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Seroprevalence of seven pathogens transmitted by the *Ixodes ricinus* tick in forestry workers in France

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Abstract

In order to assess the level of occupational exposure to the main pathogens transmitted by the \textit{Ixodes ricinus} tick, a seroprevalence study was performed on serum samples collected in 2003 from 2,975 forestry workers of North-Eastern France. The global seroprevalence estimated for the seven pathogens studied was 14.1\% (419/2,975) for \textit{B. burgdorferi} s.l, 5.7\% (164/2,908) for \textit{F. tularensis}, 2.3\% (68/2,941) for TBEV, 1.7\% (50/2,908) for \textit{A. phagocytophilum}, and 1.7\% (48/2,908) for \textit{B. henselae}. The seroprevalence of \textit{B. divergens} and \textit{B. microti} studied in a subgroup of subjects seropositive for at least one of these latter pathogens was 0.1\% (1/810) and 2.5\% (20/810) respectively. \textit{B. burgdorferi} s.l seroprevalence was significantly higher in Alsace and Lorraine and \textit{F. tularensis} seroprevalence was significantly higher in Champagne-Ardenne and Franche-Comté. The results of this survey also suggest low rates of transmission of \textit{B. henselae} and \textit{F. tularensis} by ticks and a different West/East distribution of \textit{Babesia} species in France. The frequency and potential severity of these diseases justify continued promotion of methods of prevention of \textit{I. ricinus} bites.

Key-Words: \textit{Ixodes ricinus}, zoonotic agents, occupational exposure, seroprevalence, France

Introduction

Ticks are blood-feeding arthropods responsible for the transmission of numerous infectious agents that are pathogenic for man and/or animals. \textit{Ixodes ricinus} is the most widespread tick species in Europe and constitutes...
the vector of numerous pathogens, especially *Borrelia burgdorferi* sl, *Anaplasma phagocytophilum*, tick-borne encephalitis virus (TBEV), *Babesia* spp., and some *Rickettsia* species [1]. It is also involved in the transmission of *Francisella tularensis* and possibly *Bartonella henselae* [2, 3].

*I. ricinus* is present in almost all regions of France, in biotopes with persistent relative humidity (leafy forest floor, tall grasses, broadleaf and conifer forests) [4-6]. The high level of infestation of *I. ricinus* by infectious agents plays a leading role in the epidemiology of tick-borne diseases [7]. The estimated incidence of Lyme borreliosis in France from 2009 to 2012 was 42 per 100,000 inhabitants (an estimated average of 26,500 cases per year) [8]. Several human cases of tick-borne encephalitis are regularly reported, and cases of human anaplasmosis, babesiosis and tularemia after tick bites have been published in France [9-13].

In Europe, Lyme borreliosis is the most common tick-borne disease, with a mean annual number of cases, estimated by a review in 2009, between 65,500 and 80,500 and an incidence rate that may be higher than 200 cases per 100,000 inhabitants in some countries such as Slovenia [14-15]. The second most common disease is tick-borne encephalitis with an average of 3,000 cases per year for all European endemic countries and an incidence exceeding 10 cases per 100,000 inhabitants in Estonia and Slovenia [16]. Between 70 and 100 confirmed or probable cases of anaplasmosis have been reported since 1997, especially in Slovenia and Scandinavia and almost 60 cases of babesiosis (including about 40 cases of *B. divergens*) have been reported [10, 12, 17, 18]. Ticks represent an important vector of tularemia in some European countries (Switzerland, Slovakia) [19]. Recent data have shown that ticks could be able to transmit *B. henselae* to man: detection of *B. henselae* DNA in ticks removed from infected subjects, description of a few possible human cases of *B. henselae* infection after tick bites, demonstration of the capacity of *I. ricinus* to transmit *B. henselae* by means of a tick artificial nutrition method [3, 20-22].

Human seroprevalence studies conducted in workers exposed to tick bites have essentially concerned *B. burgdorferi* sl, TBEV and *A. phagocytophilum*. Only three studies have been conducted in forestry workers in Europe to simultaneously evaluate the seroprevalence of other tick-borne pathogens [23-25]. The objective of the present study was to estimate the seroprevalence of seven pathogens transmitted by *I. ricinus* in workers exposed to ticks (*B. burgdorferi* sl, TBEV, *A. phagocytophilum*, *F. tularensis*, *B. henselae*, *B. microti* and *B. divergens*) in...
order to assess the frequency of human exposure to these different microorganisms in a region of high exposure
to tick-borne pathogens in France [7, 8].

Method

Recruitment and inclusion of subjects

This cross-sectional study was conducted among forestry workers in North-Eastern France. Subjects were
recruited by Mutualité Sociale Agricole occupational health physicians from December 2002 to December 2003
in the Alsace, Lorraine, Champagne-Ardenne, Franche-Comté and Bourgogne regions.

Forestry workers (woodcutters, silviculturists, forest rangers, fishing guards, game wardens, personnel with
multiple forestry activities at least including woodcutting or silviculture, forestry machinery operators, sawmill
personnel, wood sellers or gardeners) were invited to participate in the study at the time of occupational health
surveillance examinations performed during the study period. Subjects with an immunodeficiency were
excluded. Occupational health physicians interviewed forestry workers after obtaining their signed informed
consent by using a standardized, anonymous questionnaire to record demographic and occupational data,
including age, gender, place of residence and occupation over the previous twelve months, as well as previous
yellow fever, Japanese encephalitis and tick-borne encephalitis immunizations. A blood sample for serology was
drawn after the interview. This study was approved by our local ethical committee (CCPRB, 29 avenue du
Maréchal de Lattre de Tassigny, Nancy).

Laboratory samples

Anonymous blood samples (7 to 10 ml of blood) stored at room temperature were centrifuged within a maximum
of 12 hours after collection, sera were then frozen at -20°C within 2 hours after centrifugation and stored at -30°C within 12 hours until analysis. For each sample, a first tube was used in 2003 to perform B. burgdorferi sl
serologies. A second tube, also stored at -30°C, was thawed in 2007 and divided into five aliquots, three of
which were used to perform TBEV, A. phagocytophilum and F. tularensis serologies. Two aliquots stored at -30°C were subsequently used for B. henselae (in 2010) and Babesia (in 2011) serologies.
**Serological tests**

- Screening for anti-*Borrelia burgdorferi* IgG was performed by ELISA (Enzygnost® Borreliosis, Siemens®). Doubtful or positive sera were tested by TPHA and VDRL to eliminate cross-reactions related to *Treponema pallidum* and by Western blot for confirmation according to European guidelines [26].

- Screening for anti-TBEV IgG was performed by ELISA (Immunozym FSME, PROGEN®) on serum from subjects who declared that they had not been vaccinated against tick-borne encephalitis, Japanese encephalitis or yellow fever.

- Screening for anti-*Anaplasma phagocytophilum* IgG antibodies was performed by ELISA using a recombinant protein P44 antigen [27]. Doubtful or positive sera were retested by IFA (Anaplasma phagocytophilum IFA IgG/IgM kit, Focus®) (positive cut-off = 1/64).

- Screening for anti-*Francisella tularensis* IgG was performed by tube agglutination of *F. tularensis* antigen particles (BD®). Sera positive for *F. tularensis* were then tested for *Yersinia* (Yersinia ELISA IgA kit, Verion/Serion®) and *Brucella* by agglutination (Brucella Rose Bengal, Biorad® and Brucella Wright, Biorad® kits). Doubtful or positive sera in relation to *Yersinia* or *Brucella* were excluded from the analysis.

- Screening for anti-*Babesia microti* and anti-*Babesia divergens* IgG antibodies was performed by IFA (positive cut-offs 1/64 and 1/128, respectively). Due to the presumed low seroprevalence as reported in the literature for these 2 pathogens and the amount of human workload of IFA serology, these tests were only performed on sera positive for *B. burgdorferi* sl, TBEV, *A. phagocytophilum* or *F. tularensis*.

- Screening for anti-*Bartonella henselae* IgG was performed by ELISA. In order to increase the sensitivity of this test, the antigen used was a mixture of four strains of *Bartonella henselae*: BhI (Houston-1 G5436^T, ATCC 49882T), BhII (Marseille, strain URLLY 8, CIP 104756), Berlin-1 (isolated from the cutaneous bacillary angiomatosis lesions of an HIV-infected patient), Berlin-2 (isolated from the blood of a pet cat).

**Statistical analysis**

All data were entered with Ethnos® software and exported to Excel® 2007. For analysis of the results, doubtful serological results in relation to the various pathogens tested were classified as negative.

A descriptive analysis of the subjects included and calculation of seroprevalence rates for each pathogen were performed. Seroprevalence rates were compared by age-group, region and occupational group by Chi-square test
Results

Description of the population

Of the 5,461 subjects invited to participate in the study, 3,033 replied and 2,975 were finally included (2,916 men and 59 women). Subjects excluded from the study were personnel with little or no forestry exposure (39), subjects refusing to sign the informed consent form (11), with an intercurrent disease or receiving immunosuppressive therapy (4), or for whom the questionnaire or blood samples were not available (4). The subjects included worked in the five regions of the study, distributed throughout the territory of the study (Figure 1). The participation rate and characteristics of the subjects differed slightly according to the region (Table 1).

*B. burgdorferi* sl and TBEV serology was available for all subjects included (2,975) and *A. phagocytophilum, F. tularensis* and *B. henselae* serology was available for 2,908 subjects (the volume of serum was insufficient to perform all three additional serologies for 67 subjects).

*B. burgdorferi* sl seroprevalence

Of the 2,975 subjects tested for *B. burgdorferi* sl, 419 were positive, corresponding to a seroprevalence rate of 14.1%. Seroprevalence increased significantly with age, reaching a maximum of 25.1% (85/338) for subjects 55 years and older (p<10^{-4}) (Fig. 2a), and was significantly higher in woodcutters (17.5%, 200/1,145) compared to other occupations and in Alsace (26.9%, 171/636) and Lorraine (16.5%, 146/885) compared to the other three regions studied (Table 2).
After adjustment for age and region of residence, seroprevalence was significantly higher in woodcutters and/or silviculturists. Alsace, Lorraine and Champagne-Ardenne presented a higher seroprevalence compared to Franche-Comté (Table 3).

**TBEV seroprevalence**

After exclusion of subjects who reported having been vaccinated against TBE virus, Japanese encephalitis or yellow fever (34), 68 of the 2,941 sera were positive for TBEV, corresponding to a seroprevalence rate of 2.3%. Only age was significantly associated with the subject’s serological status ($p = 0.001$): the proportion of seropositive subjects was lower among young subjects (0.9%, 7/748 for subjects younger than 35 years) and higher among older subjects (4.8%, 16/333 for subjects 55 years and older) (Fig. 2b and Table 2).

**A. phagocytophilum seroprevalence**

Of the 2,908 subjects tested for *A. phagocytophilum*, 50 were positive, corresponding to a seroprevalence rate of 1.7%. No significant association was demonstrated between the subject’s serological status and age, occupation or region of residence (Fig. 2c and Table 2).

**F. tularensis seroprevalence**

Of the 2,908 subjects tested for *F. tularensis*, 197 were positive and none of these subjects were simultaneously positive for *Brucella*; 33 sera were also positive or doubtful for *Yersinia* and were excluded from the analysis. The seroprevalence rate was therefore 5.7% (164/2,875) and was significantly associated ($p<10^{-4}$) with occupation, reaching 9.2% (63/684) among silviculturists. Significant regional variations were also observed ($p<10^{-4}$): from 3.6% in Alsace and Lorraine to 11.2% (53/474) in Champagne-Ardenne (Table 2). After adjustment for other factors, seroprevalence was significantly higher among silviculturists and in the Champagne-Ardenne and Franche-Comté regions (Table 4).

**B. henselae seroprevalence**
Of the 2,908 subjects tested for B. henselae, 48 presented positive serology, corresponding to a seroprevalence rate of 1.7%. No significant association was demonstrated between the subject’s serological status and age, occupation or region of residence (Fig. 2e and Table 2).

**B. microti** and **B. divergens** seroprevalence

Among the 810 subjects who were seropositive for B. burgdorferi sl or TBEV or A. phagocytophilum or F. tularensis, only 1 was positive for B. divergens and 20 were positive for B. microti, corresponding to a seroprevalence rate of 0.1% (1/810) and 2.5% (20/810) respectively in this subgroup. A higher proportion of seropositive subjects was observed among subjects 55 years and older, silviculturists, and subjects living in Lorraine and Champagne-Ardenne (Fig. 2f and Table 2).

**Multiple seropositivities**

Among the subjects tested for both B. burgdorferi sl, TBEV, A. phagocytophilum, B. henselae and F. tularensis, the serology was positive for a single pathogen in 21.5% (627/2,908) subjects and for 2 pathogens (other than Babesia) in 2.0% (57/2,908), especially B. burgdorferi sl together with F. tularensis (22/57), TBEV (11/57), B. henselae (9/57) or A. phagocytophilum (7/57) (Figure 3). Age was significantly associated with the multiple serological status (p=0.001): the proportion of subjects with multiple seropositivities was lower among subjects younger than 35 years (0.8%, 6/739) and higher among subjects older than 55 years (4.6%, 15/329). Significant regional variations were also observed (p<10^{-4}): from 0.8% (4/512) in Franche-Comté to 4.0% (25/623) in Alsace.

**Discussion**

In this study, B. burgdorferi sl seroprevalence among forestry workers was 14.1%. This seroprevalence rate was considerably higher than that of the other pathogens studied: 5.7% for F. tularensis, 2.3% for TBEV, 1.7% for A. phagocytophilum and 1.7% for B. henselae, respectively. The overall seroprevalence of B. divergens and
B. microti was 2.6% but was only calculated among subjects seropositive for at least one other pathogen, which could overestimate the prevalence.

Since date of the sample collection, some factors could have possibly lead to an increase of prevalence of these pathogens in ticks: climate change, increase of the population size of wild animals hosting ticks: deers roe deers, wild boars (data from 2002/2003 to 2014/2015 provided by the Fédération des Chasseurs and the Office National de la Chasse et de la Faune Sauvage), modifications in agricultural and forest landscapes that could lead to a higher density of tick populations [28-30].

Since only one human case of Rickettsia helvetica infection has been described in France up to now and that Rickettsia slovaca infections have been reported mostly from Southern France and are transmitted by Dermacentor ticks, seroprevalence of Rickettsia spp. among forestry workers was not investigated in our study [31, 32].

Classification of seropositive subjects was optimized by the use of validated laboratory tests and by the exclusion of cases with a suspected cross-reaction with other pathogens.

This cross-sectional seroprevalence study among forestry workers mainly reflects old or recent exposure to an infectious agent, based on the generally prolonged persistence of antibodies detectable in blood, without taking into account the clinical expression of the disease and its mode of transmission. Although a transmission of F. tularensis and B. henselae by tick bites has been hypothesized in the literature, the results of this study did not confirm or infirm a possible tick transmission of these pathogens for which human infection usually occurs by direct skin contact with the animal or by inoculation (scratch or bite) respectively [13,33].

Other studies of B. burgdorferi sl seroprevalence in Europe have also been conducted among occupationally exposed workers using various techniques and kits (ELISA with or without western blot confirmation, IFA, with or without differential detection of IgG and IgM antibodies) and based on very variable sample sizes, thereby limiting comparisons. In studies using western blot analysis, the seroprevalence of anti-B. burgdorferi IgG antibodies was 9.4% in Romania and varied significantly according to regions from 0.7% to 23.2% in Italy [34-36]. The seroprevalence, without distinguishing IgG and IgM, was 18.1% in The Netherlands, 28.0% in Poland.
and 34.6% in Germany [37-39]. Studies based on the use of ELISA alone reported IgG seroprevalence rates of 23.8% in Slovenia, 33.1% in Germany and 35% in Switzerland [25, 40, 41].

The high *B. burgdorferi* sl seroprevalence observed in our study is similar to that reported by other previous surveys among occupationally exposed workers in France [42, 43]. Our study shows a predominance of this seroprevalence in the two most North-Eastern regions of France. In particular, the high prevalence in Alsace is concordant with the tick density and infestation rates reported in this region, which are among the highest in Europe, the densities of some animal hosts (we consider the number of roe deer and wild boar killed by hunters - tableau de chasse 2002/2003 provided by The Fédération des Chasseurs and The Office National de la Chasse et de la Faune Sauvage) and the incidence of human Lyme borreliosis (154 cases per 100,000 inhabitants), which is one of the highest rates of all Western and Central European countries [7, 8, 30, 44].

TBEV seroprevalence among exposed forestry workers varies considerably according to country, region and various studies: 19.8% to 81.3% in Eastern Poland [45, 46], 0% to 5.7% in Italy [35, 47], 7.3% in South-Western Germany with variations according to district ranging from 0 to 43% [39]. The TBEV seroprevalence observed in our study is similar to that reported by previous French studies conducted among subjects in contact with the forest [48]. This seroprevalence is consistent with the low and stable number of cases of human clinical TBE diagnosed in France (an average of about ten cases per year from 1968 to 2003), especially confined to Eastern France (essentially Alsace, Lorraine, Franche-Comté) and the low prevalence in *Ixodes ricinus* which varies from 0.1% to 5% in European areas where TBE is endemic [9, 49]. The incidence of TBE in France is lower than that observed in neighbouring countries such as Germany (0.3 to 0.7 cases per 100,000) or Switzerland (1.5 to 3.3 cases per 100,000), while the greatest number of clinical cases are reported in Czech Republic, Baltic States and Slovenia (incidence exceeding 10 cases per 100,000 inhabitants depending on the year) [50]. These differences in incidence rates must be analysed as a function of respective vaccine coverage rates [51].

Few data are available in the literature concerning the seroprevalence of *B. microti* and/or *B. divergens* among forestry workers: only three countries in Europe have published seroprevalence rates for these pathogens. The seroprevalence estimated in our study among workers occupationally exposed to tick bites is comparable to that reported for *B. microti* among forestry workers in Poland (4.4%) [52], but is lower than that reported for *B. divergens* in Slovenia (8.4%) [53] and in a German study, in which 9.3% of asymptomatic seropositive subjects or subjects with clinical Lyme borreliosis or who had reported a tick bite, presented anti-*B. microti* antibodies
and 4.9% presented anti-\textit{B. divergens} antibodies \cite{54}. The low seroprevalence rates for \textit{B. microti} and \textit{B. divergens} observed in our study are consistent with the small number of clinical cases of human babesiosis reported in the regions of our study (2 cases in Alsace to date), the identification of \textit{B. microti} and \textit{B. divergens}, in \textit{I. ricinus} from a forest area in Alsace and the very low prevalence rates of \textit{B. divergens} in ticks found in the French Ardennes \cite{55-57}. It is noteworthy that the higher seroprevalence of \textit{B. microti} compared to \textit{B. divergens} (20/1) suggests that \textit{B. microti} infections may be more frequent in North-Eastern France. \textit{B. divergens} infections were more often identified in Western and Central France, where they are responsible for bovine babesiosis and a dozen cases of human babesiosis \cite{58}. Despite the \textit{B. microti} seroprevalence reported in our study, no human cases related to this species have been reported to date in France and only 3 cases have been reported in Europe, suggesting that this species would be less pathogenic and more frequently responsible for asymptomatic or paucisymptomatic infections \cite{59-62}.

\textit{A. phagocytophilum} seroprevalence among subjects exposed to tick bites varies from 1% (UK) to 25% (Slovakia) \cite{63-64}. High seroprevalence rates (between 10 and 20%) have also been reported in Slovenia, Poland, Norway and Sweden \cite{65-68}. The low \textit{A. phagocytophilum} seroprevalence rate observed in our study is consistent with the existence of human cases in France, but suggests that such cases would be rare. This low seroprevalence is also compatible with the low rate of tick infestation in France, which ranges from 0.3% in Western France to 2.6% in Eastern France \cite{57, 69} and the small number of clinical cases identified (9 confirmed cases between 2003 and 2009 in North-Eastern France) \cite{10, 11}. Despite the predominance of these cases in Alsace, no significant difference in seroprevalence rates was demonstrated between the various regions of the study. Human anaplasmosis also appears to be rare in Europe (clinical cases reported essentially in Slovenia (25), Scandinavia (25) and Poland (13)), but the high \textit{I. ricinus} infestation rates (8 to 14% in Slovakia, Serbia and Poland) and the high seroprevalence rates reported in some European countries suggest that human anaplasmosis may be underestimated or asymptomatic \cite{70-74}.

In Europe, \textit{F. tularensis} seroprevalence studies among forestry workers have reported seroprevalence rates of 1.7% and 4.0% in Germany, 2.1% in Poland, and 3.4% in Austria \cite{25, 75-77}. No \textit{F. tularensis} seroprevalence study in occupationally exposed workers has been published in France to date. The seroprevalence of 5.8% observed in our study is high, with rates as high as 11.2% in Champagne-Ardenne and 7.9% in Franche-Comté. In Alsace, \textit{F. tularensis} seroprevalence (3.7%) was comparable to that reported in a study conducted on 400
forestry workers in 1996 [4.0%; Jaulhac B., personal communication]. Epizootic diseases have been regularly reported between 1993 and 2004 in Champagne-Ardenne and Franche-Comté, especially in hares, while only sporadic animal cases have been reported in Lorraine and Bourgogne [78]. On the basis of human surveillance data from 2002 to 2012, Champagne-Ardenne can also classified as one of the French regions with the highest incidence [13]. In our study, the highest seroprevalence was observed among silviculturists (9.2%).

Silviculturists, who attend to the plantation, organization and maintenance of forests, are probably also frequently exposed to this agent by direct or indirect non-vectorial contacts with the animal reservoir or contaminated soil earth. Furthermore, a history of tick bite was reported in only about 20% of cases of tularaemia in France and in the regions of the present study, while the rate of tick infestation by *F. tularensis* is low in these regions (1%) [13, 79]. Inversely, vectorial transmission of *F. tularensis* (by ticks and mosquitoes) is the predominant mode of transmission in other European countries [19].

*B. henselae* is primarily transmitted to humans by cat scratch or bite. Cat fleas are well established vectors for *B. henselae* which could be transmitted directly to humans by the bite of infected cat fleas, although this has not been proven [33]. Transmission by ticks has also been suggested [3, 20-22]. Our study demonstrated a very low *B. henselae* seroprevalence rate among forestry workers, which may reflect the low real risk of transmission of this *Bartonella* species by *I. ricinus*, at least in this part of France, especially as high prevalence levels of DNA in ticks has been found in some specific regions of Alsace (38.2%) and in Ardennes (17.6%) [21, 57].

Very few forestry workers were seropositive for at least two of the seven pathogens studied (including *B. burgdorferi* sl and TBEV), which may reflect the low seroprevalence of these pathogens observed in our study except for *B. burgdorferi* sl and the limited role of *I. ricinus* in the transmission of *F. tularensis* or *B. henselae*. Simultaneous seropositivity for *B. burgdorferi* sl and *A. phagocytophilum* or *Babesia* spp. was low in our study (0.2% for *Anaplasma*) suggesting a very low risk of human co-infection by these pathogens.

In conclusion, our study confirms occupational exposure of forestry workers to these seven pathogens transmissible by *Ixodes ricinus* and allows risk classification among forestry workers in North-Eastern France in relation to these main tick-borne pathogens. Exposure to *B. burgdorferi* sl appears to be by far the most frequent type of exposure among these workers, while the levels of exposure to *B. microti*, *B. divergens*, *A. phagocytophilum* or *B. henselae* remain low. The level of exposure to *F. tularensis* is probably related more to
the work environment than to the role of the vector. Incidence studies would allow a more accurate risk assessment, but would be difficult to perform due to the small number of cases and the nonspecific symptoms. The high density of tick populations in these regions and the potential severity of some clinical forms of these infections justify continued promotion of primary and secondary prevention messages and especially personal protection measures against this vector (systematic examination of the skin at the end of every working day and early removal of ticks with surveillance of the bite overalls, use of clothes possibly impregnated with insecticides, possible use of skin insect repellents).

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Table 1 Description of subjects included in the study according to region of residence, North-Eastern France, 2003

<table>
<thead>
<tr>
<th>Region of residence</th>
<th>TOTAL</th>
<th>Alsace</th>
<th>Lorraine</th>
<th>Champagne-Ardenne</th>
<th>Bourgogne</th>
<th>Franche-Comté</th>
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<tr>
<td>Participation rate</td>
<td>54.5%</td>
<td>56.6%</td>
<td>52.6%</td>
<td>58.4%</td>
<td>46.0%</td>
<td>59.7%</td>
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<td>2,975</td>
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<td>485</td>
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<td>Mean</td>
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<td>Median</td>
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<td>44.0</td>
<td>43.0</td>
<td>41.0</td>
<td>42.5</td>
<td>42.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>17.0</td>
<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>81.0</td>
<td>68.0</td>
<td>77.0</td>
<td>73.0</td>
<td>68.0</td>
<td>81.0</td>
</tr>
<tr>
<td>Age-group (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>761</td>
<td>118 (18.5%)</td>
<td>201 (22.7%)</td>
<td>157 (32.4%)</td>
<td>113 (28.2%)</td>
<td>164 (29.6%)</td>
</tr>
<tr>
<td>[35-44] years</td>
<td>915</td>
<td>202 (31.8%)</td>
<td>290 (32.8%)</td>
<td>142 (29.3%)</td>
<td>112 (28.0%)</td>
<td>167 (30.1%)</td>
</tr>
<tr>
<td>[45-54] years</td>
<td>961</td>
<td>243 (38.2%)</td>
<td>303 (34.2%)</td>
<td>137 (28.2%)</td>
<td>124 (31.0%)</td>
<td>151 (27.3%)</td>
</tr>
<tr>
<td>≥55 years</td>
<td>338</td>
<td>73 (11.5%)</td>
<td>91 (10.3%)</td>
<td>49 (10.1%)</td>
<td>51 (12.8%)</td>
<td>72 (13.0%)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodcutter</td>
<td>1145</td>
<td>406 (63.8%)</td>
<td>330 (37.3%)</td>
<td>145 (29.9%)</td>
<td>122 (30.5%)</td>
<td>138 (24.9%)</td>
</tr>
<tr>
<td>Silviculturist</td>
<td>715</td>
<td>100 (15.7%)</td>
<td>192 (21.7%)</td>
<td>138 (28.4%)</td>
<td>138 (34.5%)</td>
<td>145 (26.2%)</td>
</tr>
<tr>
<td>Rangers</td>
<td>347</td>
<td>52 (8.2%)</td>
<td>73 (8.2%)</td>
<td>95 (19.6%)</td>
<td>77 (19.2%)</td>
<td>47 (8.5%)</td>
</tr>
<tr>
<td>Other (machinery operators...)</td>
<td>221</td>
<td>38 (6.0%)</td>
<td>47 (5.3%)</td>
<td>49 (10.1%)</td>
<td>39 (9.8%)</td>
<td>43 (7.7%)</td>
</tr>
<tr>
<td>Multiple activities</td>
<td>547</td>
<td>40 (6.3%)</td>
<td>243 (27.5%)</td>
<td>58 (12.0%)</td>
<td>24 (6.0%)</td>
<td>181 (32.7%)</td>
</tr>
</tbody>
</table>

*a15 subjects worked in these regions, but lived outside of this zone.
Table 2 Prevalence of anti-*B. burgdorferi*, anti-TBEV, anti-*A. phagocytophilum*, anti-*Babesia*, anti-*F. tularensis*, anti-*B. henselae* antibodies in occupationally exposed workers in North-Eastern France according to occupation and region of residence, 2003

<table>
<thead>
<tr>
<th>B. burgdorferi s.l.</th>
<th>TBEV</th>
<th>A. phagocytophilum</th>
<th>R. microti, B. divergens</th>
<th>B. henselae</th>
<th>F. tularensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodcutter</td>
<td>200 (17.5)</td>
<td>27 (2.4)</td>
<td>23 (2.1)</td>
<td>5 (1.5)</td>
<td>21 (1.9)</td>
</tr>
<tr>
<td>Silviculturist</td>
<td>106 (14.8)</td>
<td>20 (2.8)</td>
<td>11 (1.76)</td>
<td>9 (4.1)</td>
<td>7 (1.0)</td>
</tr>
<tr>
<td>Ranger</td>
<td>26 (7.5)</td>
<td>6 (1.7)</td>
<td>4 (1.2)</td>
<td>2 (3.2)</td>
<td>9 (2.6)</td>
</tr>
<tr>
<td>Other (machinery operators...)</td>
<td>18 (8.2)</td>
<td>2 (0.9)</td>
<td>1 (0.5)</td>
<td>1 (2.2)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>Multiple activities</td>
<td>69 (12.6)</td>
<td>13 (2.4)</td>
<td>11 (2.1)</td>
<td>4 (2.7)</td>
<td>8 (1.5)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>419 (14.1)</td>
<td>68 (2.3)</td>
<td>50 (1.7)</td>
<td>21 (2.6)</td>
<td>48 (1.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region of residence</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsace</td>
<td>171 (26.9)</td>
<td>20 (3.2)</td>
<td>16 (2.6)</td>
<td>6 (2.5)</td>
<td>13 (2.1)</td>
</tr>
<tr>
<td>Lorraine</td>
<td>146 (16.5)</td>
<td>20 (2.3)</td>
<td>11 (1.3)</td>
<td>8 (3.2)</td>
<td>14 (1.6)</td>
</tr>
<tr>
<td>Champagne-Ardenne</td>
<td>40 (8.2)</td>
<td>8 (1.7)</td>
<td>7 (1.4)</td>
<td>4 (3.1)</td>
<td>15 (3.1)</td>
</tr>
<tr>
<td>Bourgogne</td>
<td>30 (7.5)</td>
<td>9 (2.3)</td>
<td>4 (1.0)</td>
<td>2 (2.7)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Franche-Comté</td>
<td>31 (5.6)</td>
<td>10 (1.8)</td>
<td>12 (2.3)</td>
<td>1 (0.9)</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>418 (14.1)</td>
<td>67 (2.3)</td>
<td>50 (1.7)</td>
<td>21 (2.6)</td>
<td>48 (1.6)</td>
</tr>
</tbody>
</table>
Table 3 Odds ratios of anti-*B. burgdorferi* antibody prevalence in occupationally exposed workers in North-Eastern France, adjusted for age, occupation and region of residence, 2003

<table>
<thead>
<tr>
<th>Age-group</th>
<th>TOTAL</th>
<th>Seropositive (%)</th>
<th>AOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 35 years</td>
<td>761</td>
<td>31 (4.1)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>[35-44] years</td>
<td>915</td>
<td>114 (12.5)</td>
<td>2.97 [1.96-4.50]</td>
<td>&lt; 10^-4</td>
</tr>
<tr>
<td>[45-54] years</td>
<td>961</td>
<td>189 (19.7)</td>
<td>5.00 [3.35-7.45]</td>
<td>&lt; 10^-4</td>
</tr>
<tr>
<td>≥55 years</td>
<td>338</td>
<td>85 (25.1)</td>
<td>8.02 [5.13-12.54]</td>
<td>&lt; 10^-4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>TOTAL</th>
<th>Seropositive (%)</th>
<th>AOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other (machinery operators...)</td>
<td>242</td>
<td>18 (8.2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Woodcutter</td>
<td>1145</td>
<td>200 (17.5)</td>
<td>2.00 [1.19-3.39]</td>
<td>0.0093</td>
</tr>
<tr>
<td>Silviculturist</td>
<td>715</td>
<td>106 (14.8)</td>
<td>2.22 [1.29-3.82]</td>
<td>0.0038</td>
</tr>
<tr>
<td>Ranger</td>
<td>347</td>
<td>26 (7.5)</td>
<td>1.02 [0.53-1.94]</td>
<td>0.9620</td>
</tr>
<tr>
<td>Multiple activities</td>
<td>526</td>
<td>69 (12.6)</td>
<td>2.12 [1.20-3.76]</td>
<td>0.0098</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region of residence</th>
<th>TOTAL</th>
<th>Seropositive (%)</th>
<th>AOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franche-Comté</td>
<td>554</td>
<td>31 (5.6)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Alsace</td>
<td>636</td>
<td>171 (26.9)</td>
<td>6.08 [3.98-9.28]</td>
<td>&lt; 10^-4</td>
</tr>
<tr>
<td>Lorraine</td>
<td>885</td>
<td>146 (16.5)</td>
<td>3.28 [2.17-4.95]</td>
<td>&lt; 10^-4</td>
</tr>
<tr>
<td>Champagne-Ardenne</td>
<td>485</td>
<td>40 (8.2)</td>
<td>1.76 [1.07-2.89]</td>
<td>0.0261</td>
</tr>
<tr>
<td>Bourgogne</td>
<td>400</td>
<td>30 (7.5)</td>
<td>1.46 [0.86-2.49]</td>
<td>0.1644</td>
</tr>
</tbody>
</table>

* odds ratio adjusted for age-group, occupation and region of residence
Table 4: Odds ratios of anti-*F. tularensis* antibody prevalence in occupationally exposed workers in North-Eastern France, adjusted for age, occupation and region of residence, 2003

<table>
<thead>
<tr>
<th>Age-group</th>
<th>TOTAL</th>
<th>Seropositive (%)</th>
<th>AOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 35 years</td>
<td>733</td>
<td>34 (4.6)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>[35-44] years</td>
<td>893</td>
<td>56 (6.3)</td>
<td>1.42 [0.91-2.22]</td>
<td>0.1243</td>
</tr>
<tr>
<td>[45-54] years</td>
<td>925</td>
<td>57 (6.2)</td>
<td>1.51 [0.96-2.35]</td>
<td>0.0721</td>
</tr>
<tr>
<td>≥55 years</td>
<td>324</td>
<td>17 (5.2)</td>
<td>1.20 [0.65-2.21]</td>
<td>0.5532</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>TOTAL</th>
<th>Seropositive (%)</th>
<th>AOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other (machinery operators...)</td>
<td>217</td>
<td>10 (4.6)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Woodcutter</td>
<td>1109</td>
<td>50 (4.5)</td>
<td>1.37 [0.65-2.87]</td>
<td>0.4033</td>
</tr>
<tr>
<td>Silviculturist</td>
<td>684</td>
<td>63 (9.2)</td>
<td>2.47 [1.20-5.10]</td>
<td>0.0142</td>
</tr>
<tr>
<td>Ranger</td>
<td>343</td>
<td>8 (2.3)</td>
<td>0.54 [0.20-1.43]</td>
<td>0.2172</td>
</tr>
<tr>
<td>Multiple activities</td>
<td>522</td>
<td>33 (6.3)</td>
<td>1.79 [0.83-3.87]</td>
<td>0.1398</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region of residence</th>
<th>TOTAL</th>
<th>Seropositive (%)</th>
<th>AOR* (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lorraine</td>
<td>873</td>
<td>31 (3.6)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Alsace</td>
<td>629</td>
<td>22 (3.6)</td>
<td>1.09 [0.61-1.92]</td>
<td>0.7796</td>
</tr>
<tr>
<td>Champagne-Ardenne</td>
<td>474</td>
<td>53 (11.2)</td>
<td>3.82 [2.39-6.12]</td>
<td>&lt; 10^-4</td>
</tr>
<tr>
<td>Bourgogne</td>
<td>396</td>
<td>17 (4.3)</td>
<td>1.29 [0.70-2.40]</td>
<td>0.4141</td>
</tr>
<tr>
<td>Franche-Comté</td>
<td>547</td>
<td>40 (7.9)</td>
<td>2.29 [1.40-3.72]</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

* Odds ratio adjusted for age-group, occupation and region of residence
Fig. 2 Prevalence of tick-borne pathogens antibodies in occupationally exposed workers in North-Eastern France by age-group, with 95% confidence intervals, 2003 (a anti- B. burgdorferi antibodies, b anti-TBEV antibodies, c anti- A. phagocytophilum antibodies, d anti-F. tularensis antibodies, e anti-B. henselae antibodies, f anti-Babesia antibodies)
Fig. 3 Seropositivities for different pathogens among 2,908 subjects analysed for *B. burgdorferi* sl, TBEV, *A. phagocytophilum*, *B. henselae* and *F. tularensis*

![Seropositivities for different pathogens among 2,908 subjects analysed for B. burgdorferi sl, TBEV, A. phagocytophilum, B. henselae and F. tularensis](image-url)

Bo : *Borrelia burgdorferi* sl, Ana : *Anaplasma phagocytophilum*,
Bar : *Bartonella henselae*, Fran : *Francisella tularensis*