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Prevalence of *Borrelia burgdorferi* Sensu Lato Genospecies in *Ixodes ricinus* Ticks in Europe: a Metaanalysis

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In Europe, *Borrelia burgdorferi* genospecies causing Lyme borreliosis are mainly transmitted by the tick *Ixodes ricinus*. Since its discovery, *B. burgdorferi* has been the subject of many epidemiological studies to determine its prevalence and the distribution of the different genospecies in ticks. In the current study we systematically reviewed the literature on epidemiological studies of *I. ricinus* ticks infected with *B. burgdorferi* sensu lato. A total of 1,186 abstracts in English published from 1984 to 2003 were identified by a PubMed keyword search and from the compiled article references. A multistep filter process was used to select relevant articles; 110 articles from 24 countries contained data on the rates of infection of *I. ricinus* with *Borrelia* in Europe (112,579 ticks), and 44 articles from 21 countries included species-specific analyses (3,273 positive ticks). These data were used to evaluate the overall rate of infection of *I. ricinus* with *Borrelia* genospecies, regional distributions within Europe, and changes over time, as well as the influence of different detection methods on the infection rate. While the infection rate was significantly higher in adults (18.6%) than in nymphs (10.1%), no effect of detection method, tick gender, or collection period (1986 to 1993 versus 1994 to 2002) was found. The highest rates of infection of *I. ricinus* were found in countries in central Europe. *B. afzelii* and *B. garinii* are the most common *Borrelia* species, but the distribution of genospecies seems to vary in different regions in Europe. The most frequent coinfection by *Borrelia* species was found for *B. garinii* and *B. valaisiana*.

Lyme borreliosis (LB), the most frequent tick-borne disease in the northern hemisphere, is a multisystemic disorder caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex. In Europe, the principal vector of *Borrelia* is the tick *Ixodes ricinus*. The risk to humans of infection with *Borrelia* depends on outdoor recreational activity, on the density of tick populations, and on the infection of the ticks with *Borrelia* (32). Therefore, data describing the prevalence of *Borrelia* in ticks can be used to assess the risk of LB for public health.

B. burgdorferi sensu lato is a genetically diverse group of spirochetes. In Europe, at least the following five different genospecies belonging to the *B. burgdorferi* sensu lato complex have been found: *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. valaisiana*, and *B. lusitaniae* (2, 19, 29, 43, 45, 69). Different reservoir hosts seem to harbor different genospecies of *B. burgdorferi* sensu lato, which is explained by differential properties of the hosts' complement systems that favor certain genospecies (73).

At least three species of the *B. burgdorferi* sensu lato complex (*B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto) are known to be pathogenic for humans. The pathogenicity of the other species is still unclear, although *B. valaisiana* and *B. lusitaniae* have been detected in skin biopsies of some LB patients (15, 103). There is strong evidence that infections with different genospecies of *Borrelia* correlate with different clinical symptoms of LB. Lyme arthritis is mainly attributed to *B. burgdorferi* sensu stricto, *B. garinii* infection is preferentially associated with neuroborreliosis, and skin manifestations are associated mainly with *B. afzelii* (1, 18, 121). Therefore, knowledge of the geographic distribution of different genospecies of *B. burgdorferi* sensu lato within their tick vector has not only ecological and epidemiological relevance but also clinical relevance.

Individual ticks can be infected with more than one genospecies of *B. burgdorferi* sensu lato (77, 85, 88, 97). Information on the patterns of such mixed infections may reveal important biological and ecological principles of *B. burgdorferi* sensu lato and also have clinical relevance, since such mixed infections have also been detected in patients (18, 103). However, mixed infections in ticks appear to be rather rare; thus, studies which further specify the mixed infections usually do not contain enough data to draw valid conclusions. A metaanalysis which merges the available data could provide more insight into mixed infections.

In this paper we describe a metaanalysis of epidemiological studies of the prevalence and distribution of genospecies of *B. burgdorferi* sensu lato in host-seeking ticks in various European countries based on a systematic literature review, describing (i) the distribution of *B. burgdorferi* sensu lato and (ii) the occurrence of different *Borrelia* genospecies in *I. ricinus* tick populations in Europe. The following questions concerning the distribution of *B. burgdorferi* sensu lato were examined. (i) What is the mean rate of infection of *I. ricinus* ticks with *Borrelia* in Europe? (ii) How do the rates of infection of ticks differ across Europe? (iii) Has tick infection changed during recent years? (iv) Do the methods used for detection of *Borrelia* in ticks influence the infection rate? In addition, the following questions concerning the occurrence of different *Borrelia* genospecies in *I. ricinus* tick populations in Europe were

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examined. (i) What is the predominant *Borrelia* species in Europe? (ii) Are there differences in the distribution of the *Borrelia* species in different parts of Europe? (iii) Is there a difference in *Borrelia* species distribution in tick nymphs and adults or in females and males? (iv) Which mixed infections involving different *Borrelia* species occur most often?

MATERIALS AND METHODS

We performed a computerized literature search using PubMed to identify all citations concerning rates of infection of ticks with *Borrelia* published from 1984 to 2003, using the keyword search terms "*Ixodes*" and "*Borrelia*". A copy of the abstract of each identified English language citation was obtained. A multistage assessment was used to determine which articles contained relevant data. In the first step we reviewed the abstracts to determine which articles reported epidemiological data for (i) the rate of infection of *I. ricinus* with *B. burgdorferi* sensu lato or (ii) the distribution of the different *Borrelia* genospecies in *I. ricinus*. Only articles with the following criteria were included: (i) the area of tick collection was located in Europe (without the former USSR and regions with a subtropical climate), (ii) the ticks examined were *I. ricinus*, and (iii) the ticks were unfed, host-seeking ticks. In the second step, the articles were retrieved, and their bibliographies were screened for citations not identified in the initial step of the literature search in PubMed. In the third step of the assessment, papers with incomprehensible, incomplete, or previously published data were excluded. Data on larvae were excluded, since the infection rates have consistently been reported to be very low. If this was not possible, the entire paper was excluded. The data extracted from every paper was checked twice.

Data abstraction. Every variable referring to area and period of tick collection, stage, gender, infection with *Borrelia*, and *Borrelia* species in the selected articles was documented. The specific variable analysis was limited to articles containing that variable, which eliminated the need to address missing data. These variables formed the foundation of the final database. Difficulties in abstracting data resulted from nonreported information or reported data that accounted for only a subset of the database. For data abstraction the following steps were carried out. The infection rates (p) of ticks examined in pools (containing at most five ticks per pool) were recalculated where possible, using the following formula: $p = 1 - \sqrt[k]{1 - f}$, where k is the number of specimens in each pool and f is the proportion of infected pools (17). If the number of positive ticks in the study was not given, it was calculated, if possible, based on the number of ticks examined and the reported infection rate. Papers were divided into separate records if the workers examined (i) ticks collected in different years, (ii) different ticks by different methods, (iii) ticks from different countries, or (iv) ticks from collection areas larger than 1° latitude or 2° longitude. This division was carried out only if the number of ticks examined per record was not less than 100. From papers in which the same ticks were examined with different methods leading to different results, only data obtained with PCR or an immunofluorescence assay were included. If not reported, the longitude and latitude of the sampling site of every study was approximated. If more than one collection point was given, the collection area was determined and the coordinates were averaged. Each genospecies found in a mixed infection was added to the corresponding single-infection tally. Records for species-specific analyses of pooled ticks were excluded.

Literature description. A total of 1,186 English abstracts published from 1984 to 2003 were identified in PubMed. After the abstracts were examined, 191 papers proceeded to the second stage, and their reference sections were scrutinized for missed publications, which identified another 26 articles. For analysis of infection rates 110 articles and for species-specific analysis 44 articles progressed through the third stage and to data extraction; 154 and 50 records were extracted for analysis of infection rates and species-specific analysis, respectively.

Statistics. Statistical analyses were done using GraphPad Prism 3.0 (GraphPad Software, San Diego, Calif.). The data expressed in the bar charts are the means ± standard errors of the means; the means are indicated in scatter plots by horizontal lines. An unpaired t test with Welch's correction was performed when two groups were compared. For comparison of more than two groups, analysis of variance (one-way analysis of variance) followed by Bonferroni's multiple-comparison test was used. P values of <0.05, <0.01, and <0.001 were considered significant. Linear regression was performed to analyze the relationship between two variables.

RESULTS

Infection rates. (i) Overall infection rates. Table 1 lists all of the records extracted for the analysis of rates of infection of *B. burgdorferi* sensu lato in *I. ricinus* ticks in Europe. The overall mean prevalence of *Borrelia* in ticks was 13.7% (15,423 of 112,579 ticks). Compared to nymphs (10.1%; 6,384 of 63,298 ticks), adults (18.6%; 8,051 of 43,390 ticks) had a considerably higher infection rate. There was no difference between the infection rates of females and males (18.0% [2,784 of 15,464 ticks[and 16.2% [2,321 of 14,344 ticks], respectively). For studies in which both nymphs and adults in parallel and at least 100 of each stage were examined, 28 of 64 records showed that there were at least twice as many infected adults as infected nymphs, 26 records showed that there were between one and two times more infected adults, 7 records showed that there were as many infected adults as nymphs, and the infection rate in adults was lower than that in nymphs in only 3 records.

Correlating the rates of infection of nymphs with those of adults enabled a linear regression (Fig. 1), resulting in the following formula: $I_N = 0.97 \times I_A - 7.35$, where I_N and I_A are the mean rates of infection of nymphs and adults, respectively.

(ii) Influence of detection methods. The methods generally used for detection of *Borrelia* in ticks are cultivation in BSK medium, dark-field microscopy, an immunofluorescence assay, and PCR. A comparison of the mean infection rates for studies in which at least one of these methods was used for detection of *Borrelia* in ticks revealed no significant difference in either nymphs or adults (Fig. 2). It was noteworthy that the highest infection rates (nymphs, >30%; adults, >35%) were obtained almost exclusively with PCR in Bulgaria, Croatia, southern Germany, Latvia, and Slovakia. In the analysis described below, no distinction between detection methods was made.

(iii) Infection rates in different regions of Europe. The rates of infection of ticks with *Borrelia* were correlated with the latitude or longitude of the sampling site in every study. For this purpose, the coordinates of the sites of tick collection were determined. In studies with a large collection area, the means of latitude and longitude were calculated. Coordinates were transformed to decimal values. Negative longitudes represent the zone west of Greenwich, England, and positive longitudes represent the zone east of Greenwich.

Regression analyses of the mean infection rates with the corresponding longitudes or latitudes showed that there was a significant increase in the infection rate in adult ticks from western Europe to eastern Europe (14 to 24%, as calculated from the linear regression; $P < 0.05$) (Fig. 3a), whereas no such trend was seen for nymphs (data not shown). Latitude had no effect on the prevalence of tick infection either in nymphs (data not shown) or in adults (Fig. 3b). The effect of longitude on the infection rates did not change if only studies in which at least 100 nymphs or adults were examined were included. However, this correction did lead to a significant increase in the infection rates in adult ticks from northern Europe to southern Europe (12 to 23%, as calculated from the linear regression; $P < 0.01$; $r^2 = 0.092$) (data not shown).

To calculate the infection rates in several regions in Europe, the means of the infection rates for all studies in each region were determined for nymphs and adults. To define the regions, the following criteria were taken into account. (i) The regions

TABLE 1. Records with data on rates of infection of *I. ricinus* ticks with *B. burgdorferi* sensu lato in Europe[a]

Country	Reference	Year(s)	Method(s)	Infection rate (%)	No. of ticks		
					Total	Nymphs	Adults
Austria	117	1997	DFM	20.8	1,163	853	310
	53	1989–2002	DFM	23.2	422	211	211
Belgium	85	1996	PCR	23	489	444	45
Bulgaria	9	2000	PCR	32.7	202	90	112
	8	NG	BSK	17	47		47
Croatia	100	1995	PCR	45	124	34	90
Czech Republic	4	1995–1998	PCR	4.5	779	572	207
	48	1988–1996	DFM	20.7	8,339	3,546	4,793
	128	1987–1991	IFA	15.3	5,104	4,417	687
	50	1992	DFM	27	163	34	129
	20	2000	PCR	20.3	370	261	109
	49	1995	DFM	24.3	350	150	200
	49	1996	DFM	22	350	150	200
	49	1997	DFM	26	350	150	200
	49	1998	DFM	24.9	350	150	200
	49	1999	DFM	17.7	350	150	200
	49	2000	DFM	17.7	350	150	200
	49	2001	DFM	24	350	150	200
	53	1989–2002	DFM	21.8	1,094	586	508
	51	1991	DFM	15.2	350	150	200
	51	1992	DFM	23	350	150	200
	51	1993	DFM	22.3	350	150	200
	51	1994	DFM	19.8	350	150	200
	52	1988	GS	8.5	378		378
	47	1988–1989	DFM	20.2	460	209	251
	68	NG	DFM	7.9	570	NG	NG
	114	1997	DFM	5.4	555	145	410
	114	1998	DFM	11	163	13	150
Denmark	74	1990–1991	IFA	18.6	317	202	115
	59	1995	IFA	5.3	396	396	
	59	1996	IFA	3.1	829	829	
	59	1997	IFA	2.4	327	327	
	60	1996	IFA	5.1	1,151	1,045	106
Estonia	79	2000	PCR	15	100		100
Finland	62	1996	PCR, DFM, BSK	32.2	726	303	423
	63	1992	BSK	5.5	1210	392	818
	79	2000	PCR	5.1	454	343	111
France	94	1994	PCR	12	249	249	
	95	1995	PCR	8.2	461	461	
	31	1992	IFA	9.8	1,556	1,014	542
	31	1992	IFA	6.4	1,822	1,422	400
	31	1992	IFA	4.7	1,295	811	484
	129	1991	IFA	10.7	383	314	69
	96	1994–1995, 1998	PCR	14.3	638	419	219
Germany	25	1985–1986	IFA	14.6	2,369	1,157	1,212
	26	1994	IFA	19.1	472	157	315
	27	1997	PCR	36.2	492	91	401
	97	1999–2000	PCR	35.2	1,055	507	548
	38	1992–1993	DFM	10.4	279	68	211
	38	1992–1992	DFM	4.7	1,185	1,185	
	38	1992–1993	BSK	8.4	598	485	113
	38	1992–1993	IFA	12.4	194	147	47
	64	1991	IFA	18.4	414	414	
	126	1992	DFM	16	100		100
	126	1993	BSK	18	100		100
	126	1994	PCR	22	100		100
	111	1996	PCR	9	3,926	3,520	406
	69	NG	PCR	18.1	226		226
	71	1987–1988	IFA	6.3	1,954	1,954	
	88	1998–2000	PCR	15.8	3,138	NG	NG
	44	1999–2000	PCR	11.1	305	243	62
	65	1986	BSK	5.8	173	49	124
	98	1994	PCR	9	112	112	
	90	NG	PCR	31.5	165	NG	NG
	125	1993–1994	BSK	16.3	559		559
	81	1991	DFM, IFA	17.9	756	531	225

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TABLE 1—*Continued*

Country	Reference	Year(s)	Method(s)	Infection rate (%)	No. of ticks		
					Total	Nymphs	Adults
Ireland	36	1997–1998	PCR	12	183	164	19
	37	NG	IFA	12	100	100	
	66	1995	PCR	14.9	915	686	229
	34	1992–1993	IFA	2.6	2,587	2,518	69
	33	1990–1991	IFA	6.5	612	509	103
	67	1995	PCR	18.4	512	411	101
Italy	13	1994	PCR	40	227	218	9
	11	NG	PCR	4.4	420	NG	NG
	105	1997–1998	PCR	2.9	1,503	1,475	28
	12	NG	PCR	19.8	86	84	2
	24	1998	PCR	11.4	55	55	
	106	NG	PCR	4.3	141	125	16
	80	1995	BSK	0	80	80	
	80	1995	PCR	0	110	110	
Latvia	69	NG	PCR	31.3	300		300
	23	1999	PCR	31.2	205	55	150
	23	2000	PCR	25.7	300	150	150
Lithuania	86	1988–1991	DFM	10.1	3,820	287	3,533
The Netherlands	121	NG	BSK	10.5	248	176	72
	102	1994	PCR	15.6	96	39	57
	102	1994	IFA	19.5	210	120	90
	101	1988–1993	IFA	24.6	463	341	122
	17	1991	DFM	12.5	522	476	46
Norway	58	1999	PCR	15.8	341	185	156
Poland	110	1997–1998	PCR	21.6	616	48	568
	113	2000	PCR	11.6	424		424
	108	1999	PCR	7.8	1,710	1,160	550
	122	1993	IFA	11.5	1,666	1,070	596
	7	2000	IFA	11.3	1,387	1,264	123
	7	2001	IFA	9.6	993	931	62
	93	1996	DFM, BSK	20.7	92	6	86
	93	1996	DFM, BSK	12.8	815	110	705
	83	1998	IFA	18.6	587	538	49
	83	1998	IFA	13.6	536	464	72
	107	1999	PCR	18.9	514	234	280
	127	1998	PCR	4	1,262	835	427
	127	1999	PCR	5.7	1,379	1,006	373
	127	2000	PCR	4.8	1,356	955	401
	127	2001	PCR	3.3	1,439	894	545
	14	2000	DFM	8.8	114	50	64
	14	2001	PCR	5.3	549	265	284
	112	1996	IFA	8.1	1333	554	779
	123	1994	IFA	8.8	3,958	2,395	1,563
Portugal	18	1998	PCR	75	55		55
	69	NG	PCR	14.7	217		217
Slovakia	22	1991	DFM	18.5	2,857		2,857
	29	1998	IFA	49.1	114		114
	115	1994	DFM	4.8	809	149	660
	115	1995	DFM	17.2	805	215	590
	115	1996	DFM	15.6	805	155	650
	115	1996	DFM	14.2	399	113	286
	69	NG	PCR	40.5	585		585
	21	2002	PCR	43.3	60	20	40
	42	2000	PCR	28.2	177	93	84
	43	1999	PCR	32.2	966	382	584
	43	2000	PCR	33.2	328	174	154
Slovenia	104	1990	IFA	7.7	496	411	85
	116	NG	BSK	19	363	206	157
Spain	3	1992–1993	PCR	1.5	134		134
Sweden	40	1988–1991	IFA	14.4	2974	397	2,577
	119	1994–1995	PCM	13	1,908	1,908	
	28	1999	PCR	11	301		301
	120	1991	PCM	15.4	408	408	
	120	1992	PCM	20.6	471	471	
	120	1993	PCM	24.1	551	551	
	82	1988	PCM	12.8	539	459	80

Continued on following page

TABLE 1—*Continued*

Country	Reference	Year(s)	Method(s)	Infection rate (%)	No. of ticks		
					Total	Nymphs	Adults
	82	1989	PCM	8.6	1,005	946	59
	5	1991	IFA	20.8	149	92	57
	56	1991	PCM	9.1	396	396	
	6	1989	PCM	2	42	42	
	57	1992	PCM	12.6	381	381	
	57	1993	PCM	11	581	581	
	57	1994	PCM	14.2	604	604	
	39	NG	IFA	29.6	54	16	38
Switzerland	92	1987–1993	IFA, DFM, BSK	30.4	727		727
	54	1993	BSK	13.7	95	22	73
	84	1987	IFA	30.8	182	94	88
	75	1991	BSK	16.5	133		133
	76	NG	PCR	49	100		100
	61	1999–2001	IFA	10.9	460	415	45
	55	1993–1994	BSK	19.1	235	93	142
	109	NG	BSK	13.6	118	NG	NG
	30	1990	DFM	21.4	56	56	
United Kingdom	16	1997	BSK	8.5	128	NG	NG
	72	1995–1996	PCR	4.6	680	580	100
	41	NG	PCR	7.7	65		65
	89	1994	IFA or PCR	8.3	109	73	36
	89	1995	IFA or PCR	3.9	333	221	112

[a] The total infection rate is indicated. The number of nymphs and adults examined shows the stage composition of all ticks examined (total) for each record. BSK, cultivation in BSK medium; DFM, dark-field microscopy; GS, Giemsa-stained smears; IFA, immunofluorescence assay; PCM, phase-contrast microscopy; NG, not given. The average infection rate was 13.7%, and a total of 112,579 ticks were examined.

were defined so that they were large enough to include the studies with large collection areas completely; and (ii) geographic conditions were taken into consideration. The available data are summarized and mapped in Fig. 4. We distinguished regions with low infection rates (nymphs, ≤11%; adults, ≤20%) and high infection rates (nymphs, >11%; adults, >20%). In three regions (regions A, R, and S) the mean rate of infection of adults was extremely high (>30%) (Fig. 4b).

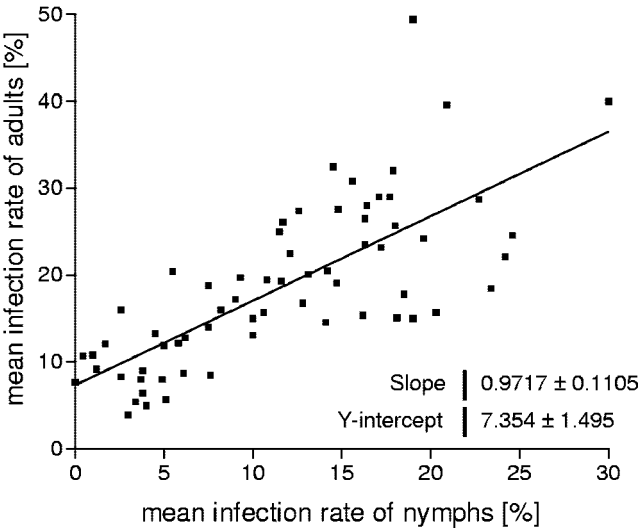


FIG. 1. Regression of mean rates of infection of *Borrelia* in nymphs and adults. Only studies in which both nymphs and adults and at least 100 individuals of each stage were examined were included. Each data point represents one record.

The limitation to studies in which at least 100 adults or 100 nymphs were examined led to considerably different results for four of the regions. In regions A and M the rates of infection of adults decreased from 30 to 8% and from 21 to 11%, respectively. In region R the rate of infection of adults increased from 31 to 46% and for nymphs no data could be used. In region S, an increase from 17 to 23% in the rate of infection of nymphs occurred.

(iv) Years of tick collection. To compare the course of infection rates over the years, studies with a collection period longer than 1 year were excluded unless the data could be separated into years. As the rates of infection of ticks in several regions in Europe vary significantly (Fig. 4), a comparison of the rates of infection per year requires a representative profile of regions with high and low infection rates in each year. Since such data were not available, data for ranges of years (1986 to 1993 and 1994 to 2001) were merged. Data for regions with extremely high infection rates (regions A, R, and S) were excluded to obtain similar proportions of areas with high and low infection rates in both periods. A comparison of the two periods revealed no significant difference in the means of the rates of infection of nymphs or adults (Fig. 5).

Species-specific analysis. (i) Overall ratio of *Borrelia* species in Europe. The data from studies in which there were species-specific analyses of *B. burgdorferi* sensu lato in *I. ricinus* ticks are summarized in Tables 2 and 3. The following problems were noticed. (i) The number of ticks examined per study was extremely low in some cases (3 of 50 records included less than 10 ticks). Therefore, we first checked in every analysis to determine whether these studies distorted the results. If this was the case, the studies were excluded, as mentioned below. (ii) The stage and gender of the ticks examined were not stated in

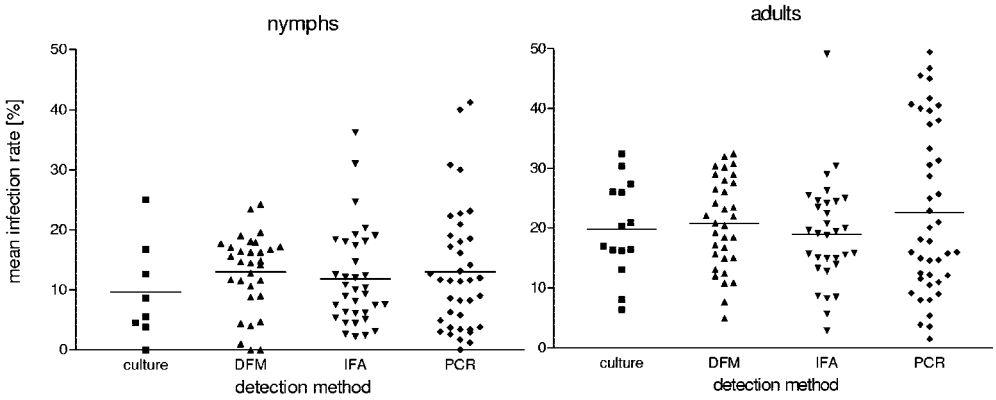


FIG. 2. Influence of detection method on infection rates. DFM, dark-field microscopy; IFA, immunofluorescence assay.

every study, which reduced the number of records considerably. In Table 2 each species found in a mixed infection reported separately was added to the appropriate single-infection tally in order to harmonize results (therefore, the sum of the percentages may result in values greater than 100%). To avoid overrepresentation of single studies in which high numbers of ticks were examined, the percentages of positive ticks for each species (combination) of every single study were used for the analyses (Fig. 6 to 8).

Figure 6 shows the overall ratio of *Borrelia* species in Europe. No distinction was made between nymphs and adults. The mean percentages of *B. afzelii*-, *B. garinii*-, *B. burgdorferi* sensu stricto-, *B. valaisiana*-, and *B. lusitaniae*-infected ticks were 38, 33, 18, 19, and 7%, respectively. Five percent of the borreliae were untypeable, and 13% of the ticks had a mixed infection. There was no significant difference between *B. afzelii* and *B. garinii*, but the means for both species were significantly different from the means for the other three species.

Since all the studies distinguished between the genospecies *B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto (50 records), but not every study tested for *B. valaisiana* (33 records) and only a few studies tested for for *B. lusitaniae* (20 records), the data for the latter two species are weaker. Also, in records which did not determine *B. valaisiana* and *B. lusitaniae*, these species might be recognized as "nontypeable" or be falsely recognized as one of the other species. For example, we reported this problem in a study employing real-time PCR which distinguished species by different numbers of mismatches with a fluorescent probe. The PCR product of *B. valaisiana* had the same melting point as that of *B. afzelii* and was therefore recognized as *B. afzelii* (97).

To check if studies containing *B. lusitaniae*- and nontypeable *Borrelia*-positive ticks influenced the results, we repeated the analysis after these ticks were excluded. The mean percentages of *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, and *B. valaisiana* positive ticks remained similar to those shown in Fig. 6 (36, 34, 15, and 21%, respectively).

(ii) Stage- and gender-dependent distribution of *Borrelia* species in Europe. No significant difference was seen when the prevalence of each *Borrelia* species in nymphs was compared to that in adults (Fig. 7). This result did not change when we included only studies which distinguished between the genospecies *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, and *B. valaisiana*.

Only 11 records for a total of 156 female and 97 male ticks were available for a direct gender-based comparison of *Borrelia* species distribution. Since no information on gender was given for the ticks with mixed infections, we were not able to add

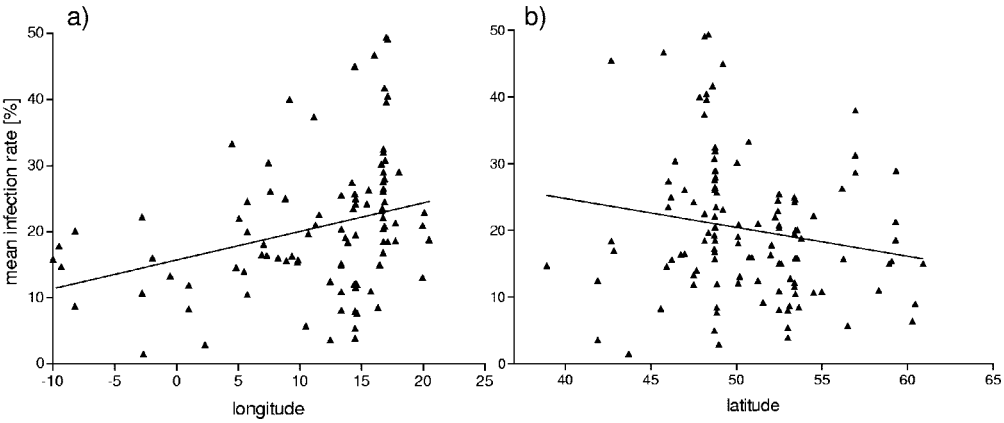
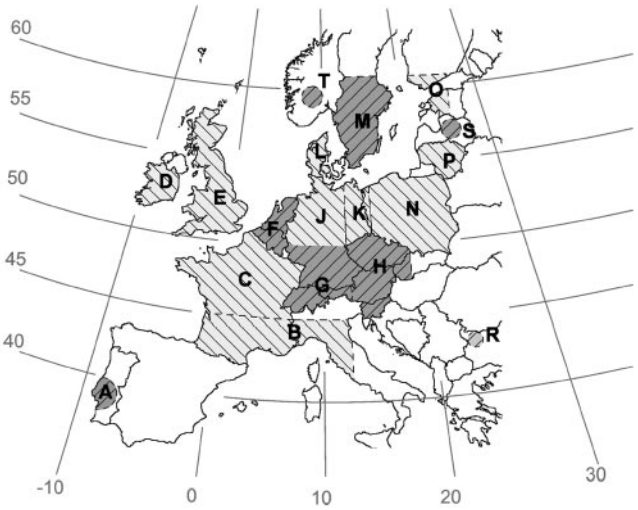


FIG. 3. Regression analysis of the mean rates of infection in adults with the corresponding longitude ($P < 0.05$; $r^2 = 0.102$) (a) or latitude ($P > 0.05$; $r^2 = 0.031$) (b).

a)



b)

region	Inf. N [%]	# records	Inf. A [%]	# records
A	–	0	30.4 §	3
B	2.1	3	8.1	2
C	8.3	8	10.8	6
D	10.6	6	15.7	5
E	1.5	3	16.3	3
F	20.0	5	22.1	5
G	17.5	10	25.0	10
H	16.3	29	27.5	33
J	8.0	4	17.9	4
K	8.3	17	14.5	16
L	7.2	5	10.7	2
M	15.6	12	21.2	5
N	9.0	14	15.8	14
O	3.8	2	10.1	3
P	1.0	1	10.8	1
R	17.2	1	31.3 §	2
S	17.7	2	32.7 §	3
T	16.2	1	15.4	1

FIG. 4. (a) Map of the defined regions. Areas with low infection rates (nymphs, ≤11%; adults, ≤20%) are indicated by light gray; areas with higher infection rates are indicated by dark gray. (b) Regions and means of the rates of infection of nymphs (Inf. N) and adults (Inf. A). The number of records used for every region is indicated. A section sign indicates that the infection rates were extremely high (>30%).

each species in a mixed infection to the appropriate single-infection tally. Hence, for gender-specific comparisons, only infections with a single species were included. Data were extracted from 11 records (156 females and 97 males). There was no significant difference in the mean percentage between females and males for any species (data not shown). Insufficient data were available for analysis with *B. valaisiana* and *B. lusitaniae*.

(iii) Mixed infections. The data for the 13% mixed infections are shown in Table 3, which indicates the summarized frequencies for the combinations reported. The occurrence of mixed infections in nymphs (12.1%) was not statistically different from that in adults (13.6%). The distribution of combinations of mixed infections is shown in Fig. 8. If the analysis was restricted to studies which distinguished between *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, and *B. valaisiana* (21 records), the combination of *B. garinii* and *B. valaisiana* occurred 51% more often than all other species combinations.

A mixed infection with *B. lusitaniae* was described only once in combination with *B. garinii* (9). Combinations of three species occurred only rarely.

A direct comparison of species combinations for nymphs and adults revealed no significant differences (data not shown), although only a few records were available (10 records for nymphs and 14 records for adults).

(iv) *Borrelia* genospecies distribution in different parts of Europe. To compare the distributions of *Borrelia* species in various parts of Europe, we merged the data from different countries. For this purpose, the following inclusion criteria were taken into account. (i) The countries had to be adjacent (except group 3). (ii) The ratios of genospecies in the records were similar. (iii) There had to be at least four records for each group. Based on these criteria, seven groups were defined (Table 4). Because of the low number of records, studies in which less than 10 ticks were examined were excluded to avoid distortion. Since only a few data are available for *B. lusitaniae*, this organism was also excluded from this analysis. The results clearly revealed diverse patterns of species distribution in the different areas of Europe. In groups 1, 2, and 3 significantly more ticks were infected with *B. afzelii* than with *B. garinii*, while in groups 4 and 5 *B. garinii* seemed to predominate. In group 4, ticks seemed to be equally frequently infected with *B. valaisiana* and *B. garinii*. The data for groups 6 and 7 revealed that there was no significant difference between *B. afzelii* and *B. garinii*.

As mentioned above, some studies distinguished only between the genospecies *B. afzelii*, *B. garinii*, and *B. burgdorferi*

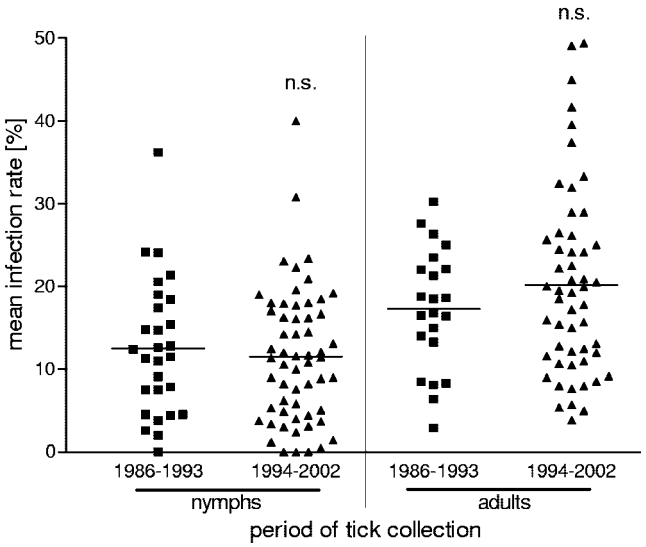


FIG. 5. Comparison of rates of infection of nymphs and adults in two collecting periods. Each data point represents one record. To obtain a similar proportion of areas with high and low infection rates in both periods, data for extremely high infection rates (>30%) were excluded. n.s., not significant.

TABLE 2. Records extracted for analysis of *B. burgdorferi* sensu lato genospecies in *I. ricinus* ticks in Europe[a]

Country	Reference	Total no.	No. of *Borrelia*-positive ticks					
			Nontypeable	*B. afzelii*	*B. garinii*	*B. burgdorferi* sensu stricto	*B. valaisiana*	*B. lusitaniae*
Austria	117	29	0	11	17	2		
	118	50	0	25	21	5		
Belgium	85	114	17	14	79	56		
Bulgaria	9	24	1	18	2	5	2	1
	8	8	0	0	4	4		
	10	46	6	26	3	15	3	1
	10	22	2	11	5	0	5	0
Croatia	100	56	8	37	5	2	15	
Czech Republic	4	34	0	15	21	0		
	20	76	1	45	16	8	9	
	114	9	0	2	3	7		
Estonia	79	15	0	12	3	0	0	0
	62	142	5	101	38	0		
Finland	79	23	0	12	2	9	0	0
France	94	25	0	19	9	3		
	95	38	12	17	9	3		
	129	3	0	0	1	2		
	96	91	5	36	29	5	27	0
Germany	97	371	5	261	126	46		
	69	41	3	18	15	0	6	0
	124	67	0	6	26	27	8	
	78	17	1	3	13	0		
	44	34	0	6	19	11	2	
	98	10	0	4	5	1	0	0
Ireland	36	22	4	1	10	0	8	
	66	136	8	9	47	27	64	
	67	94	2	16	26	21	47	
Italy	13	91	21	8	42	56	1	
Latvia	69	94	1	39	34	5	19	0
	23	64	1	20	29	3	15	0
	23	77	8	35	20	10	10	0
The Netherlands	121	26	0	26	0	0		
	102	15	0	6	7	0	5	
	87	63	13	2	19	29		
Norway	58	54	0	49	1	14		
Poland	110	121	14	57	45	48		
Portugal	18	41	0	0	0	0	0	41
	69	32	5	1	14	0	18	0
Slovakia	29	37	0	25	5	0	5	2
	69	237	26	129	59	5	42	0
	42	50	0	21	5	0	23	1
	43	311	0	186	79	7	68	6
	43	109	0	45	8	0	53	3
Slovenia	116	60	0	32	20	8		
Sweden	28	32	2	14	10	4	2	
Switzerland	92	50	0	3	19	26	2	
	61	20	2	0	13	0	3	3
	55	41	2	14	8	21	0	0
	109	28	0	6	12	8	2	
United Kingdom	72	23	2	0	15	2	7	

[a] The numbers of *B. afzelii*-, *B. garinii*-, *B. burgdorferi* sensu stricto-, *B. valaisiana*-, and *B. lusitaniae*-positive ticks and the total numbers of ticks examined are shown. Each species found in a coinfection reported separately was added to the corresponding single-infection tally.

sensu stricto. Since *B. valaisiana*- and untypeable *Borrelia*-infected ticks are also included in Table 4, this may have resulted in a disproportion in the ratio of the first three species. Due to the low number of records it was not possible to compare only studies in which all four species were examined. Therefore, we repeated the analysis and excluded untypeable *Borrelia*- and *B. valaisiana*-positive ticks, which allowed direct comparison of *B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto. There was no significant difference in the results compared to the results shown in Table 4 (data not shown).

DISCUSSION

A metaanalysis provides a versatile alternative to the more traditional review methods and allows quantitative conclusions to be drawn. The prevalence of *Borrelia* infection in ticks is one of the most essential components of risk assessment for LB. In recent years, many studies of the rate of infection of ticks with *Borrelia* have been reported. Analysis of data from 155 records of studies conducted in Europe showed that the overall mean infection rate was 13.6% and that the rate of infection of adult ticks was significantly higher than

TABLE 3. Records of mixed infections with *B. burgdorferi* sensu lato genospecies

Country	Reference	Total	No. of ticks infected with:							Triple infection
			B. afzelii and *B. garinii*	*B. afzelii* and *B. burgdorferi* sensu stricto	*B. garinii* and *B. burgdorferi* sensu stricto	*B. afzelii* and *B. valaisiana*	*B. garinii* and *B. valaisiana*	*B. burgdorferi* sensu stricto and *B. valaisiana*	*B. garinii* and *B. lusitaniae*	
Austria	117	1	1	0	0					0
	118	1	1	0	0					0
Bulgaria	9	5	1	2	0	1	0	0	1	0
Croatia	100	11	0	1	0	10	0	0		0
Czech Republic	4	2	2	0	0					0
	20	4	0	2	0	0	2	0		0
	114	3	2	0	1					0
Finland	62	2	2	0	0					0
France	94	6	3	1	2					0
	95	3	2	1	0					0
	96	11	2	1	0	2	6	0	0	0
Germany	97	66	59	6	0					1
	44	4	0	0	4	0	0	0		0
	69	1	0	0	0	0	1	0	0	0
Ireland	66	18	0	1	0	2	14	0		1
	67	17	0	3	0	3	10	0		1
	36	1	0	0	0	0	1	0		0
Italy	13	31	0	1	23	0	1	0		6
	106	1	1	0	0	0	0	0		0
Latvia	69	4	0	2	0	0	2	0	0	0
	23	4	0	1	0	0	2	1	0	0
	23	6	0	3	0	0	3	0	0	0
The Netherlands	102	3	0	0	0	3	0	0		0
Norway	58	10	0	10	0					0
Poland	110	41	11	17	11					2
Portugal	69	6	0	0	0	0	6	0	0	0
Slovakia	69	24	2	2	0	0	20	0	0	0
	43	34	2	2	0	2	26	0	0	2
Switzerland	55	3	0	2	0	0	0	0	0	1
	61	1	0	0	0	0	1	0	0	0
United Kingdom	72	3	0	0	0	0	3	0		0

that of nymphs, which was observed in the majority of the studies and is explained by the fact that host-seeking adult ticks had had two blood meals on different hosts. These data are in line with the data reviewed by other workers (35, 46). Recently, a *B. miyamotoi*-like *Borrelia* species was detected in *I. ricinus* ticks in Europe (28, 99). Therefore, the rate of infection of ticks with spirochetes belonging to the *B. burgdorferi* sensu lato complex may be overestimated by some detection methods.

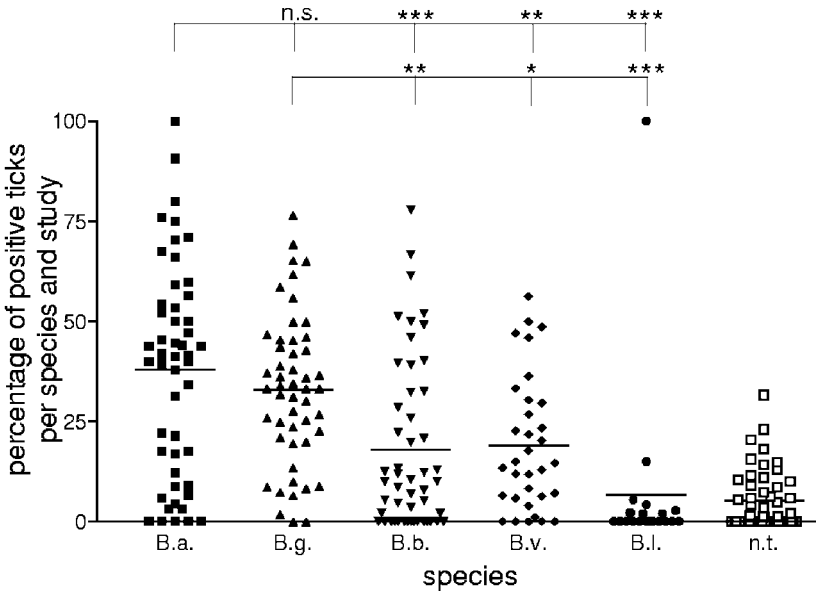


FIG. 6. Overall ratio of the *Borrelia* species *B. afzelii* (B.a.), *B. garinii* (B.g.), *B. burgdorferi* sensu stricto (B.b.), *B. valaisiana* (B.v.), and *B. lusitaniae* (B.l.) in *I. ricinus* ticks in Europe. Fifty records with 3,273 positive ticks were included. The percentages of positive ticks per species for every single study are given. n.t., nontypeable; n.s., not significant; one asterisk, $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

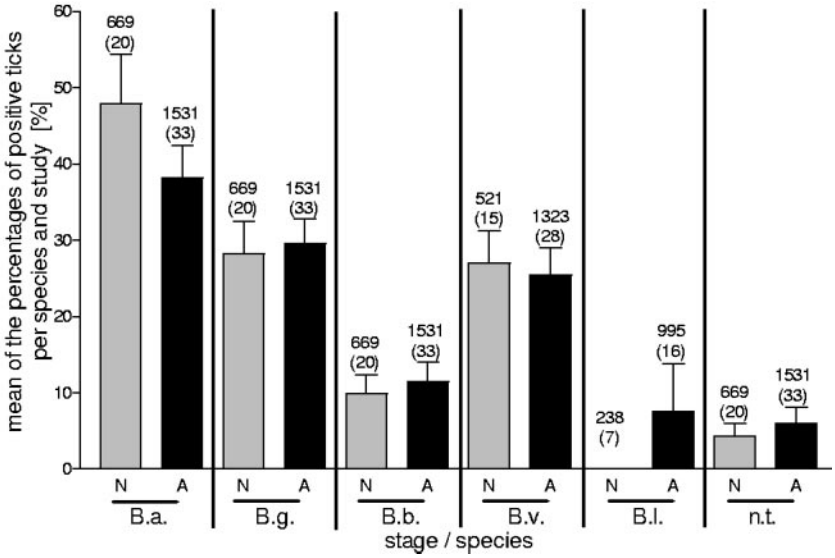


FIG. 7. Distribution of the *Borrelia* genospecies *B. afzelii* (B.a.), *B. garinii* (B.g.), *B. burgdorferi* sensu stricto (B.b.), *B. valaisiana* (B.v.), and *B. lusitaniae* (B.l.) and nontypeable *Borrelia* (n.t.) in nymphs (N) and adults (A). The average of the percentages of positive ticks per species for every single study is given. The numbers of infected ticks examined are indicated above the bars, and the numbers of records are indicated in parentheses.

The correlation of rates of infection of adult ticks with the coordinates of sampling sites showed a significant trend, with the rates increasing from western Europe to eastern Europe. No effect was seen for nymphal ticks. The effect of longitude on the rates of *B. burgdorferi* sensu lato infection of unfed *I. ricinus* ticks was also examined by Gray et al. in 1998. These authors reported that the rates of infection for both nymphal and adult *I. ricinus* ticks were significantly higher in eastern Europe than in the west (35). In contrast to our analyses, they restricted the area to the coordinates from 5°W to 20°E. Indeed, a restriction of our data to this area also led to a significant increase in the rates of infection of nymphs (7 to 15%, calculated from regression; $P < 0.05$; $r^2 = 0.037$) from western Europe to eastern Europe. The map in Fig. 4 shows that the regions with the highest infection rates are located in central Europe (Austria, Czech Republic, southern Germany, Switzerland, Slovakia, and Slovenia). The high infection rates in central Europe combined with the large number of studies carried out in these regions probably led to the overall trend from western Europe to eastern regions of Europe.

Including only studies in which at least 100 adults and/or nymphs were examined led to different results in only four of the regions, indicating that most of the values are robust. Since in these four regions only a few records were available, they are vulnerable to outliers. In region A, for example, the decrease in the infection rate is based on the loss of a single record with an infection rate of 75%. Therefore, more studies with a higher number of ticks examined are required in such regions to obtain more robust results.

The available data did not allow analysis of the tick infection rate by years. Therefore, we compared two time periods (1986 to 1993 versus 1994 to 2001). In the first of the two periods, ticks were examined by a immunofluorescence assay, dark-field microscopy, or culture. From 1994 on, PCR, which is generally considered to be the most sensitive method, was the most commonly used detection method. Nevertheless, this did not lead to any differences in reported infection rates between the two periods. It was noteworthy that the highest infection rates reported for nymphs (>25%) and adults (>30%) were found only in the period from 1997 to 2001 and were almost all detected with PCR. Not enough data were available to break down the data by year and method, but exclusion of PCR from the analysis made no difference (data not shown). Taken together, there is no indication that there was an increase in the tick infection rates over time or due to introduction of the more sensitive PCR method. For future analyses, and if a sufficient sampling population is available, it would be interesting to study the effect that a significant seasonal event can have on the rate of infection of *I. ricinus*.

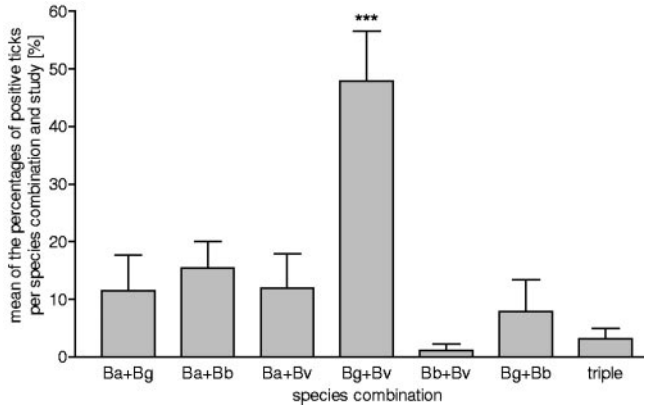


FIG. 8. Distribution of mixed infections in ticks. Only records in which *B. afzelii* (Ba), *B. garinii* (Bg), *B. burgdorferi* sensu stricto (Bb), and *B. valaisiana* (Bv) were examined were included (21 records). The average of the percentages of positive ticks per species combination for every single study is given. Three asterisks, $P < 0.001$.

TABLE 4. Distribution of *Borrelia* genospecies in different parts of Europe[a]

Group	Countries	% of positive ticks (mean ± SEM)					No. of records for *B. afzelii, B. garinii, B. burgdorferi* sensu stricto, and nontypeable *Borrelia*	No. of records for *B. valaisiana*
		B. afzelii	*B. garinii*	*B. burgdorferi* sensu stricto	*B. valaisiana*	Nontypeable *Borrelia*		
1	Southern Germany, Czech Republic, Slovakia	55 ± 4	25 ± 6[b]	3 ± 2[b]	27 ± 7[c]	2 ± 1	8	6
2	Norway, Sweden, Finland, Estonia	68 ± 9	18 ± 5[b]	16 ± 8[b]	2 ± 2	2 ± 1	5	3
3	Bulgaria, Croatia, Slovenia	60 ± 5	16 ± 5[b]	14 ± 6[b]	16 ± 5[b]	8 ± 3	5	4
4	United Kingdom, Ireland	7 ± 4[c]	43 ± 8	13 ± 5[c]	41 ± 5	9 ± 3	4	4
5	Central and northern Germany	26 ± 7	52 ± 7	17 ± 8[c]	8 ± 3[c]	3 ± 2	5	4
6	Austria, Switzerland	25 ± 8	44 ± 7	25 ± 9	7 ± 3	3 ± 2	6	4
7	The Netherlands, Belgium, northern France	46 ± 15	34 ± 9	19 ± 9	33	11 ± 5	6	1

[a] Seven different groups were defined as described in the text. The *P* values are the *P* values for the species with the highest mean percentage (for groups 1 to 3, *B. afzelii*, for groups 4 and 5, *B. garinii*).
[b] $P < 0.001$.
[c] $P < 0.01$.

The fact that the isolated variables are interconnected should be taken into account. A solution to this problem would be a multiple linear or logistic regression, but the heterogeneity of data reporting precluded such an approach.

In Europe there are at least three species that are known to be pathogenic for humans, *B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto, the last of which is the only species found in the United States (2). As the different *Borrelia* species appear to be associated with different clinical manifestations, accurate knowledge of the distribution of these species in Europe might be helpful. Analyses of data from 53 records revealed a distinct pattern of *Borrelia* species distribution in Europe: *B. afzelii* and *B. garinii* are the most common species, followed by *B. valaisiana* and *B. burgdorferi* sensu stricto. *B. lusitaniae* was tested in only a few studies but seems to be rather rare or nonexistent in most regions, since it was found in only 8 of the 20 records, which showed that *B. lusitaniae* was present at mostly a low frequency. The occurrence of *B. bissettii* was described once in combination with *B. afzelii* and *B. garinii* (43) in Slovakia. The prevalence of *Borrelia* genospecies in *I. ricinus* ticks seems to vary in different parts of Europe. This could be explained by the prevalence of different reservoir hosts, although considerably more data are necessary before robust patterns can be determined and a correlation between ecological data and clinical treatment manifestations can be carried out. Not enough data were available to break down the species differences by method of detection. Therefore, the possibility that the differences in species distribution by region may be determined in part by the different accuracies of the methods used by the different investigators in each region could not be ruled out.

Various studies showed that there are specific associations between *Borrelia* species and reservoir hosts. *B. afzelii* is preferentially transmitted by small rodents, and *B. valaisiana* and *B. garinii* are mainly associated with birds, although the latter genospecies is very heterogeneous and some strains preferentially infect ticks via rodents. *B. burgdorferi* sensu stricto seems to occur in both birds and rodents (42, 43, 55, 70, 72, 91). This host association can be explained by the specific effect of the host's complement system on each *B. burgdorferi* sensu lato genospecies (73). Direct comparison of questing nymphs and adults showed no significant difference in the *Borrelia* species distribution, suggesting that the infected hosts for larvae and nymphs are similar.

Thirteen percent of *Borrelia*-positive *I. ricinus* ticks showed infection with multiple genospecies. Such mixed infections in individual ticks can be explained by (i) superinfection of ticks that are already infected transovarially, (ii) cotransmission of multiple *Borrelia* species from an infected tick to an uninfected tick feeding on the same host, (iii) cotransmission of several strains from a host infected by more than one *Borrelia* species, or (iv) consecutive infectious blood meals. In contrast to a questing nymph, which has had only one previous blood meal as a larva, an adult has fed on two different hosts. Therefore, it appeared likely that adults would exhibit more mixed infections than nymphs, although this was not the case. A possible explanation for this could be that the effect of complement may occur not only in the host, which selects for *Borrelia* species, but also in the midgut of the tick by complement taken up during the blood meal, as discussed by Kurtenbach et al. (69, 73). In fact, the most frequent combination of *Borrelia* species found in Europe was *B. garinii* and *B. valaisiana*, both of which occur most commonly in birds.

In conclusion, the prevalence of different *B. burgdorferi* sensu lato genospecies was deduced for various regions in Europe. While the infection rate was about twofold higher in adult ticks than in nymphs, no effect of detection method, tick gender, or collection period (1986 to 1993 versus 1994 to 2002) was found.

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