

# Antibodies against *Borrelia burgdorferi* sensu lato among Adults, Germany, 2008–2011

Hendrik Wilking, Volker Fingerle, Christiane Klier, Michael Thamm, and Klaus Stark

To assess *Borrelia burgdorferi* sensu lato (the cause of Lyme borreliosis) seropositivity in Germany, we tested serum samples from health survey (2008–2011) participants. Seroprevalence was 5.8% among women and 13.0% among men; infection risk was highest among persons  $\geq 60$  years of age. Public health interventions, including education about risk factors and preventive measures, are needed.

Lyme borreliosis, the most common tickborne disease in the Northern Hemisphere, is caused by infection with spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex. Five genospecies are known to be pathogenic for humans: *B. burgdorferi* sensu stricto (s.s.), *B. afzelii*, *B. garinii*, *B. bavariensis*, and *B. spielmanii* (1). In Europe, the bacterium is transmitted to humans through the bite of *Ixodes ricinus* ticks; in eastern Europe, *I. persulcatus* ticks can also transmit the bacterium.

In Europe, where the most common clinical manifestation of Lyme borreliosis is erythema migrans, followed by Lyme neuroborreliosis and Lyme arthritis (2), data are sparse regarding *B. burgdorferi* s.l. infection rates and risk factors (3). Persons of all ages are at risk for infection; however, surveillance data and prospective studies in Europe and the United States suggest that children and the elderly are particularly at risk (4–6). Population-based surveillance data suggest that Lyme borreliosis is endemic in eastern Germany: annual incidence is 20–35 cases/100,000 inhabitants (7). Regional differences in incidence are observed, but data cannot be easily compared because of reporting biases and differences in infection awareness.

The limited representativeness and comparability of Lyme borreliosis surveillance data are well documented (8). Under such conditions, population-based serosurveys with high representativeness can provide valid estimates of the force of infection (rate at which susceptible persons acquire Lyme borreliosis) and the lifetime risk for infection; however, seroprevalence estimates do not necessarily represent cases of clinical disease. In a population-based seroprevalence study among 1- to 17-year-old children in

Germany, seroprevalence increased cumulatively by age (9). We present data on the prevalence and determinants of *B. burgdorferi* s.l. seropositivity among adults in Germany during 2008–2011.

## The Study

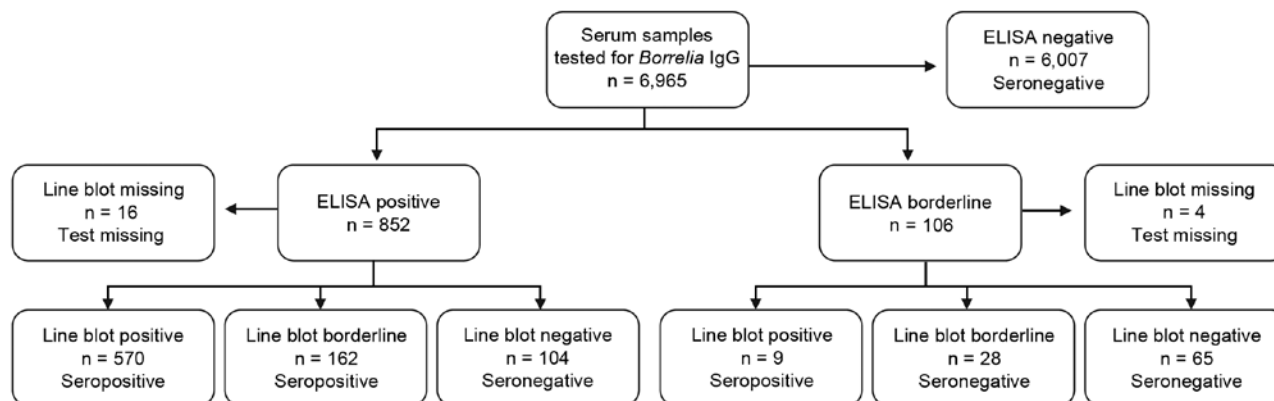
We estimated *B. burgdorferi* s.l. seroprevalence among participants of the German Health Interview and Examination Survey for Adults (DEGS). This nationwide cross-sectional survey assessed the health status of 18- to 79-year-old persons in Germany during 2008–2011 (10). The response rate was 48.4%; analysis of nonresponder questionnaires revealed high population representativeness. Data from standardized interviews were used to assess potential risk factors for seropositivity. Survey weights based on age, sex, residence in western or eastern Germany, and nationality (German vs. non-German) were calculated to correct for deviations from the German population statistics (December 31, 2010; <http://www.destatis.de>) and used throughout the analyses. The study was approved by the Ethical Review Board of the Medical School Charité, Berlin, Germany.

As recommended for serologic confirmation of clinical cases, serum samples were tested for the presence of *Borrelia burgdorferi* s.l. IgG. For screening, we used an ELISA based on *B. afzelii* extract antigen enriched with recombinant VlsE (an outer-surface protein) from *B. burgdorferi* s.s., *B. afzelii*, and *B. bavariensis*. ELISA-positive results were confirmed by line blot testing, which included purified antigens OspC, DbpA, and p83 from *B. afzelii*; recombinant VlsE from *B. burgdorferi* s.s. and *B. garinii*; and BmpA and DbpA from *B. garinii*, *B. bavariensis*, and *B. spielmanii*. Details regarding the tests are available in the online Technical Appendix (<http://wwwnc.cdc.gov/EID/article/21/1/14-0009-Techapp1.pdf>). All tests were performed/interpreted according to the manufacturer's recommendations. To categorize samples by test results, we applied the rules shown in Figure 1.

We used sampling weights for all statistical analyses and accounted for the 2-stage sampling structure. Age-related prevalence was graphed and the values were smoothed by using the Lowess procedure of Stata 12.1 (StataCorp LP, College Station, TX, USA). We assessed differences between group prevalences (explanatory variables) by using the Wald test (univariable logistic regression) with 2-sided p values. Independent risk factors for seropositivity were investigated by using stepwise multivariable logistic regression. All plausible 2-way interactions were tested.

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**Figure 1.** Categorization, according to ELISA and line blot test results, of serum samples tested for *Borrelia burgdorferi* sensu lato IgG, Germany, 2008–2011.

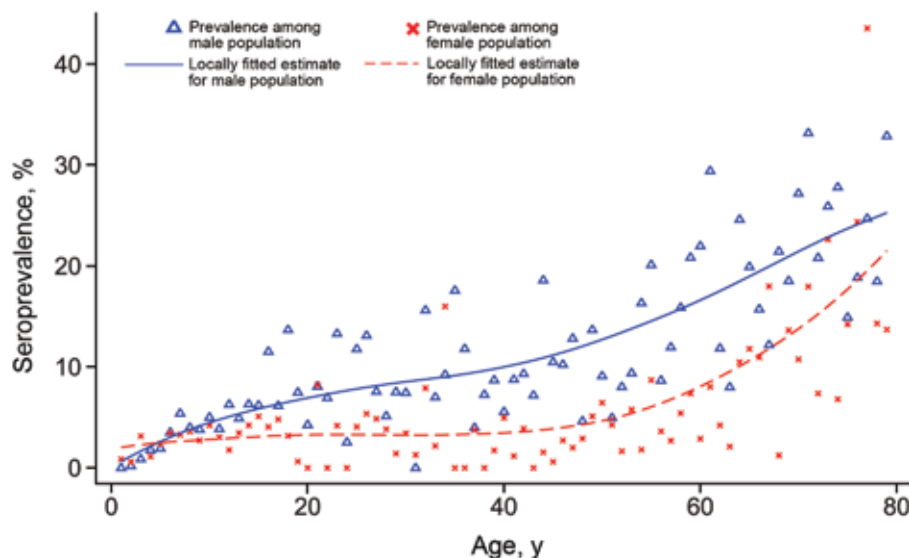
A total of 6,945 adults, representing 97.6% of the survey population with available blood samples, were included in the analysis. The overall weighted seroprevalence for *B. burgdorferi* s.l. was 9.4% (95% CI 8.4%–10.0%); seroprevalence was significantly higher among men (13.0%) than women (5.8%) ( $p < 0.01$ ). Seroprevalence among both sexes increased by age (Figure 2); the increase was low among 18- to 50-year-old participants, most pronounced among participants >59 years of age, and higher among women than men >59 years of age. Seropositivity reached 20.0% (95% CI 16.9%–23.6%) in 70- to 79-year-old participants.

Among participants  $\geq 18$  years of age, more than twice as many men than women were seropositive for *B. burgdorferi* s.l. (odds ratio 2.44, 95% CI 2.01–2.96) (Table). No significant interaction between sex and age was found ( $p = 0.075$ ). Independent risk factors for seropositivity were residence in a rural area ( $p < 0.001$ ) and in southern Germany ( $p = 0.032$ ). Non-German citizenship was negatively associated ( $p = 0.004$ ) with seropositivity; having a dog/cat

in the house was not associated with a higher risk for seropositivity. To facilitate comparison of our data with data from serosurveys lacking confirmatory testing, we have made our ELISA results available online (online Technical Appendix Table).

## Conclusions

*B. burgdorferi* s.l. infections are common in Germany; Lyme borreliosis is endemic in all regions, but case numbers are highest in southern Germany. Previously identified risk factors for *B. burgdorferi* s.l. seropositivity in children (male sex and living in rural areas, small-sized towns, or southern Germany) were identified as risk factors for seropositivity among adults in our study. Holding a cat was previously shown to be a risk factor for children/adolescents (9), but was not a risk factor in our study. Seroprevalence among the oldest age group indicates that at least one fifth of the German population becomes infected with *B. burgdorferi* s.l. during their lifetime.



**Figure 2.** Estimated seroprevalence of *Borrelia burgdorferi* sensu lato IgG among the male and female population, Germany, 2008–2011. For comparison, results of Dehnert et al. (9), a previous study among children/adolescents <18 years of age, were added to the graph.

**Table.** Stratified seroprevalence of *Borrelia burgdorferi* sensu lato IgG detected by combined ELISA and line blot testing in adults and results of weighted logistic regression analysis of potential risk factors for seropositivity, Germany, 2008–2011\*

Characteristic†	No. positive/ no. total†	Prevalence (95% CI)	Univariable analysis		Multivariable analysis	
			OR (95% CI)	p value	OR (95% CI)	p value
<b>Sex</b>						
F	240/3,614	5.8 (4.9–6.7)	Ref	Ref	Ref	Ref
M	501/3,331	13.0 (11.4–14.8)	2.44 (2.01–2.96)	<0.001	2.61 (2.15–3.16)	<0.001
<b>Age group, y</b>						
18–29	62/1,043	6.0 (4.5–8.0)	Ref	Ref	Ref	Ref
30–39	50/829	6.3 (4.4–9.0)	1.05 (0.64–1.69)	0.854	1.07 (0.67–1.72)	0.779
40–49	83/1,263	6.4 (5.0–8.2)	1.07 (0.72–1.58)	0.737	1.04 (0.69–1.55)	0.856
50–59	126/1,373	8.5 (6.8–10.7)	1.46 (1.01–2.10)	0.043	1.39 (0.97–1.99)	0.069
60–69	186/1,361	13.2 (10.9–15.9)	2.37 (1.65–3.40)	<0.001	2.37 (1.65–3.45)	<0.001
70–79	234/1,076	20.0 (16.9–23.6)	3.91 (2.77–5.51)	<0.001	4.02 (2.84–5.70)	<0.001
<b>Residence location</b>						
West‡	484/4,748	9.1 (8.0–10.4)	Ref	Ref	–	–
East§	257/2,197	10.4 (8.5–12.6)	1.15 (0.89–1.49)	0.273	–	–
North¶	181/1,767	9.0 (7.2–11.0)	1.11 (0.82–1.51)	0.479	1.16 (0.86–1.57)	0.318
Middle#	304/3,087	8.1 (6.7–9.8)	Ref	Ref	Ref	Ref
South**	256/2,091	11.2 (9.4–13.3)	1.43 (1.08–1.88)	0.011	1.34 (1.03–1.75)	0.032
<b>Population of residence municipality</b>						
<5,000	189/1,258	15.4 (12.8–18.4)	2.50 (1.85–3.30)	<0.001	2.13 (1.54–2.97)	<0.001
5,000 to <20,000	185/1,685	10.0 (8.0–12.5)	1.51 (1.11–2.07)	0.010	1.33 (0.96–1.84)	0.082
20,000 to <100,000	193/2,030	8.4 (6.9–10.2)	1.24 (0.92–1.66)	0.154	1.21 (0.88–1.67)	0.231
>100,000	174/1,972	6.9 (5.6–8.4)	Ref	Ref	Ref	Ref
<b>Foreign national††</b>						
No	721/6,528	10.0 (8.9–11.2)	Ref	Ref	Ref	Ref
Yes	18/396	4.3 (2.5–7.5)	0.41 (0.22–0.75)	0.004	0.54 (0.30–0.90)	0.041
<b>Pet in household</b>						
None	502/4,596	9.5 (8.4–10.7)	Ref	Ref	–	–
Any	217/2,182	9.3 (7.8–11.0)	0.98 (0.80–1.20)	0.834	–	–
<b>Dog</b>						
No	639/5,909	9.4 (8.4–10.6)	Ref	Ref	–	–
Yes	80/858	9.3 (7.0–12.2)	0.98 (0.71–1.35)	0.909	–	–
<b>Cat</b>						
No	622/5,886	9.2 (8.2–10.3)	Ref	Ref	–	–
Yes	119/1,077	10.3 (8.1–13.0)	1.13 (0.87–1.47)	0.356	–	–
<b>Other animals</b>						
No	655/6,001	9.7 (8.8–10.6)	Ref	Ref	–	–
Yes	64/766	7.7 (5.8–10.1)	0.78 (0.56–1.07)	0.127	–	–
<b>Total</b>	<b>741/6,945</b>	<b>9.4 (8.4–10.0)</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>

\*OR, odds ratio; Ref, reference; –, not included in the final model.

†Unweighted.

‡Western states: Baden-Württemberg, Bavaria, Bremen, Hamburg, Hesse, Lower Saxony, Northrhine-Westfalia, Rhineland-Palatinate, Saarland, Schleswig-Holstein.

§Eastern states: Berlin, Brandenburg, Mecklenburg–West Pomerania, Saxony, Saxony-Anhalt, Thuringia.

¶Northern states: Schleswig-Holstein, Hamburg, Lower Saxony, Bremen, Berlin, Brandenburg, Mecklenburg-West Pomerania.

#Middle states: Northrhine-Westfalia, Hesse, Saxony, Saxony-Anhalt, Thuringia.

\*\*Southern states: Rhineland-Palatinate, Baden-Württemberg, Bavaria, Saarland.

††Defined as persons holding a foreign citizenship.

*B. burgdorferi* s.l. IgG seroprevalence among blood donors in Italy (4.9%; n = 365) (11) and Romania (4.3%; n = 1,598) (12) was lower than the seroprevalence in our study. Prevalences higher than those in our study have been shown in serosurveys in areas of high disease endemicity in southwestern Germany (16.9%; n = 1,228) (13) and Finland (19.3%; n = 3,248) (14). In serosurveys of persons with high exposure to ticks (e.g., forestry and agricultural workers), similar or higher seroprevalence rates have been described.

Seroprevalence rates among men in our study were strikingly higher than rates among women, indicating that tick contact/spirochete transmission is more frequent

among men. Prospective studies in Germany and Sweden and surveillance data from Germany show no differences in clinical cases (except only a slight preponderance among women) that would point to substantial sex-specific differences in the development of clinical disease (5–7).

The age distribution for seropositivity reflects the population's cumulative exposure to *B. burgdorferi* s.l.. An increased risk for infection among children and persons >59 years of age suggests that leisure activities/behaviors rather than occupational exposure are the main risk factor for infection. Alternatively, these findings might be explained by a birth-cohort effect, in which the force of infection was lower during 1950–1990.

Persons living in urbanized areas had a lower probability for *B. burgdorferi* s.l. seropositivity, suggesting that exposure to infected ticks is higher in rural areas. However, urban populations are also at substantial risk for infection. Seropositivity is not equivalent to clinical disease; thus, seropositivity rates among the different population groups may not necessarily reflect the true effect of infection on disease burden. Furthermore, a US study showed that persons can be consecutively infected by different *B. burgdorferi* strains and experience clinical manifestations with each infection (15).

Our seroprevalence estimates can be used, within the context of clinical diagnoses, to assess the likelihood of Lyme borreliosis in persons with test results positive for *B. burgdorferi* s.l. IgG. To reduce the incidence and disease burden of Lyme borreliosis, enhanced public health interventions are needed, including education campaigns targeted to parents, children, and the elderly about potential risk factors and preventive measures for Lyme borreliosis.

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Dr. Wilking is an infectious disease epidemiologist in the unit for Gastrointestinal Infections, Zoonoses and Tropical Infections at the Robert Koch Institute. His research interests include population-based disease studies.

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## Investigating *Listeria* Outbreaks

Dr. Emily Cartwright, Infectious Disease fellow at Emory University and former EIS Officer with CDC's Division of Foodborne, Waterborne, and Environmental Diseases discusses foodborne *Listeria* outbreaks.

<http://www2c.cdc.gov/podcasts/player.asp?f=8626870>



# Antibodies against *Borrelia burgdorferi* sensu lato among Adults, Germany, 2008–2011

## Technical Appendix

The serum samples were tested at the National Reference Centre for *Borrelia* for the presence of anti-*Borrelia burgdorferi* sensu lato IgG antibodies. As an initial test an ELISA (Enzygnost Lyme link VlsE/IgG; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) was applied. It is based on a detergent extract from cultured *B. afzelii* (strain PKo) mixed with recombinant VlsE from *B. burgdorferi* sensu stricto (strain B31), *B. afzelii* (strain PKo), and *B. bavariensis* (strain PBi). The test was automatically processed on a BEP III (Siemens Healthcare Diagnostics) and interpreted as recommended by the manufacturer. Validation studies for this ELISA have been published (1,2).

For confirmation of ELISA test-positive and borderline tests samples, a line blot was performed (Borrelia Europe plus TpN17 LINE IgG; Virotech, Rüsselsheim, Germany). It includes the purified antigens OspC, DbpA, and p83 (all from *B. afzelii* strain PKo) and additionally recombinant antigens VlsE (from *B. burgdorferi* sensu stricto strain B31 and *B. garinii* strain IP90), BmpA (PKo), DbpA (from *B. garinii* strain PBr, *B. bavariensis* strain PBi, and *B. spielmanii*). Antigens are bound separately to a nitrocellulose membrane either as single antigens or, in the case of VlsE and DbpA, as a mix of the respective antigens. The test was performed and interpreted according to the manufacturer's recommendations.

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<http://dx.doi.org/10.1007/s10096-007-0445-7>

Technical Appendix Table. Stratified seroprevalence of *Borrelia burgdorferi* sensu lato IgG detected by ELISA (initial test only) in adults 18- to 79-years if age and results of weighted bivariate logistic regression analysis of potential risk factors for seropositivity, Germany, 2008–2011\*

Characteristics†	No. positive/no. total†	Prevalence (95% CI)	Univariable analysis		Multivariable analysis	
			OR (95% CI)	p value	OR (95% CI)	p value
<b>Sex</b>						
F	295/3,619	7.1 (6.2–8.2)	Ref	Ref	Ref	Ref
M	557/3,346	14.5 (12.8–16.4)	2.21 (1.84 – 2.66)	<0.001	2.34 (1.95–2.82)	<0.001
<b>Age group, y</b>						
18–29	68/1,044	6.9 (5.3–8.9)	Ref	Ref	Ref	Ref
30–39	66/831	8.2 (6.1–11.1)	1.22 (0.79–1.87)	0.374	1.25 (0.82–1.91)	0.303
40–49	98/1,270	8.0 (6.4–10.0)	1.18 (0.83–1.67)	0.351	1.15 (0.80–1.64)	0.454
50–59	149/1,375	9.8 (7.8–12.1)	1.46 (1.04–2.06)	0.029	1.40 (1.01–1.95)	0.046
60–69	209/1,364	14.4 (12.0–17.2)	2.28 (1.64–3.16)	<0.001	2.28 (1.64–3.16)	<0.001
70–79	262/1,081	21.9 (18.6–25.7)	3.80 (2.76–5.23)	<0.001	3.84 (2.77–5.32)	<0.001
<b>Residence location in Germany</b>						
West‡	569/4,761	10.5 (9.3–11.9)	Ref	Ref	–	–
East§	283/2,204	11.8 (9.8–14.2)	1.14 (0.89–1.46)	0.273	–	–
North¶	200/1,771	10.1 (8.3–12.2)	1.05 (0.78–1.39)	0.761	1.08 (0.81–1.44)	0.587
Middle#	358/3,094	9.7 (8.1–11.5)	Ref	Ref	Ref	Ref
South**	294/2,100	12.7 (10.9–14.9)	1.37 (1.05–1.77)	0.020	1.30 (1.00–1.69)	0.049
<b>Population of residence municipality</b>						
<5,000	207/1,264	16.8 (13.9–20.3)	2.23 (1.69–2.95)	<0.001	1.88 (1.37–2.60)	<0.001
5,000 to <20,000	205/1,687	11.2 (9.0–13.8)	1.38 (1.03–1.86)	<0.001	1.21 (0.89–1.64)	0.220
20,000 to <100,000	237/2,037	10.0 (8.3–12.1)	1.23 (0.94–1.61)	0.154	1.19 (0.89–1.58)	0.236
>100,000	203/1,977	8.3 (7.0–9.8)	Ref	Ref	Ref	Ref
<b>Foreign national††</b>						
No	829/6,547	11.6(10.4–12.8)	Ref	Ref	Ref	Ref
Yes	21/397	4.5 (2.6–7.7)	0.36 (0.20–0.64)	0.001	0.45 (0.25–0.80)	0.007
<b>Pet in household</b>						
No pet	589/4,613	11.0 (9.9–12.2)	Ref	Ref	–	–
Any pet	240/2,185	10.6 (9.0–12.5)	0.96 (0.80–1.16)	0.676	–	–
<b>Dog</b>						
No	741/5,928	11.0 (9.9–12.2)	Ref	Ref	–	–
Yes	88/859	10.2 (7.8–13.1)	0.92 (0.68–1.23)	0.562	–	–
<b>Cat</b>						
No	698/5,708	10.7 (9.6–11.9)	Ref	Ref	–	–
Yes	131/1,079	11.8 (9.4–14.8)	1.12 (0.86–1.45)	0.394	–	–
<b>Other animals</b>						
No	758/6,020	11.2 (10.0–12.4)	Ref	Ref	–	–
Yes	71/767	8.8 (6.7–11.5)	0.77 (0.57–1.04)	0.086	–	–
<b>Total</b>	<b>852/6,965</b>	<b>11.0 (9.7–12.0)</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>

\*OD, odds ratio; Ref, reference, –,not included in the final mode.

†Unweighted.

‡Western states: Baden-Württemberg, Bavaria, Bremen, Hamburg, Hesse, Lower Saxony, Northrhine-Westfalia, Rhineland-Palatinate, Saarland, Schleswig-Holstein.

§Eastern states: Berlin, Brandenburg, Mecklenburg-West Pomerania, Saxony, Saxony-Anhalt, Thuringia.

¶Northern states: Schleswig-Holstein, Hamburg, Lower Saxony, Bremen, Berlin, Brandenburg, Mecklenburg-West Pomerania.

#Middle states: Nordrhine-Westfalia, Hesse, Saxony, Saxony-Anhalt, Thuringia.

\*\*Southern states: Rhineland-Palatinate, Baden-Württemberg, Bavaria, Saarland.

††Defined as persons holding a foreign citizenship.