Global seroprevalence and sociodemographic characteristics of Borrelia burgdorferi sensu lato in human populations: a systematic review and meta-analysis

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ABSTRACT

Introduction Borrelia burgdorferi sensu lato (Bb) infection, the most frequent tick-transmitted disease, is distributed worldwide. This study aimed to describe the global seroprevalence and sociodemographic characteristics of Bb in human populations.

Methods We searched PubMed, Embase, Web of Science and other sources for relevant studies of all study designs through 30 December 2021 with the following keywords: ‘Borrelia burgdorferi sensu lato’ AND ‘infection rate’; and observational studies were included if the results of human Bb antibody seroprevalence surveys were reported, the laboratory serological detection method reported and be published in a peer-reviewed journal. We screened titles/abstracts and full texts of papers and appraised the risk of bias using the Cochrane Collaboration-endorsemed Newcastle-Ottawa Quality Assessment Scale. Data were synthesised narratively, stratified by different types of outcomes. We also conducted random effects meta-analysis where we had a minimum of two studies with 95% CIs reported. The study protocol has been registered with PROSPERO (CRD42021261362).

Results Of 4196 studies, 137 were eligible for full-text screening, and 89 (158277 individuals) were included in meta-analyses. The reported estimated global Bb seroprevalence was 14.5% (95% CI 12.8% to 16.3%), and the top three regions of Bb seroprevalence were Central Europe (20.7%, 95% CI 13.8% to 28.6%), Eastern Asia (15.9%, 95% CI 6.6% to 28.3%) and Western Europe (13.5%, 95% CI 9.5% to 18.0%). Meta-regression analysis showed that after eliminating confounding risk factors, the methods lacked western blotting (WB) confirmation and increased the risk of false-positive Bb antibody detection compared with the methods using WB confirmation (OR 1.9, 95% CI 1.6 to 2.2). Other factors associated with Bb seropositivity include age ≥50 years (12.6%, 95% CI 8.0% to 18.1%), men (7.8%, 95% CI 4.6% to 11.9%), residence of rural area (8.4%, 95% CI 5.0% to 12.6%) and suffering tick bites (18.8%, 95% CI 10.1% to 29.4%).

Conclusion The reported estimated global Bb seropositivity is relatively high, with the top three regions as Central Europe, Western Europe and Eastern Asia. Using the WB to confirm Bb serological results could significantly improve the accuracy. More studies are needed to improve the accuracy of global Lyme borreliosis burden estimates.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Borrelia burgdorferi sensu lato (Bb) infection, the most frequent tick-transmitted disease in Europe and North America, is distributed worldwide.
⇒ The Northern Hemisphere residents have the highest Lyme borreliosis (LB, also called Lyme disease) burden, but no consensus exists regarding the reported global seroprevalence and specific risk factors of Bb infection.

WHAT THIS STUDY ADDS

⇒ This systematic review and meta-analysis of the literatures addressed this knowledge gap.
⇒ Reported seroprevalence was highest in the LB-like symptoms population and lowest in the general population.
⇒ Meta-regression analyses showed that the reported pooled Bb seroprevalence of studies using methods confirmed by western blotting (WB) was lower than that of studies using methods not confirmed by WB after eliminating confounding risk factors.
⇒ Potential risk factors associated with Bb infection were male sex, age ≥40 years, residence in rural area and suffering tick bites.

INTRODUCTION

Lyme borreliosis (LB, also called Lyme disease) is caused by the tickborne spirochete Borrelia burgdorferi sensu lato (Bb). The complex biology and multiple immune escape mechanisms of LB make it the most common vectorborne disease in temperate...
North America, Europe and Asia.1–3 The most common clinical manifestation of LB is migrating erythema (an enlarged erythema on the skin, usually at the site of a tick bite), and the infecting agent can spread to other tissues and organs, resulting in manifestations that can involve the nervous system, joints, heart and skin.4 LB has continued to spread globally in recent years as a chronic, multisystemic vectorborne disease.5 Such vectorborne diseases, which are characterised by specificity of geographical distribution and frequent emergence and introduction of pathogens, pose a significant and growing public health problem and are major causes of disease and death worldwide.5 A strong worldwide push for continuous surveillance (including global epidemiological surveys of LB), diagnosis and control of vectors of tickborne diseases is essential for the development of effective new LB treatments and prevention methods.

*Bb* is one of several extracellular pathogens capable of establishing a persistent infection in mammals, and laboratory diagnosis of LB depends on the detection of IgM and IgG antibodies against *Bb*, the causative agent of the disease.4–7 Several laboratory tests are available for the diagnosis of LB, including serological, microscopic and molecular-based methods.8 Standard two-stage tests (STTT) based on immunoblotting, ELISA, IFA test, protein biochip, chemiluminescence immunoassay, passive haemagglutination assays, line blot and western blotting (WB); laboratory molecular detection methods used as additional detection methods in some studies: PCR, real-time PCR, PCR–restriction fragment length polymorphism and sequencing assays; (c) results of human studies including *Bb* antibody seroprevalence surveys; (d) original articles presenting surveillance reports or cross-sectional or case–control or cohort studies.

Exclusion criteria were as follows: (a) animal/insect studies (eg, ticks, sheep, cattle, dogs, etc); (b) serological *Bb* antibody detection method not described or detection methods did not match description; (c) incomplete data (eg, only reported total *Bb* seroprevalence while the total number of participants was not indicated or geographical information and population categories not described) and studies lacking primary data (eg, full-text study was not found); (d) systematic review, meta-analysis, conference presentation, case report, repeated publication (the highest quality publication was retained). This phase involved a group of three reviewers (YD, WC and YZ)
who independently catalogued all reports using the set criteria. Outcome of this initial categorisation was then
crosschecked by a different reviewer within this group
to ensure its accuracy with a 90% level of agreement
(detailed in online supplemental appendix 3).

Data screening and extraction
Data extraction was assessed independently, with
conflicts of opinion and uncertainties discussed and
resolved by consensus with third-party reviewers (YD, JC
and GZ). Data were extracted from each included study
and entered into a database. Data pertaining to the first
author, publication year period, country, area of resi-
dence, serological screening test used, population cate-
gories, sex, age, sample size, tick bite, and number of
seropositive results, type of antibody and other relevant
information were extracted.

Risk of bias
Full-text papers were obtained for all identified potential
reports for detailed risk of bias assessment (by YD, JK and
WC), and assessment inconsistencies were discussed and
disagreements resolved by consensus. Data quality scores
were rated with the Cochrane Collaboration-endorsed
Newcastle-Ottawa Quality Assessment Scale, which was
specifically designed to assess aspects of population-
based studies of prevalence.13 14 The assessment
was based on three main criteria relating to (a) selection
bias, (b) confounding, and (c) outcome measurement
bias. The checklist included seven items, each option as
‘a’, ‘b’, ‘c’ or ‘d’. The assessment options correspond to
a ‘star system’ that allows us to rate the overall quality
(low, moderate or high) after assessing the risk of bias
in the three main criteria. Option scoring ‘***’ received
20% scores; option scoring ‘*’ received 10% scores;
others received 0 point. Thus, final scores for each study
could range from 0% to 100%: studies with a score of
0%–49% were defined as low quality; those with a score of
50%–69% were considered moderate quality; and those
with a score of 70%–100% were deemed high quality.
Studies with a score of ≥50% were included in the final
analysis (detailed in online supplemental appendix 4).

Data synthesis and analysis
A meta-analysis was conducted using the ‘meta’ package
in R (V.4.0.5) to estimate Bb seroprevalence. A random
effects model was used to calculate the reported pooled
seroprevalence. An I² value of more than 50% was consid-
ered to indicate significant heterogeneity, and more than
75% was considered to indicate high heterogeneity. The
global seropositivity rate was calculated, as this was the
objective of the study. For each reported seroprevalence,
an exact binomial 95% CI was calculated. Significant
differences in estimated seroprevalence between pairs of
risk factors for Bb seroprevalence were evaluated based
on 95% CIs. Differences were considered statistically
significant if the 95% CIs did not overlap.

Overall, heterogeneity among studies was assessed
using the I² test, and seroprevalence estimates were
stratified by population category (general, high-risk,
tick-bitten, LB-like symptoms) and other potential risk
factors (sex, age, years of publication, continent, country,
tick bites, residence, serological screening test used), as
these variables were considered a priori potential predic-
tors of Bb seroprevalence.15 The effect of heterogeneity
on seroprevalence estimates was examined by subgroup
and meta-regression analyses. Reported pooled ORs and
95% CIs were calculated from raw data of the included
studies using the random effects model, and reported
subgroup pooled ORs were generated for different risk
factors. Logit transformation, arcsine transformation and
Freeman-Tukey methods (different methods used for
propportion meta-analyses) were compared via sensitivity
analysis. Publication bias was detected using Egger’s test
and funnel plots.

Patient and public involvement
Patient and public involvement statement is not appli-
cable in this paper since the patients or the public were
not involved in either the design, conduct, reporting or
dissemination plans of our research.

RESULTS
Search results and eligible studies
We retrieved 4196 studies from three databases (the
PubMed, Embase and Web of Science abstract data-
bases) and grey literatures. A total of 89 observational
studies (cross-sectional, cohort, case–control studies)
that met inclusion requirements were included after full-
text review (online supplemental appendix 5).16–104 The
studies involved 158287 participants, and the reported
estimated Bb seroprevalence was 14.5% (95% CI 12.8%
to 16.3%). Details regarding article screening procedures
and reasons for exclusion are summarised in figure 1.
According to the Newcastle-Ottawa Quality Assessment
Scale, the 89 studies were graded as moderate to high
quality. Online supplemental appendix 6 presents the
details of the risk of bias assessment.

Meta-analysis of global Bb seroprevalence
The reported pooled seroprevalence was 14.5% (95% CI
12.8% to 16.3%) according to the random effects model
(online supplemental appendix 7). Of the 89 studies, 31
lacked WB confirmation of serological testing, and 58
had WB confirmation, with reported pooled Bb seroposi-
tivity rates of 16.3% (95% CI 13.8% to 18.9%) and 11.6%
(95% CI 9.5% to 14.0%), respectively (online supple-
mental appendix 8).

Forty of the included studies were unique, in that they
documented the results of serological testing techniques
with/without WB confirmation in the same cohort,
thus allowing a better comparison of the two methods
for determining Bb seropositivity. The reported pooled
Bb seropositivity was 17.5% (95% CI 14.2% to 21.0%)
without WB confirmation and 9.8% (95% CI 7.5% to
12.3%) with WB confirmation (online supplemental appendix 9). The two methods were also compared after eliminating confounding risk factors such as sex, age, specified antibody type (IgM/IgG/IgM+IgG), publication year period, tick bite history, population category and residence region (rural/urban) (table 1, figure 2). Our results suggested that using only one-step methods such as ELISA/IFA to determine Bb seropositivity has limitations and that serological testing for Bb seropositivity with WB confirmation is more reliable. These results further support the use of standard two-stage testing (STTT, ELISA or IFA assay followed by WB confirmation) as a more reliable means of supporting the laboratory diagnosis of LB and assessing its development.

Based on the above results, the 58 studies that used WB confirmation to determine Bb seropositivity were used to analyse factors predictive of LB. By gender, the reported pooled Bb seropositivity rates were 5.3% (95% CI 3.2% to 8.0%) for females and 7.8% (95% CI 4.6% to 11.9%) for males. By age, the reported pooled Bb seropositivity rates were 7.1% (95% CI 5.1% to 9.5%) for those 0–39 years of age, 10.1% (95% CI 4.6% to 17.6%) for those 40–49 years of age and 12.6% (95% CI 8.0% to 18.1%) for those ≥50 years of age. By place of residence, the reported pooled Bb seropositivity rates were 8.4% (95% CI 5.0% to 12.6%) for rural populations and 5.4% (95% CI 3.2% to 8.1%) for urban populations. According to tick bite history, the reported pooled Bb seropositivity rates were 18.8% (95% CI 10.1% to 29.4%) for the tick-bitten population and 10.5% (95% CI 2.1% to 24.3%) for those not tick bitten. For the four population categories, the reported pooled Bb seropositivity rate in the general population was 5.7% (95% CI 4.3% to 7.3%), which was significantly lower than the reported pooled Bb seropositivity rate of 14.7% (95% CI 9.9% to 20.2%) for the high-risk population, 18.8% (95% CI 10.1% to 29.4%) for the tick-bitten population and 21.3% (95% CI 14.1% to 29.4%) for the LB-like symptoms population. Two time periods (2001–2010 and 2011–2021) were examined to analyse the Bb prevalence.

Subgroup analysis
A subgroup analysis was performed according to possible predictors of LB. By gender, the reported pooled Bb seropositivity rates were 5.3% (95% CI 3.2% to 8.0%) for females and 7.8% (95% CI 4.6% to 11.9%) for males. By age, the reported pooled Bb seropositivity rates were 7.1% (95% CI 5.1% to 9.5%) for those 0–39 years of age, 10.1% (95% CI 4.6% to 17.6%) for those 40–49 years of age and 12.6% (95% CI 8.0% to 18.1%) for those ≥50 years of age. By place of residence, the reported pooled Bb seropositivity rates were 8.4% (95% CI 5.0% to 12.6%) for rural populations and 5.4% (95% CI 3.2% to 8.1%) for urban populations. According to tick bite history, the reported pooled Bb seropositivity rates were 18.8% (95% CI 10.1% to 29.4%) for the tick-bitten population and 10.5% (95% CI 2.1% to 24.3%) for those not tick bitten. For the four population categories, the reported pooled Bb seropositivity rate in the general population was 5.7% (95% CI 4.3% to 7.3%), which was significantly lower than the reported pooled Bb seropositivity rate of 14.7% (95% CI 9.9% to 20.2%) for the high-risk population, 18.8% (95% CI 10.1% to 29.4%) for the tick-bitten population and 21.3% (95% CI 14.1% to 29.4%) for the LB-like symptoms population. Two time periods (2001–2010 and 2011–2021) were examined to analyse the Bb prevalence.

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of search strategy for selecting eligible studies. conf WB, confirmatory western blotting.
The Bb prevalence after 2011 was higher than that before, with the Bb seropositivity rate increasing from 8.1% (95% CI 5.7% to 10.8%) to 12.2% (95% CI 9.6% to 15.0%) (table 2). Depending on the continental plate, the reported pooled Bb seropositivity rates for the Americas, Europe, the Caribbean, Asia and Oceania (only Australia reported) were 9.4% (95% CI 3.5% to 17.7%), 10.3% (95% CI 7.5% to 14.1%), 2.0% (95% CI 0.0% to 4.1%), 6.6% (95% CI 3.3% to 10.9%) and 4.1% (95% CI 0.0% to 14.1%), respectively (table 3). Forest plots of pooled Bb prevalence stratified by subgroup are shown in online supplemental appendices 10–23. The compositions of the four population cohorts in each region are summarised in figure 3. The reported pooled seropositivity rate is summarised by cohort according to region and population group in figure 4.

### Sensitivity analyses

Sensitivity analyses involved omitting each study in turn and comparing the reported pooled Bb seropositivity rate using an inverse sine transformation. After omitting each study in turn, the reported pooled seropositivity rate of the remaining studies was approximately 12%, indicating

Table 1  Meta-regression analysis of Borrelia burgdorferi sensu lato seroprevalence determined using methods confirmed by WB and methods not confirmed by WB after eliminating confounding risk factors

<table>
<thead>
<tr>
<th></th>
<th>Methods conf WB</th>
<th>Methods not conf WB</th>
<th>Random effects model</th>
<th>P value</th>
<th>Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td>1.9 (1.6 to 2.2)</td>
<td>&lt;0.0001</td>
<td>38</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5.1% (3.5%; 7.1%)</td>
<td>10.4% (7.1%; 14.2%)</td>
<td>1.7 (1.3 to 2.2)</td>
<td>0.0002</td>
<td>14</td>
</tr>
<tr>
<td>Male</td>
<td>5.4% (2.6%; 9.2%)</td>
<td>11.1% (5.1%; 18.9%)</td>
<td>1.8 (1.2 to 2.8)</td>
<td>0.0055</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>8.1% (3.7%; 14.0%)</td>
<td>15.9% (8.2%; 25.6%)</td>
<td>1.6 (1.2 to 2.19)</td>
<td>0.0030</td>
<td>6</td>
</tr>
<tr>
<td>40–49</td>
<td>6.2% (0.0%; 26.4%)</td>
<td>18.0% (2.6%; 43.1%)</td>
<td>2.4 (1.6 to 3.6)</td>
<td>&lt;0.0001</td>
<td>2</td>
</tr>
<tr>
<td>≥50</td>
<td>8.8% (1.2%; 22.6%)</td>
<td>18.0% (7.2%; 32.4%)</td>
<td>1.9 (1.4 to 2.4)</td>
<td>&lt;0.0001</td>
<td>6</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>9.5% (3.6%; 17.7%)</td>
<td>13.4% (3.1%; 29.3%)</td>
<td>1.4 (1.1 to 1.9)</td>
<td>0.0082</td>
<td>3</td>
</tr>
<tr>
<td>Urban</td>
<td>5.3% (1.0%; 12.8%)</td>
<td>8.9% (0.8%; 24.6%)</td>
<td>1.5 (1.0 to 2.2)</td>
<td>0.0451</td>
<td>3</td>
</tr>
<tr>
<td>Tick bites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not suffering</td>
<td>3.2% (0.8%; 6.9%)</td>
<td>14.3% (8.7%; 20.9%)</td>
<td>4.5 (1.6 to 12.9)</td>
<td>0.0052</td>
<td>1</td>
</tr>
<tr>
<td>Suffering</td>
<td>16.2% (4.6%; 33.1%)</td>
<td>38.0% (2.7%; 67.5%)</td>
<td>2.4 (1.1 to 5.0)</td>
<td>0.0215</td>
<td>4</td>
</tr>
<tr>
<td>Different continents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>10.3% (7.5%; 14.1%)</td>
<td>17.2% (11.7%; 23.8%)</td>
<td>1.7 (1.5 to 1.9)</td>
<td>&lt;0.0001</td>
<td>12</td>
</tr>
<tr>
<td>America</td>
<td>4.1% (0.7%; 10.1%)</td>
<td>10.6% (6.7%; 15.4%)</td>
<td>3.8 (1.9 to 7.6)</td>
<td>0.006</td>
<td>6</td>
</tr>
<tr>
<td>Asia</td>
<td>6.6% (3.3%; 10.9%)</td>
<td>13.8% (8.2%; 20.6%)</td>
<td>2.3 (1.7 to 3.1)</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
<tr>
<td>Different populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>5.3% (3.7%; 7.3%)</td>
<td>9.7% (7.5%; 12.1%)</td>
<td>1.9 (1.5 to 2.3)</td>
<td>&lt;0.001</td>
<td>22</td>
</tr>
<tr>
<td>High risk</td>
<td>10.9% (6.6%; 16.2%)</td>
<td>22.0% (16.1%; 28.7%)</td>
<td>1.5 (1.3 to 1.7)</td>
<td>&lt;0.001</td>
<td>12</td>
</tr>
<tr>
<td>Tick bitten</td>
<td>16.2% (4.6%; 33.1%)</td>
<td>38.0% (12.7%; 67.5%)</td>
<td>2.4 (1.1 to 5.0)</td>
<td>0.0215</td>
<td>4</td>
</tr>
<tr>
<td>LB-like symptoms</td>
<td>18.9% (10.7%; 28.7%)</td>
<td>26.5% (14.6%; 40.6%)</td>
<td>1.4 (1.1 to 1.8)</td>
<td>0.007</td>
<td>6</td>
</tr>
<tr>
<td>Antibodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>7.8% (4.8%; 11.4%)</td>
<td>12.8% (7.8%; 18.9%)</td>
<td>1.7 (1.4 to 2.0)</td>
<td>&lt;0.001</td>
<td>12</td>
</tr>
<tr>
<td>IgM</td>
<td>4.2% (2.7%; 5.9%)</td>
<td>12.2% (9.2%; 15.4%)</td>
<td>3.1 (2.1 to 4.4)</td>
<td>&lt;0.001</td>
<td>12</td>
</tr>
<tr>
<td>IgG+IgM</td>
<td>0.2% (0.0%; 0.7%)</td>
<td>2.2% (0.4%; 5.4%)</td>
<td>4.8 (2.0 to 11.5)</td>
<td>&lt;0.001</td>
<td>3</td>
</tr>
<tr>
<td>Two time periods</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>2001–2010</td>
<td>7.1% (3.6%; 11.6%)</td>
<td>14.3% (11.2%; 17.7%)</td>
<td>2.5 (1.4 to 4.4)</td>
<td>0.0017</td>
<td>9</td>
</tr>
<tr>
<td>2011–2021</td>
<td>10.1% (7.3%; 13.4%)</td>
<td>17.9% (13.8%; 22.4%)</td>
<td>1.9 (1.6 to 2.3)</td>
<td>&lt;0.001</td>
<td>26</td>
</tr>
</tbody>
</table>

Methods conf WB group was considered the reference group when conducting meta-regression analysis. LB, Lyme borreliosis; WB, western blotting.
that the meta-analysis results were robust and reliable (online supplemental appendix 24).

**Meta-regression**

To assess potential sources of heterogeneity, a random effects model meta-regression analysis was conducted, which revealed significant heterogeneity with pooled analyses (online supplemental appendix 25) ($I^2=0.99$; $p<0.001$).

**Publication bias**

The Egger’s test and a funnel plot were constructed to assess publication bias. According to the Egger’s test, the $p$ value was 0.04, and the funnel plot was clearly asymmetric; thus, the review had some publication bias (figure 5).

**DISCUSSION**

*Bb* is a zoonotic tickborne spirochete and pathogen of LB. Since its identification in 1975, LB has become the most common tickborne zoonotic disease worldwide. The incidence and distribution of LB have increased over the last four decades. Therefore, there is a need for preventive measures, which necessitates understanding the dynamics of tickborne disease transmission and the lack of effective disease prevention strategies to reduce the risk of contracting the disease. This is the most comprehensive and up-to-date systematic review of the worldwide seroprevalence of *Bb*. We estimated a reported global seroprevalence of 14.5% (95% CI 12.8% to 16.3%) and confirmed wide variation in *Bb* prevalence between regions and countries, with the reported prevalence highest in Central Europe (20.7%), followed by Eastern Asia (15.9%), Western Europe (13.5%) and Eastern Europe (10.4%). In contrast, the reported prevalence was lowest in the Caribbean (2.0%), Southern Asia (3.0%) and Oceania (5.3%).

The global seroprevalence rates assessed in our meta-analysis should be considered preliminary estimates because of the large heterogeneity of the included studies. After stratification by potentially important predictors (eg, population category, continental distribution, detection test), heterogeneity across populations, continents and detection methods remained high. No specific sources of heterogeneity were identified by various means (subgroup, sensitivity or meta-regression analyses). The high heterogeneity after specified stratification suggests that (1) heterogeneity could be due to the limited data, indicating that more data are needed to address heterogeneity and obtain more globally representative estimates of *Bb* prevalence; or (2) heterogeneity could be due to other possible sources: differences in study design, inclusion/exclusion criteria, population size, recruitment/sampling methods, test kits.

Furthermore, the publication bias of the included studies should not be overlooked. First, in areas where LB is endemic, clinicians routinely use the *Bb* antibody test and are therefore more likely to report higher seropositivity rates relative to LB-non-endemic areas; thus, the reported seropositivity rate in the general population may be overestimated and non-representative of the global population. Second, whether the study’s sample size was representative of the region’s total population and whether small samples were used for estimation could have impacts that cannot be ignored. The funnel plot and Egger’s test results showed some publication bias in this review, so the global seroprevalence that we assessed should be considered a preliminary estimate. The population was therefore divided into four subpopulations (general, high-risk, tick-bitten and LB-like symptoms populations), and each analysed separately.

Jointly improving and standardising testing methods is of great value in providing accurate epidemiological data on LB and identifying potential risk factors for LB. The possibility of false-positive cross-reactivity with pathogens of other infectious diseases (eg, Epstein-Barr virus) in one-step tests such as ELISA has been reported. The reported pooled prevalence rate in this study was based primarily on WB-confirmed results due to concerns over results comparability and reliability. This conclusion was based on our results after comparing the seroprevalence of WB-confirmed and non-WB-confirmed results; the seropositivity rate with WB confirmation, which exhibited high consistency after excluding confounding factors, was more reliable than that without WB confirmation. These results suggest that WB confirmation could reduce false positivity to some degree and improve specificity. However, WB confirmation has limitations, such as low sensitivity of serological assays in the early stages of *Bb* infection, the subjectivity and complexity of the techniques associated with secondary immunoblotting and high relative expense. Other improved secondary serological assays (eg, whole-cell ultrasound enzyme immunoassay (EIA)+C6 EIA) and molecular diagnostics (eg,
next-generation sequencing) are developing rapidly, which could improve LB diagnosis.

To identify potential risk factors associated with anti-\textit{Bb} antibody positivity, we conducted meta-regression analyses according to reported demographic characteristics for the 58 studies confirmed by WB. Our limited results showed that the prevalence of people who suffered tick bites was higher than that of those not suffering from tick bites. The high-risk population was defined in terms of occupation (farmers, skilled and unskilled workers, police officers, soldiers, housewives and retirees), and the specificity of these occupations has greatly increased the exposure to ticks and intermediate host animals (eg, dogs, sheep) related to LB. The general, high-risk, tick bite and LB-like symptoms populations showed a progressive increase in seropositivity over time. Numerous investigations have shown that the prevalence of tickborne diseases has doubled in the last 12 years. Our results indicate that the prevalence of \textit{Bb} in 2010–2021 was higher than that in 2001–2010. LB is the most prominent tickborne disease, and tick populations (carriers of microbial pathogens second only to mosquitoes) have expanded globally and geographically in recent years, thereby greatly increasing the risk of human exposure to ticks. This may be related to ecological changes and anthropogenic factors, such as longer summers and warmer winters, changes in precipitation during dry months, animal migration, fragmentation of arable land

### Table 2: Meta-regression analysis of the potential risk factors associated with \textit{Borrelia burgdorferi sensu lato} (\textit{Bb}) infection in 58 included studies determining \textit{Bb} seroprevalence confirmed by WB

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cohort</th>
<th>Seroprevalence (95% CI)</th>
<th>Random effects model</th>
<th>P value</th>
<th>Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>58</td>
<td>11.5% (9.4% to 13.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>5.5% (3.2% to 8.0%)</td>
<td>Reference</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>7.8% (4.6% to 11.9%)</td>
<td>1.4 (1.2 to 1.9)</td>
<td>0.001</td>
<td>18</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>18</td>
<td>7.1% (5.1% to 9.5%)</td>
<td>Reference</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>5</td>
<td>10.1% (4.6% to 17.6%)</td>
<td>1.3 (1.0 to 1.6)</td>
<td>0.049</td>
<td>5</td>
</tr>
<tr>
<td>≥50</td>
<td>14</td>
<td>12.6% (8.0% to 18.1%)</td>
<td>2.0 (1.5 to 2.7)</td>
<td>&lt;0.001</td>
<td>9</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>9</td>
<td>8.4% (5.0% to 12.6%)</td>
<td>Reference</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>9</td>
<td>5.4% (3.2% to 8.1%)</td>
<td>0.7 (0.6 to 0.9)</td>
<td>0.002</td>
<td>8</td>
</tr>
<tr>
<td>Tick bites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not suffering</td>
<td>10</td>
<td>10.5% (2.1% to 24.3%)</td>
<td>Reference</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Suffering</td>
<td>5</td>
<td>18.8% (10.1% to 29.4%)</td>
<td>1.8 (1.0 to 3.2)</td>
<td>0.036</td>
<td>5</td>
</tr>
<tr>
<td>Different continents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>35</td>
<td>14.0% (11.2% to 17.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>America</td>
<td>10</td>
<td>9.4% (3.5% to 17.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>10</td>
<td>7.4% (3.7% to 12.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caribbean</td>
<td>1</td>
<td>2.0% (0.6% to 4.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Different populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>35</td>
<td>5.7% (4.3% to 7.3%)</td>
<td>Reference</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>22</td>
<td>14.7% (9.9% to 20.2%)</td>
<td>1.6 (1.3 to 2.2)</td>
<td>&lt;0.001</td>
<td>7</td>
</tr>
<tr>
<td>Tick bitten</td>
<td>10</td>
<td>18.8% (10.1% to 29.4%)</td>
<td>2.5 (1.7 to 3.8)</td>
<td>&lt;0.001</td>
<td>2</td>
</tr>
<tr>
<td>LB-like symptoms</td>
<td>13</td>
<td>21.3% (14.1% to 29.4%)</td>
<td>5.8 (2.7 to 13.6)</td>
<td>&lt;0.001</td>
<td>2</td>
</tr>
<tr>
<td>Methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methods not conf WB</td>
<td>41</td>
<td>16.3% (13.8% to 18.9%)</td>
<td>Reference</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Methods conf WB</td>
<td>40</td>
<td>11.6% (9.5% to 14.0%)</td>
<td>0.6 (0.6 to 0.7)</td>
<td>&lt;0.001</td>
<td>36</td>
</tr>
<tr>
<td>Two time periods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001–2010</td>
<td>12</td>
<td>8.1% (5.7% to 10.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011–2021</td>
<td>45</td>
<td>12.2% (9.6% to 15.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LB, Lyme borreliosis; WB, western blotting.
Table 3  Range of Borrelia burgdorferi sensu lato (Bb) seroprevalence in five populations from different continents

<table>
<thead>
<tr>
<th>Continent</th>
<th>General population</th>
<th>High-risk population</th>
<th>Tick-bitten population</th>
<th>LB-like symptoms population</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>1.2% (0.0%–5.4%)</td>
<td>27.8% (4.6%–60.8%)</td>
<td>30.4% (0.9%–77.2%)</td>
<td>14.2% (12.4%–16.2%)</td>
<td>9.4% (2.6%–19.8%)</td>
</tr>
<tr>
<td>South America</td>
<td>–</td>
<td>4.6% (1.9%–8.5%)</td>
<td>–</td>
<td>12.2% (1.8%–29.9%)</td>
<td>8.7% (3.2%–16.6%)</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern Europe</td>
<td>4.4% (2.1%–6.8%)</td>
<td>12.3% (6.4%–16.9%)</td>
<td>–</td>
<td>–</td>
<td>11.1% (5.2%–18.8%)</td>
</tr>
<tr>
<td>Western Europe</td>
<td>7.5% (5.2%–10.1%)</td>
<td>13.1% (1.7%–32.7%)</td>
<td>23.2% (6.1%–46.9%)</td>
<td>47.9% (34.3%–61.6%)</td>
<td>13.5% (9.5%–18.0%)</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>8.6% (5.3%–12.6%)</td>
<td>9.3% (7.6%–11.1%)</td>
<td>3.2% (1.6%–5.4%)</td>
<td>14.3% (5.5%–26.3%)</td>
<td>7.6% (4.3%–11.7%)</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>4.8% (3.4%–6.5%)</td>
<td>–</td>
<td>10.7% (7.8%–13.9%)</td>
<td>35.5% (27.3%–44.1%)</td>
<td>10.4% (5.3%–16.9%)</td>
</tr>
<tr>
<td>Central Europe</td>
<td>10.9% (8.8%–13.2%)</td>
<td>35.7% (23.0%–49.4%)</td>
<td>5.5% (2.2%–10.1%)</td>
<td>11.2% (6.6%–16.8%)</td>
<td>20.7% (13.8%–28.6%)</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Asia</td>
<td>–</td>
<td>22.5% (14.1%–32.2%)</td>
<td>71.4% (46.0%–91.2%)</td>
<td>11.1% (7.5%–15.7%)</td>
<td>15.9% (6.6%–28.3%)</td>
</tr>
<tr>
<td>Southern Asia</td>
<td>–</td>
<td>3.0% (1.6%–4.7%)</td>
<td>–</td>
<td>–</td>
<td>3.0% (1.6%–4.7%)</td>
</tr>
<tr>
<td>Western Asia</td>
<td>5.2% (1.5%–10.9%)</td>
<td>7.8% (1.6%–18.2%)</td>
<td>17.1% (9.5%–26.3%)</td>
<td>–</td>
<td>6.3% (2.3%–12.2%)</td>
</tr>
<tr>
<td>Caribbean</td>
<td>–</td>
<td>2.0% (0.6%–4.1%)</td>
<td>–</td>
<td>–</td>
<td>2.0% (0.6%–4.1%)</td>
</tr>
<tr>
<td>Oceania</td>
<td>0.0% (0.0%–21.5%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.1% (0.4%–15.4%)</td>
</tr>
<tr>
<td>All</td>
<td>5.7% (4.3%–7.3%)</td>
<td>14.7% (9.7%–19.5%)</td>
<td>18.8% (10.1%–29.4%)</td>
<td>21.3% (14.1%–29.4%)</td>
<td>11.3% (9.2%–13.6%)</td>
</tr>
</tbody>
</table>

LB, Lyme borreliosis.
and forest cover due to human activities and the prevalence of outdoor activities (eg, more time spent in public green spaces and increasingly frequent pet contact).116117

In addition, our limited results regarding gender showed that the higher seropositivity rate in men relative to women was closely associated with the greater likelihood of males to engage in high-risk occupations. Older age is also a risk factor. Regarding residence, seropositivity rates were higher in rural than urban areas, suggesting that residence in rural areas is a risk factor of Bb infection, and other studies have reported increases in the proportion of seropositivity in urban populations over time, highlighting the need to raise awareness of Bb pathogens in cities.118 We believe that these differences may have a predictive value for assessing Bb risk factors as more data become available.

CONCLUSIONS

In conclusion, this systematic review provides a global estimate of the epidemiology of Bb infection in humans. With a high reported pooled seropositivity rate in the total population, Bb infection was most common in Europe. Subgroup analysis showed that the pooled seroprevalence increased steadily in these four subpopulations (the general population, the high-risk population, the tick-bitten population and the LB-like symptoms population).
population). This report further elaborates on the public health implications of the increasing prevalence of \( Bb \) infection. We confirmed that results confirmed by WB are more reliable than those not confirmed by WB when assessing human \( Bb \) infection. For risk factors, male sex, age >40 years, residence in rural areas and suffering from tick bites might increase the risk of \( Bb \) infection. However, future studies should be undertaken to verify these conclusions. \( Bb \) is a widely distributed infectious disease, but it has not received much attention worldwide. One of the major public health challenges regarding \( Bb \) is the ability to predict when and where there is a risk of \( Bb \) infection. A more accurate characterisation of the global distribution of \( Bb \) infection would guide the circulating epidemiology of \( Bb \) and identify risk factors for the disease, which could inform the development of public health response policies and \( Bb \) control programmes.

**Contributors**

BFK, guarantor. BFK, LAH, and DY conceived and designed the study. DY, CJW, and ZY conducted the database search and screening. DY, CJJ, and ZGZ interpreted the data and drafted the manuscript. BFK and LAH revised and approved the manuscript.

**Funding**

This work was supported by grants from the National Natural Science Foundation of China (No 32060180, 82168304, 81860644, 81560596 and 31560505) and the Joint Foundation of Yunnan Province Department of Science and Technology-Kuming Medical University (No 2019FE001 (2019FE002) and 2017FE467 (2017FE001)) and the Science Research Fund Project of Yunnan Provincial Department of Education (2021Y323).

**Disclaimer**

The funding institutions had no involvement in the design of the study or review of the manuscript.

**Competing interests**

None declared.

**Patient and public involvement**

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication**

Not required.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**Data availability statement**

Data are available in public, open access repository.

**Supplemental material**

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