Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 1, 43–50
www.aaem.pl

Zoonotic occupational diseases in forestry workers – Lyme borreliosis, tularemia and leptospirosis in Europe

Stéphanie Richard[1], Anne Oppliger[1]

[1] *Institute for Work and Health, University of Lausanne, Switzerland*

Richard S, Oppliger A. Zoonotic occupational diseases in forestry workers – Lyme borreliosis, tularemia and leptospirosis in Europe. Ann Agric Environ Med. 2015; 22(1): 43–50. doi: 10.5604/12321966.1141368

Abstract

Introduction. Forestry workers and other people who come into close contact with wild animals, such as hunters, natural science researchers, game managers or mushroom/berry pickers, are at risk of contracting bacterial, parasitological or viral zoonotic diseases. Synthetic data on the incidence and prevalence of zoonotic diseases in both animals and humans in European forests do not exist. It is therefore difficult to promote appropriate preventive measures among workers or people who come into direct or indirect contact with forest animals.

Objectives. The objectives of this review are to synthesise existing knowledge on the prevalence of the three predominant bacterial zoonotic diseases in Europe, i.e. Lyme borreliosis, tularemia and leptospirosis, in order to draw up recommendations for occupational or public health.

Methods. 88 papers published between 1995–2013 (33 on Lyme borreliosis, 30 on tularemia and 25 on leptospirosis) were analyzed.

Conclusions. The prevalences of these three zoonotic diseases are not negligible and information targeting the public is needed. Moreover, the results highlight the lack of standardised surveys among different European countries. It was also noted that epidemiological data on leptospirosis are very scarce.

Key words

leptospirosis, Lyme borreliosis, tularaemia, zoonose, occupational health, forester

INTRODUCTION

Most of the infectious agents causing zoonotic diseases can be considered as occupational hazards since they occur sporadically or chronically in the occupational environments of diverse professions. In fact, any workers who are occasionally or permanently in contact with forest environments are at risk of contracting one or more zoonotic diseases. There are multiple routes of transmission for zoonotic diseases. Humans can be infected following direct contact with infected live or dead birds and mammals, by means of a vector – tick or insect, by contact with contaminated water, soil, urine or saliva, or by inhalation of dust containing infectious agents. Zoonotic diseases can have severe health and economic impacts on human society [1]. In Europe, Lyme borreliosis, tularemia and leptospirosis are considered as emerging or re-emerging infection risks [2, 3, 4]. The presented study therefore focuses attention on these three bacterial diseases known to be transmissible to forestry workers. 83 papers published between 1995–2011 (33 on Lyme borreliosis, 30 on tularemia and 25 on leptospirosis) were analyzed. The database PubMed, Web of Sciences and Up ToDate were used to find all scientific papers published with the followed key words: *Borrelia burgdorferi*, Lyme borreliosis, *Francisella tularensis*, Tularemia, *Leptospira interrogans*, Leptospirosis, Occupational disease, forestry workers and seroepidemiological study. The web sites of some national or international organisations involved in occupational or public health were also consulted, e.g. the World Organisation for Animal Health (OIE), L'Institut National de Recherche et de Sécurité (INRS) in France, Federal Veterinary Office (OVF) in Switzerland, and the European Union Concerted Action on Lyme Borreliosis (EUCALB). To better understand the eco-epidemiology of these bacterial zoonoses, some basic knowledge about their taxonomy, ecology, vectors and life-cycle are first presented for each of the three dieases.

Address for correspondence: Anne Oppliger, Institute for Work and Health, University of Lausanne, CH-1011 Lausanne, Switzerland
E-mail: Anne.Oppliger@hospvd.ch

Received: 12 September 2013; Accepted: 21 October 2013

LYME BORRELIOSIS (LYME DISEASE)

Lyme borreliosis (LB) is a group of frequently diagnosed zoonotic disease variants. Its agents in Europe are five species of a group of related spirochaetes: *Borrelia burgdorferi sensu lato*. These are: *B. afzelii*, *B. garinii*, and more rarely, *B. burgdorferi sensu stricto*, *B. spielmanii*, and *B. bavariensis*. *B. afzelii* is mostly associated with dermatological symptoms, *B. garinii* seems to be the most neurotropic, and *B. burgdorferi* seems to be the most arthritogenic.

Ecology, vectors and transmission. The vector of LB in Europe is the *Ixodes ricinus* tick, which is widely present in forest and woodland environments. The larvae hatch from eggs laid on the ground and attach themselves to small animals and birds (but not humans) to take their first meal. The next stage – the nymph – is responsible for most cases of human LB. Due to their small size (< 2 mm) they are difficult to spot, giving them ample opportunity to transmit *B. burgdorferi* while feeding. Although more adult ticks than nymphs carry *B. burgdorferi*, the adult ticks are much larger, more easily noticed and more likely to be removed before the 24 hours or more of continuous feeding needed to transmit the disease. A study has demonstrated that 66% of nymphs remained attached for more than 24 h, whereas only 38% of female adults remained attached for more than 24 h [5]. The tick has to remain attached for 24–48 hours before transmission of the bacteria can occur [6]. It has been observed that *B. afzelii* can be transmitted within 24 hours, while 48 hours are needed for the transmission of *B. burgdorferi s.s.* [7].

Epidemiological data. Numerous studies carried out in several European countries have estimated the rate of infection of *I. ricinus*. Results are heterogenous, for example, the regions of Ile-de-France (France), Roztocze and Lublin (Poland), respectively reported 11%, 12% and 13% [8, 9, 10] of ticks infected by *B. burgdorferi s.l.*, while the region of Friuli-Venezia-Giulia (Italy) reported up to 70% [11]. Studies have shown that more than 80% of forestry workers report having been bitten by a tick (83% in France, 86% in Friuli-Venezia, Italy, and 90%–95% in Lublin, Poland) [11, 12, 13, 14].

Table 1 lists 22 studies published between 1995–2010 which determined the percentage of forestry workers (lumberjacks, gamekeepers, hunters) in specific geographic regions having IgM and/or IgG antibodies against *B. burgdorferi s.l.* The IgM antibody appears in the blood approximately 1–2 weeks after contamination and disappears afterwards. Consequently, their presence reflects a recent infection. The IgG antibody is present 2–6 weeks after contamination. Although generally these antibodies disappear after eradication of the bacterium [15], approximately 10% – 20% of cured human subjects were still seropositive 10–20 years later [15].

Antibodies were detected using three different methods: the enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence assay (IFA), and the western blot. The ELISA and IFA assays are more sensitive than the western blot test, and were more often used to detect antibodies to *B. burgdorferi s.l.* Due to its greater specificity, the western blot technique was often used to confirm ELISA or IFA results, allowing the elimination of false positive results. Fifteen of these studies also measured the seroprevalence of anti-*B. burgdorferi* in a control population (blood donors inhabiting the same region as the forestry workers or workers of administrative offices).

Results from these 22 studies must to be interpreted and compared with caution due to non-standardised methodologies, differences in the sample sizes of populations studied, and the lack of control populations in seven studies. However, these data highlight an over-representation of positive seroprevalences for *B. burgdorferi s.l* in forestry workers. The observed data vary considerably, not only between European countries, but also within them. For instance, Poland is particularly affected by *B. burgdorferi s.l*, with a seroprevalence in forestry workers of between 20% – > 60%, while in Italy only 5% – 23% of forestry workers show a positive serology for *B. burgdorferi s.l.* In France, the situation is homogeneous across the three different regions, showing that 14% – 20% of forestry workers are seropositive. These differences are difficult to interpret since no data are available

Table 1. Seroprevalence of B. *burgdorferi s.l.* in forestry workers and control group (blood donors) in European countries. WB. western blot ; IFA : indirect fluorescent assay ; ELISA : enzyme-linked immunosorbent assay

Country, region	Seroprevalence in forestry workers	Seroprevalence in control group	Detection method	P value	Reference
France					
East	14.1% (419/2975)	-	WB (IgG)	-	Thorin et al. 2008 [12]
East Rhine and Centre	20.2%	1.9%	IFA	< 0.05	Nübling et al. 2002 [58]
Ile de France	14,3% (25/175)	-	ElISA (IgM/IgG)	< 0.05	Christiann et al. 1997 [59]
	15.2% (32/211)	3.2% (1/31)	IFA		Zhioua et al. 1997 [16]
Italy					
Lazio	13.1% (19/45)	8.2% (23/282)	WB (IgM/IgG)	> 0.05	Di Renzi et al. 2010 [60]
Tuscany	7% (29/412)*	3.6% (13/365)	WB (IgM/IgG)	> 0.05	Tomao et al. 2005 [17]
Friuli-Venezia-Giulia	23.2% (42/181)	-	WB (IgG)	-	Cinco et al. 2004 [11]
Centre	5.4% (2/37)	0.6% (1/180)	ELISA (IgG)	< 0.05	Santino et al. 2004 [18]
South	14.5% (21/145)	2.1% (3/145)	ELISA (IgG)	< 0.05	Santino et al. 2004 [18]
Germany					
Southwest	13.4%	1.8%	IFA	< 0.05	Nübling et al. 1999 [61]
Brandenburg	8%	4%	IFA	< 0.05	Rath et al. 1996 [62]
Baden-Württemberg	0-43%	-	ELISA (IgM/IgG)	-	Oehme et al. 2002 [83]
Netherlands	19.3% (39/202)	1.4% (5/356)	WB	< 0.05	Moll van Charante et al. 1998 [63]
Poland					
South	19.6% (226/1155)	-	ELISA (IgG)	-	Buczek et al. 2009 [64]
Roztocze (SE)	40.7% (46/113)	7.1% (4/56)	ELISA (IgM/IgG)	< 0.05	Cisak et al. 2005 [9]
Dobrzany	61.5% (32/52)	-	ELISA (IgM/IgG)	-	Niscigorska et al. 2003 [65]
East, Lublin	24.5% (59/241)	-	ELISA IgM/ IgG	-	Cisak et al. 2001 [66]
Lublin	39%	6%	ELISA IgM/ IgG	< 0.05	Chmielewska-Badora 1998 [13]
Lublin	41%, 31% (26/63 ; 8/27)	21.4% (6/27)§	ELISA IgG,	< 0.05	Cisak et al 2012 [67]
Lublin	19.2%, 15.4%	0% (0/27)§	ELISA IgM		Cisak et al. 2012 [67]
Slovenia	23.8% (29/122)	-	ELISA IgG	-	Rojko et al. 2005 [68]
Romania	9.4% (99/1053)	4.3% (69/1598)	WB IgG	< 0.05	Hrista et al. 2002 [69]
Turkey					
Duzce (NW)	10.9% (38/349)*	2.6% (5/193)	ELISA IgG	< 0.05	Kaya et al. 2008 [70]
Hungary	37% (622/1670)	-	ELISA (IgM/IgG)	-	Lakos et al. 2012 [71]

* – population including farm workers; § – control group = employee of forestry service with mainly office tasks

concerning the density of ticks and their infection rates in the different regions. Information about the prevention measures implemented in each region are also lacking; therefore, estimation of impact on the prevalence of LB is not possible. However, it is surprising that the proportion of infected ticks does not correlate with seroprevalence of the forestry workers. Indeed, while the proportion of infected ticks in Paris and the surrounding area (11%) is almost the same as in the Roztocze region of Poland (12%) [8, 9], seropositivity of forestry workers in Roztocze is almost twice that of those from the Paris area. In the Friuli-Venezia region in Italy, only 23% of forestry workers are seropositive, while 70% of the ticks were infected [11]. Lyme borreliosis is considered as an occupational risk for forestry workers [16], even if human infection by *B. burgdorferi s.l* does not necessarily lead to LB.

In America, it is considered that 90% of infected individuals develop LB [15], while other studies report that only 5% of infected human show symptoms [16]. Some seropositive forestry workers showed no symptoms of the disease [9, 16, 17, 18] and it is impossible to determine whether they are true asymptomatic cases or whether they are latent or cured infected individuals. Some authors advance the hypothesis that asymptomatic infections would be more frequent in individuals who are repeatedly exposed [17, 18]. A French study observed a positive relationship between the age of forest workers and the seropositivity of *B. burgdorferi s.l.* This can be explained by a continuous stimulation of their immune system due to the repeated exposure to infected ticks [12]. LB is not systematically reported by the authorities, which imply an underestimation of the occurrence. Only one study [19] has investigated LB from an occupational health point of view. It reported that, from 2000–2007, the province of Wielkopolska in Poland recognised 218 cases of LB as occupational diseases. Workers between the ages of 40 and 60 being the most frequently affected. This trend was also observed in the Podkarpackie province in Poland [20].

TULAREMIA

Tularemia is caused by the *Francisella tularensis* bacterium, a coccobacillus present almost exclusively in the northern hemisphere [21]. Three subspecies are considered as pathogenic for humans: *F. tularensis tularensis*, *F. tularensis holartica* and *F. tularensis novicida*.

Mainly found in North America, *F. tularensis tularensis* is the most virulent of the three subspecies and classified in the WHO Risk Group 3. The infectious dose is very low since only 10–50 bacteria inhaled or injected intradermally can reliably cause the disease in human [22]. *F. tularensis holartica* is the most frequent subspecies in Europe [23, 22]. *F. tularensis novicida* is the only subspecies isolated in the southern hemisphere [24, 25] although it is mainly present in North America.

Ecology, vectors and transmission. *F. tularensis holartica* can infect a wide variety of animals living in forests and the principal hosts are voles and brown hares. Direct cutaneous contact with the bacterium is the main mode of human contamination in Central Europe [25, 26]. Individuals mostly become infected by manipulating the meat and fur of the brown hare. The bacterium is capable of penetrating healthy skin [27]. Bites and scratches by contaminated animals represent an additional danger [26]. Vector-borne transmission is the main route of transmission to humans in the USA [22] where human infections predominate in late summer and autumn, associated with arthropod inoculation [25]. Inhalation of contaminated dusts aerosolized from soil, faecal matter and dead animals is another frequent route of transmission, while ingestion of contaminated meat or water is also reported.

Lagomorphs seem to be an important reservoir of the pathogen [25, 28, 26]. However, protozoons living in fresh water could also be a reservoir for the bacterium [28]. Water contamination could be maintained by the faecal matter of infected amphibians, rodents and other animals, or by infected animal carcasses. This hypothesis is supported by the fact that a clear relationship between *F. tularenis holartica* and mosquitoes was found in Scandinavia, and by the presence of the bacterium in samples of water taken in several endemic regions [29]. Ticks can also serve as a reservoir since *F. tularensis* has been isolated in several species of ticks in Europe, in particular *I. ricinus* and *Dermacentor reticulatus* [26].

Epidemiological data. Despite the mandatory reporting of tularemia (for both humans and animals in most European countries) epidemiological data are very sparse. Data on the prevalence and geographical distribution of *F. tularensis* are very imprecise. Once established, sources of tularemia in animals are reported to the World Organisation for Animal Health (OIE: www.oie.int). The distribution of the disease in Europe in 2005, 2008 and 2010, in both the first and second half of each year, shows that Romania, Ukraine, Belarus and Lithuania do not seem affected by this disease, having not reported any cases to the OIE. In 2010, cases of tularaemia are reported in Finland, Sweden, Norway, Austria, Germany and France. In Spain and Switzerland cases are regionally reported, and infection seems to be well established in Italy. In 2010, Sweden and Norway were affected by tularemia only during the second part of each year. Several factors could explain this trend. On the one hand, vectors such as ticks, mites, tabanid flies and mosquitoes are more active in the summer and could be responsible for a larger number of inter-animal transmissions in these periods. On the other hand, more rigorous surveys of game animals by the competent authorities during the hunting season could also explain this increase of reported cases.

One weakness of these results is the lack of regional data. *F. tularensis* is not found across countries in a homogeneous way. Sources of the disease are often very regional. For example, in France, between 1993–2004, tularemia in hares was found every year in certain departments, while no cases were reported during the same period in surrounding regions. Studies of the prevalence in wild animals are sparse. Table 2 lists 18 studies published from 1995–2009, having examined infections in hares, wild boars, foxes, rodents and ticks. These studies were carried out in Germany, Austria, Norway, Czech Republic, Slovakia, Serbia, Switzerland and Portugal. They include studies of seroprevalence – indicating a recent or former infection – with the detection of antibodies by standard agglutination test (SAT), microagglutination test (MAT) or western blot (WB). They include also studies of current infection (bacteria) prevalence, detected by culture or polymerase chain reaction (PCR). Analyses were conducted

Table 2. Seroprevalence or presence of *F. tularensis* in brown hare, wild boar,fox, small mammal and tick in european countries ; MAT : microagglutination test, MIR :[minimum infection rate ; SAT : standard agglutination test, WB: western blot, PCR: polymerase chain reaction

Country, region	Brown hare	Wild boar	Fox	Small mammal	Tick	Detection method	Reference
Czech Republic							
Moravian	6.6% (69/1051)				2.2% (20/918) (*D.reticulatus*)	MAT, MIR	Treml et al. 2007 [76]
Breclav, Znojmo	1.4% (1/73)					SAT	Hubalek et al 1998 [34]
Breclav		10.8% (22/204)				MAT	Winkelmayer et al. 2005 [32]
Drnholec, Pritluky, Breclav, Lanzhot					0.2% (1/504) (*I.ricinus*) and 2.6% (20/924) (*D. reticulatus*)	SAT	Hubalek et al. 2002 [77] Hubalek et al. 1996 [36]
Austria							
Northeastern	60.8% (62/109)		7.5% (29/385)			Culture-MAT	Hofer et al. 1997 [30]
	4.5% (5/110)					Culture-MAT	Hoflechner-Poltl et al. 2000 [31]
	7.1% (22/311)					SAT	Winkelmayer et al 2005 [32]
Hohenau				2.8% (12/423)		Culture	Vyrostekova et al. 2002 [78]
Hohenau				1.2% (12/1033)	1.5% (18/1217) (*D.reticulatus*) 0% (0/1977) (*I. ricinus*)	Culture	Gurycova et al. 2001 [35]
					2.3% (25/1098) (*D. reticulatus*)	Culture	Hubalek et al. 1998 [34]
Mistelbach							
Germany							
Several places				4.9% (19/386)		PCR	Kaysser et al. 2008 [29]
Northeastern		3.1% (24/763)				WB	Dahouk et al. 2005 [79]
Schleswig-Holstein	0% (0/299)					WB	Frölich et al. 2003 [80]
Norway							
Narwick Est				7.7% (2/13)		SAT	Berdal et al. 1996 [75]
Slovakia							
Zahorie lowland				7% (15/2714)		Culture	Gurycova et al. 2001 [35]
Serbia					3.8% (11/287) (*I.ricinus*)	PCR	Milutinovic et al. 2008 [38]
Switzerland					0.12% (7/602) (*I.ricinus*)	PCR	Wicki et al 2005 [37]
Portugal					1.3% (1/79) (*D. reticulatus*)	PCR	Lopes de Carvalho et al. 2007 [33]

in healthy animals (hares, wild boars and foxes), animals killed in hunting, or, in the case of rodents, trapped. Only ywo studies [30, 31] were based on analyses performed on dead or sick hares.

In the presented study it was noted that wild boars, foxes and rodents showed seropositivity comparable to those of hares (up to 11%). Consequently, these hosts are also significantly affected by tularemia. One Austrian study showed the presence of *F. tularensis* in 60% of the hares examined. This very high rate compared to those obtained in other studies is certainly due to the selection bias of the hares analyzed since these animals were sick or killed by hunters. The second Austrian study [32] included 14 sick or dead hares and 96 healthy animals. Results showed that 14% (2/14) of the sick or dead hares were affected by tularemia and/or had a positive seroprevalence while only 3% (3/96) of the healthy hares were affected. It can be concluded that tularemia was overrepresented in the population of sick or dead hares in both studies.

Studies on ticks' seroprevalence were carried out in two species – *D. reticulatus* and *I. ricinus* – collected from vegetation by using the flagging method. The prevalence of infection in *D. reticulatus* was 1.3% in Portugal [33] and 2.8% in Austria [34]. The rate of infection of *I. ricinus* was lower, with no infected ticks in Austria [35], 0.2% in the Czech Republic [36], 1.2% in Switzerland [37] and 3.8% in Serbia [38]. These results suggest that *D. reticulatus* is a non-negligible vector of tularemia among animals in Europe, and that in certain regions *I. ricinus* could also have a non-negligible role in human transmission. However, it can be concluded that, on average, only 1% of the cases of human tularemia are transmitted by ticks.

Table 3 shows the number of cases of human tularemia reported per year in different countries. However, this disease remains extremely rare in European countries. Nevertheless, due to the non-specific symptoms (a flu-like syndrome) sometimes associated with tularemia, it is possible that the number of real cases is underestimated. For instance, the data from Sweden show a far higher number of cases (10 times greater) than in other European countries. This excess of cases can be explained by the transmission by mosquitoes, particularly during the summer season. The number of cases can also vary considerably from one year to the next within the same country. For example, in France and Sweden between 2007–2008, an unexplained twofold increase in cases was observed. As mentioned previously, all cases of human tularemia are not diagnosed. A study carried out in 2004 in Germany revealed that 0.2% of the general population is

Table 3. Number of human cases of tularemia / leptospirosis reported yearly in european countries 2005–2010.
- = no information (data extracted from WAHID, OIE : www.oie.int)

	2005	2006	2007	2008	2009	2010
Germany	15 / 58	1 / 46	20 / -	15 / _	10 / -	31 / -
Austria	- / -	- / -	4 / 9	- / -	2 / 8	5 / 16
France	23 / -	24 /192	47 / 327	108 / -	31 / 161	41 / -
Norway	- / -	11 / -	49 / -	66 / -	13 / -	33 / -
Czech Republic	- / -	86 / 17	- / 24	113 / 17	65 / 32	53 / 41
Poland	- / 5	- / -	1 / 12	4 / 5	1 / 6	4 / 4
Slovakia	- / 35	45 / 22	- / -	- / -	- / -	- / -
Sweden	246 / 3	241 / 2	174 / 1	382 / 6	244 / 4	484 / 4
Switzerland	- / -	- / -	7 / -	1 / -	- / -	17 / -

Table 4. Seroprevalence of *F.tularensis* and *L.interrogans* in forestry workers and control group (blood donors) in european countries. MAT – microagglutination test; SAT – standard agglutination test; WB – western blot; ELISA – enzyme-linked immunosorbent assay

Country	Tularemia: Seroprevalence in forestry workers	Tularemia : Seroprevalence in control group	Leptospirosis : Seroprevalence in forestry workers	Leptospirosis : Seroprevalence in control group	Detection method	P value	Reference
Austria							
Styria, Burgenland	3.4% (5/149)*	0/50	10% (15/149)	0/50	MAT		Deutz et al. 2003 [72]
Germany							Jenzora et al. 2008 [39]
Dortmund	1.7% (5/286)*	0.2%			WB	< 0.05	Porsch-Ozcurumez et al. 2004 [73]
Poland							Rastawicki et al. 2006 [74]
Northeast	2.1% (20/765)				SAT		
Norway							Berdal et al. 1996 [75]
Telemark	9.1% (5/55)*				SAT		
Netherland			0% (0/202)	0.2% (1/356)	ELISA		Moll van Charante et al. 1998 [63]

*- population of hunters

seropositive. This result confirmed that human tularemia prevalence is underestimated.

Only four studies have determined the seroprevalence of *F.tularensis* in forestry workers or hunters (Tab. 4). Only one study has used a control group, which is the only way to show a significant difference between occupationally-exposed persons and the general population. The study [39] showed that between 1.7% – 9.1% of forestry workers were seropositive, in comparison to 0.2% in the general population. It can therefore be concluded that forestry workers face a greater risk of infection by *F. tularensis* than individuals without close contact with forests. Additional studies are necessary to determine more precisely the occupational risk associated with forestry work.

LEPTOSPIROSIS

The group of bacterium responsible for leptospirosis, the spirocheta *Leptospira interrogans sensu lato,* possess more than 200 serovars. Among them, the most frequently found are: *L. interrogans icterohaemorrhagiae* and *L. interrogans grippotyphosa* [40, 41, 42, 43, 44]. Leptospirosis is a worldwide zoonotic disease, present in developing and industrialised countries [41, 45, 46].

Ecology, vectors and transmission. *L. interrogans* can infect a large spectrum of mammals. The bacterium can survive for several days or months in water or soil, for as long as the temperature is favourable (20–30 °C) [47]. Some serovars are associated with specific hosts [46, 44, 48]. Humans become infected after exposure to environmental sources, such as animal urine, contaminated water or soil or infected animal tissue [41, 46]. As leptospires can penetrate the skin, mucous or conjunctival tissue, any cutaneous wound, in particular a scratch, wound or animal bite, can considerably increase the risk of contamination [40, 41, 45, 49].

Epidemiological data. Table 5 lists nine studies of the seroprevalence of *L. interrogans* in animals. All the analyses were performed by MAT – the gold standard for the immunological diagnosis of the most common serovars of leptospirosis (gryppotyphosa, icterrohaemorrhagiae, pomona and australis). Seven of the studies investigated healthy wild boars killed in hunting. Seropositivity varies from 6% in Italy [50] to 45.5% in Slovenia [51]. Further studies are needed to determine which factors caused this difference. In the Czech Republic in 2003, 6.4% of hares were seropositive [32], while between 1999–2002, about 17% of wild boars were seropositive [52]. Hares and wild boars from these two studies came from different districts and were consequently difficult to compare. A study performed in Croatia [53] showed a high seroprevalence in wild boars (43.8%) and foxes (46.4%) coming from the same region and collected during the same period. Another Croatian study [54] showed that the seroprevalence in wild boars (26%)

Table 5. Seroprevalence of *L.interrogans* in brown hare, wild boar, fox and small mammal in European countries ; MAT – microagglutination test

Country, region	Brown hare	Wild boar	Fox	Small mammal	Detection method	Reference
Czech Republic						
Breclav					MAT	Treml et al. 2003 [52]
Moravian	7.5% (79/1051)	16.9% (52/307)			MAT	Treml et al. 2007 [76]
Breclav, Znojmo, Olomouc	16.4% (12/73)				MAT	Winkelmayer et al. 2005 [32]
Austria						
Northeastern	6.4% (20/311)				MAT	Winkelmayer et al. 2005 [32]
Germany						
Westphalia		24 % (59/245)			MAT	Schönberg et al. 1999 [81]
Slovenia		45.5% (200/437)			MAT	Vengust et al. 2008 [51]
Croatia					MAT	Cvetnic et al. 2003 [54]
North		26% (40/154)	46.4% (52/112)	12.7% (48/379)	MAT	Slavica et al. 2008 [53]
Several places		35% (151/431)	33.8% (121/358)		MAT	Slavica et al. 2011 [82]
Italy						
Tuscany		6% (34/562)			MAT	Ebani et al. 2003 [50]

was higher than in rodents (12.7%). This could be due to the presence of serovars other than icterohaemorrhagiae in the region.

Table 3 shows the number of cases of human leptospirosis declared per year in different countries since 2005. These numbers do not reflect the reality since, in numerous countries, leptospirosis is no longer subject to mandatory reporting, although it continues to be reported to public health agencies in several countries. The significant number of cases declared in France in comparison to the other European countries must be stressed. Leptospirosis is an occupational risk for any persons working in close contact with potentially contaminated animal carcasses or live animals, or in close contact with humid environments favourable to the survival of the bacteria. For a long time, occupational exposure was considered to be the main risk factor for this disease, but today, in the western world, incidences of occupational exposure are decreasing, while incidences of recreational exposure are increasing. Indeed, fresh water sports such as swimming, canoeing, rafting, kayaking and tropical holidays are resulting in an increased number of cases. In Bulgaria, 50% of the cases are considered as a consequence of occupational exposure [55]; in France [56] and Germany [44], epidemiological estimations suggest that 30% of leptospirosis cases are due to occupational exposure and, respectively, only 5% (3/62) and 6.5% (2/31) of all cases concerned forestry workers (hunters and gamekeepers). A study carried out in Austria [30] showed that 10% of hunters and 0% of a control group were seropositive (Table 4). These results clearly show that hunters are frequently exposed to *L. interrogans*. However, due to the relatively high rate of seropositivity, compared with the relatively low number of declared cases, it is possible that leptospirosis remains asymptomatic or sub-diagnosed in a certain number of cases. Other studies are necessary to confirm this hypothesis.

PREVENTIVE MEASURES

As detailed previously, zoonotic diseases have mulitple transmission routes. Forestry workers are therefore strongly encouraged to apply certain preventive measures and they can act at various levels. By wearing simple protective clothing such as long trousers and a long-sleeved shirt, by tucking trousers into socks and by thoroughly inspecting their entire body at the end of the day in order to remove any attached ticks, they can greatly reduce their risk of infection. Use of repellent sprayed on clothing and exposed skin is efficient against ticks and other vectors such as tabanid flies and mosquitoes [see review 57]. One study carried out in France [12] showed that 70% of forestry workers inspect their body at the end of the working day and that > 90% remove ticks quickly if necessary. Only 26% used repellents. Protective clothing was worn by < 50% of forestry workers (41% wore boots and trousers tucked into socks and 33% wore long-sleeved jackets). In Poland, in the region of Podkapackie, inspection of the body at the end of work is most frequently made by workers of aged 30–45 (55%) than by the older workers (40%) [20], while in the Lublin region, 65% of workers inspect their body [14]. In that last study, it was also observed that a large proportion (41.3%) of the forestry workers removed the ticks improperly, using the fingers. Forestry workers should avoid direct contact with potentially contaminated water, animals, soil, etc. The use of protective gloves is highly recommended, and the use of boots and impermeable clothing is recommended to avoid contact with water. To prevent oral contamination, forestry workers should avoiding drinking untreated water and picking and eating forest mushrooms or berries. Game meat must be well cooked before consumption.

To prevent inhalation of contaminated dust or aerosols, wearing appropriate respiratory protection is recommended when manipulating animal carcasses, or working in a dusty environment. No vaccine against LB exists, and the vaccine against tularemia is forbidden in certain countries. A vaccine against leptospirosis is available but it offers protection only against the *L. interrogans icterohaemorrhagiae* serovar [43].

CONCLUSIONS

Lyme borreliosis, tularemia and leptospirosis are zoonoses present in the forests of Europe, as shown by the sero-epidemiological studies performed on animals and forestry workers. They exhibit different prevalencies, mortality and risks of transmission. Lyme borreliosis is the most frequent, while tularemia and leptospirosis are the most lethal. Forestry workers are a population at risk for all three diseases. Studies have highlighted a significantly higher occurrence of these zoonotic diseases in forestry workers compared to control populations. However, the occupational risk remains very difficult to quantify. These zoonotic diseases are certainly under-diagnosed and under-reported as occupational diseases. For these reasons it is difficult to estimate their number and their economic consequences. Preventive measures are relatively easy to apply and not very expensive; however, it seems that the majority of forestry workers do not even apply basic measures. Better information on the risks of these diseases should be communicated, in particular to young forestry workers, and qualitative studies should be carried out to understand why preventive measures are not adhered to by a high proportion of exposed workers.

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