.57 to 17.69°C. The two years with the highest temperature were 2009 (16.89°C) and 2011 (17.69°C). The median rainfall was significantly higher in 2011 than 2009 during this period (72.4 versus 56.1 mm in July, (p=0.0099) and 71.0 versus 27.0 mm in August, (p=0.0011)). The Figure (3) shows a parallel evolution between the mean of maximum temperature in the nine regions and the age-standardized PLS rate observed in CERBA laboratory.

DISCUSSION

Between 2010 and 2011, 83,528 patient samples were analyzed and 5,800 patients (6.9%) had positive serology for Lyme disease in France. The standardized rate of PLS observed in our study was 4.63 cases per 100,000 person-year in 2010-2011, which is well below the 9.4 cases per 100,000 people observed in a study conducted in France in 1999-2000 [9,13]. Several reasons may explain this difference. First, large variations can be observed from one year to the next. In our study, the observed PLS rate is actually increased from 3.98 in 2010 to 5.28 cases per 100,000 person-years in 2011. The year 2010 was one of the coldest years of the past two decades in France, reducing probably human exposure due to lower recreational activities. Secondly, the observed incidence in our study is probably underestimated due to the lack of consideration of all erythema migrants while they represent 85% of cases of Lyme borreliosis cases. This result is based on the recommendations of [10], who advocate not making any serology in patients with erythema migrans. However, our studies and others have shown that 50 to 72% of patients presenting erythema migrans have positive Lyme serologies [14]. Finally, although CERBA and Biomnis laboratories cover a major part of France, the samples from hospitals especially University Hospitals that have their own analytical laboratory are not taken into account. In our study, only 23% of patients were from hospitals.

Our study showed that patients with PLS were older than patients with negative Lyme serology (57 [44-67] versus 49 [33-62], p<0.001). This observation was also reported by Letrilliard et al. [13], and might be explained by recreational activities (walks in forest, fishing, and gardening activities) that are more developed in the senior population making them more likely to be in contact with infected ticks [15]. Although the majority of cases concern an elderly population, 8.8% of patients younger than 15 years old had a Lyme serology and 5.2% were positive. 25% of children with a positive result came from the Rhône-Alpes.

The regions of Limousin, Auvergne, Rhône-Alpes and Alsace had a very high incidence of PLS whatever the year. These regions correspond to the areas with the maximum measure of the photosynthetic activity of the vegetation [16]. Therefore, these areas with a dense forest cover represent a favorable habitat for ticks as well as for human outdoor activities. Moreover, surveillance activities on tick have been done in the past in some regions of France from 2003 to 2011 (Reference Centre for *Borrelia*, Institut Pasteur). Figure (4) shows the values of the density of infected nymphs in the investigated regions in parallel with the crude PLS rate. We can see that the incidence of PLS rate and the density of infected nymphs per 100m^2 collected in the same regions follow a similar trend.

A strong increase of the incidence was observed in 4 of the 5 regions with the highest incidence (Rhône-Alpes, Lorraine, Auvergne, and Limousin) with the exception of Alsace, as well as in Picardie and Champagne-Ardenne in 2011. While a single department was involved by the increase in Picardie (Oise), all departments were concerned in Champagne-Ardenne, Auvergne, Lorraine and Rhône-Alpes with different levels. This increase is mainly explained by climate changes with an average temperature increase of 2°C. Indeed, Eisen et al. [17],

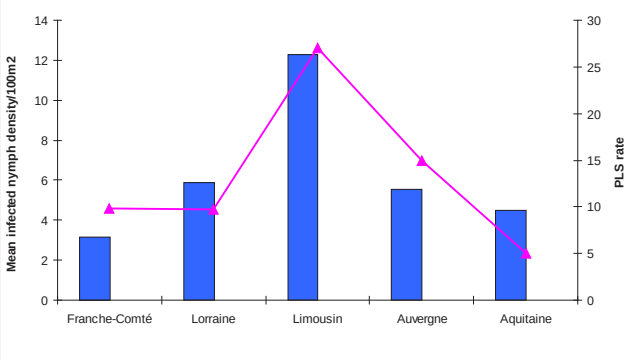

Figure 4 Comparison of PLS rate and the density of infected nymphs in some of the investigated regions.

pointed out those meteorological variables are most influential in determining host-seeking behavior. The increase in Picardie is also explained by the increase in the number of laboratories working with CERBA in 2011 (+ 6 laboratories) in Oise. Although most of PLS cases concern an elderly population with a sedentary lifestyle, some cases could be in contact with infected ticks in a region different from the region where the request was analyzed.

Our study describes the geographical distribution and evolution of 5 years (between 2007 and 2011) of positive serology for Lyme disease in 9 regions in France. Percentage of positive patients over year was correlated with the annual temperatures. The same trend of reported cases of Lyme disease was observed in United States between 2007 and 2011 [18]. The exception is however observed in 2011 during which the high annual temperature recorded was not associated with a higher PLS rate than the one observed in 2009. Beside temperature, rainfall is also key parameter impacting Lyme disease incidence. July and August are usually correlated with high tick bite incidence rates. In 2011, the increase in rainfall observed during this period could have resulted in a diminution of the recreational activities leading to a lower human exposure to tick bites. These results show that climate change may impact human-ticks interactions leading to variation in disease incidence [16]. We have no information on the role played by birds and rodents in the variation of tick numbers and thus the incidence rates. No correlation was found between the rate of PLS and population of large ungulates.

CONCLUSION

A low rate of patients with positive Lyme borreliosis serology was observed in France between 2010 and 2011 but with large differences between regions and between years. The regions of Limousin, Auvergne, Rhône-Alpes and Alsace had a very high incidence of Lyme disease whatever the year. Prevention strategies should be established in these regions particularly in the elderly population. A strong increase of the incidence was observed in Champagne-Ardenne and Picardie in 2011, requiring an enhanced monitoring in the future. Climate change is one of the factors that may impact interaction between ticks and humans in the near future. Therefore, prevention strategies should be established in the regions at risk where optimal conditions (high temperature, dense forest cover, rainfall ...) for an increase of Lyme incidence are present.

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