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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-endorsed 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 (158 287 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.

PROSPERO registration number CRD42021261362.

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 as 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

HOW MIGHT IT IMPACT ON CLINICAL PRACTICE IN THE FORESEEABLE FUTURE?

⇒ We confirmed that results confirmed by WB are more reliable than those not confirmed by WB when assessing human Bb infection.
⇒ Using WB to confirm Bb serological results could significantly improve the accuracy.
⇒ For risk factors, male sex, age >40 years, residence in rural areas, and suffering tick bites might increase the risk of Bb infection.
⇒ We provided a more accurate characterizationcharacterisation of the global distribution and sociodemographic factors of Bb infection, which would guide the global epidemiology of LB and identify risk factors for the disease, and could inform the development of public health response policies and LB control programsprogrammes.

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.[6 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 or indirect fluorescent antibody (IFA) assay followed by immunoblot confirmation) currently serve as the primary supports for the laboratory diagnosis of LB and assessment of disease development.[9 10] Positive serological reactions indicating the presence of anti-*Bb* IgM or IgG reflect active or previous infection, respectively.[11 12]

This review provides the first meta-analysis of literature regarding seropositivity to anti-*Bb* antibodies in different countries and among different populations worldwide aimed at enhancing understanding of the global epidemiology of LB over the last 36 years. In addition, the detection of different antibodies is compared and analysed based on two different serological testing protocols. Finally, the distribution of *Bb* seropositivity rates is discussed in conjunction with analyses of potential risk factors, including population categories (general population, defined high-risk population, tick-bitten population and LB-like symptoms population), population characteristics (sex, age, geographical residence, tick bite status), geographical factors (continental plates), testing methods and publication year in order to identify factors associated with *Bb* seropositivity.

METHODS

This article was prepared according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (2020) guidelines (detailed in online supplemental appendix 1) and registered with PROSPERO (CRD42021261362).

Search strategy

We performed systematic, internet-based searches using the PubMed, Embase, Web of Science and the grey literature abstract databases with the following keywords: '*Borrelia burgdorferi sensu lato*' OR 'Lyme Disease Spirochete' OR '*Borrelia burgdorferi sensu stricto*' AND 'infection rate' OR 'prevalence' OR 'seroprevalence' OR 'serological survey' OR 'sero-prevalence' OR 'seroepidemiology' OR 'sero-epidemiology'. The search was not limited by language; reports not written in English were translated using Google Translate or by colleagues proficient in that language. To minimise publication bias, reference lists of included studies were manually retrieved and searched for grey literature that met our inclusion criteria. If data were missing, we contacted the corresponding authors of the relevant studies. The search was carried out between January 1984 and December 2021 (detailed in online supplemental appendix 2).

Inclusion and exclusion criteria

Inclusion criteria were as follows: (a) samples were human serum; (b) laboratory serological detection methods with the following standard laboratory tests for identifying *Bb*, including conventional serological methods such as 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 residence, serological screening test used, population categories, 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 I^2 value of more than 50% was considered 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 I^2 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 predictors 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 proportion 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 applicable 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 databases) 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 158 287 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* seropositivity rates of 16.3% (95% CI 13.8% to 18.9%) and 11.6% (95% CI 9.5% to 14.0%), respectively (online supplemental 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

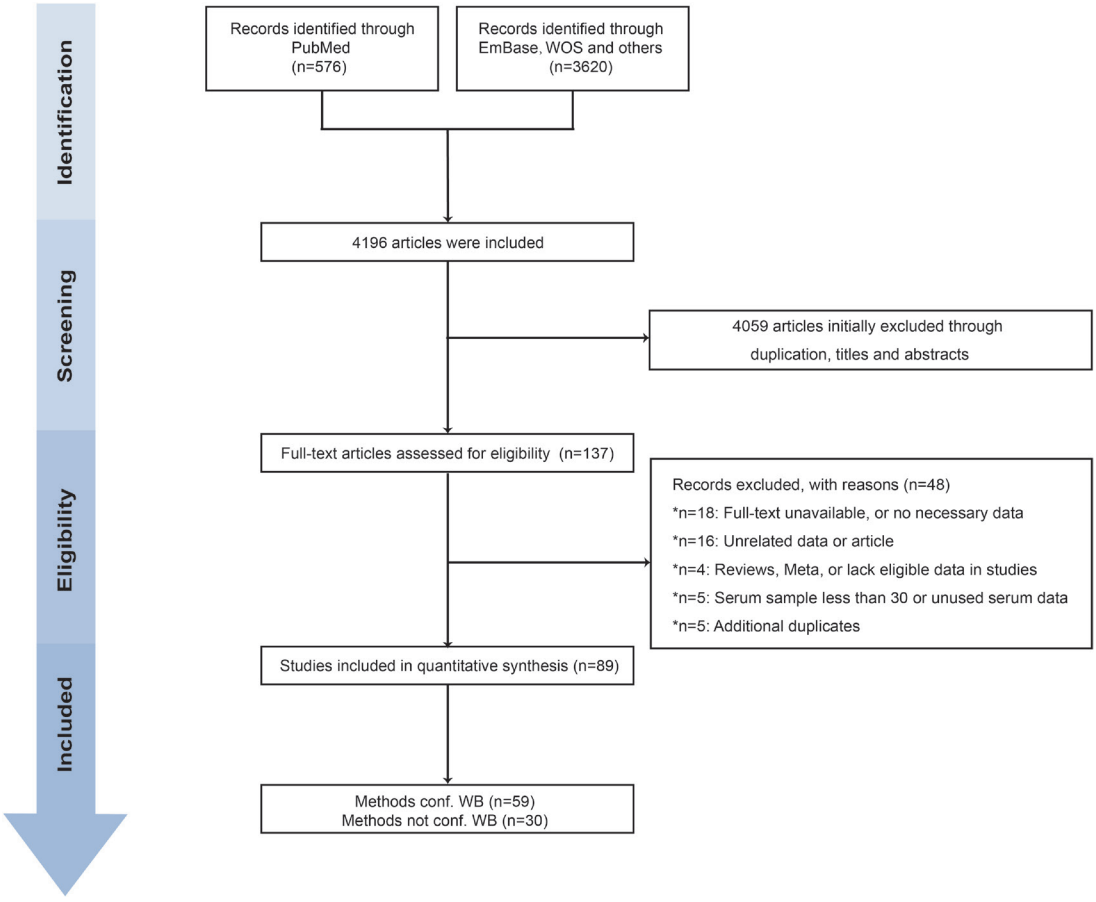

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of search strategy for selecting eligible studies. conf WB, confirmatory western blotting.

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 *Bb* infection, as our data showed *Bb* seropositivity results were more reliable with WB confirmation. Therefore, a subgroup analysis of the data from the 58 studies was conducted to survey possible predictors for *Bb* infection. The 58 selected articles were published between 1999 and 2021 and conducted in 28 countries from the Americas (5), Europe (17), Asia (4), Australia (1) and the Caribbean region (1).

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

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

	Methods conf WB	Methods not conf WB	Random effects model OR (95% CI)	P value	Cohort
Overall	9.8% (7.5%; 12.3%)	17.5% (14.2%; 21.1%)	1.9 (1.6 to 2.2)	<0.0001	38
Sex					
Female	5.1% (3.5%; 7.1%)	10.4% (7.1%; 14.2%)	1.7 (1.3 to 2.2)	0.0002	14
Male	5.4% (2.6%; 9.2%)	11.1% (5.1%; 18.9%)	1.8 (1.2 to 2.8)	0.0055	7
Age (years)					
<40	8.1% (3.7%; 14.0%)	15.9% (8.2%; 25.6%)	1.6 (1.2 to 2.19)	0.0030	6
40–49	6.2% (0.0%; 26.4%)	18.0% (2.6%; 43.1%)	2.4 (1.6 to 3.6)	<0.0001	2
≥50	8.8% (1.2%; 22.6%)	18.0% (7.2%; 32.4%)	1.9 (1.4 to 2.4)	<0.0001	6
Residence					
Rural	9.5% (3.6%; 17.7%)	13.4% (3.1%; 29.3%)	1.4 (1.1 to 1.9)	0.0082	3
Urban	5.3% (1.0%; 12.8%)	8.9% (0.8%; 24.6%)	1.5 (1.0 to 2.2)	0.0451	3
Tick bites					
Not suffering	3.2% (0.8%; 6.9%)	14.3% (8.7%; 20.9%)	4.5 (1.6 to 12.9)	0.0052	1
Suffering	16.2% (4.6%; 33.1%)	38.0% (2.7%; 67.5%)	2.4 (1.1 to 5.0)	0.0215	4
Different continents					
Europe	10.3% (7.5%; 14.1%)	17.2% (11.7%; 23.8%)	1.7 (1.5 to 1.9)	<0.0001	12
America	4.1% (0.7%; 10.1%)	10.6% (6.7%; 15.4%)	3.8 (1.9 to 7.6)	0.0001	6
Asia	6.6% (3.3%; 10.9%)	13.8% (8.2%; 20.6%)	2.3 (1.7 to 3.1)	<0.0001	10
Different populations					
General	5.3% (3.7%; 7.3%)	9.7% (7.5%; 12.1%)	1.9 (1.5 to 2.3)	<0.001	22
High risk	10.9% (6.6%; 16.2%)	22.0% (16.1%; 28.7%)	1.5 (1.3 to 1.7)	<0.001	12
Tick bitten	16.2% (4.6%; 33.1%)	38.0% (12.7%; 67.5%)	2.4 (1.1 to 5.0)	0.0215	4
LB-like symptoms	18.9% (10.7%; 28.7%)	26.5% (14.6%; 40.6%)	1.4 (1.1 to 1.8)	0.007	6
Antibodies					
IgG	7.8% (4.8%; 11.4%)	12.8% (7.8%; 18.9%)	1.7 (1.4 to 2.0)	<0.001	12
IgM	4.2% (2.7%; 5.9%)	12.2% (9.2%; 15.4%)	3.1 (2.1 to 4.4)	<0.001	12
IgG+IgM	0.2% (0.0%; 0.7%)	2.2% (0.4%; 5.4%)	4.8 (2.0 to 11.5)	<0.001	3
Two time periods					
2001–2010	7.1% (3.6%; 11.6%)	14.3% (11.2%; 17.7%)	2.5 (1.4 to 4.4)	0.0017	9
2011–2021	10.1% (7.3%; 13.4%)	17.9% (13.8%; 22.4%)	1.9 (1.6 to 2.3)	<0.001	26

Methods conf WB group was considered the reference group when conducting meta-regression analysis.
LB, Lyme borreliosis; WB, western blotting.

trend over time. 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%), 13.5% (95% CI 10.9% to 16.3%), 2.0% (95% CI 0.6% to 4.1%), 7.4% (95% CI 3.7% to 12.2%) 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

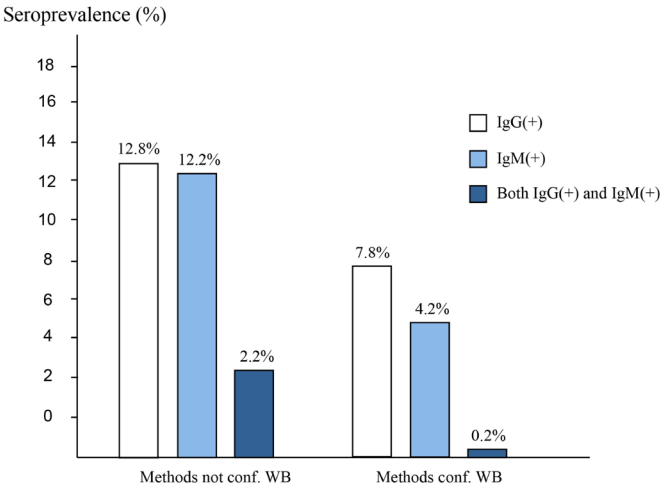

Figure 2 Prevalence of specific anti-IgG and anti-IgG antibodies against *Borrelia burgdorferi sensu lato* among extracted studies that reported seropositivity confirmed by western blotting (WB) relative to seropositivity not confirmed by WB.

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.[105] Since its identification in 1975, LB has become the most common tickborne zoonotic disease worldwide.[106 107] The incidence and distribution of LB have increased over the last four decades.[108] 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.[109] 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.[9 110] 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,[111] the subjectivity and complexity of the techniques associated with secondary immunoblotting and high relative expense.[112] Other improved secondary serological assays (eg, whole-cell ultrasound enzyme immunoassay (EIA)+C6 EIA)[113] and molecular diagnostics (eg,

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

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

LB, Lyme borreliosis; WB, western blotting.

next-generation sequencing)[113] are developing rapidly, which could improve LB diagnosis.

To identify potential risk factors associated with anti-*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),[15] 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.[114] Our results indicate that the prevalence of *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.[115] 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 3 Range of *Borrelia burgdorferi sensu lato* (*Bb*) seroprevalence in five populations from different continents

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

LB, Lyme borreliosis.

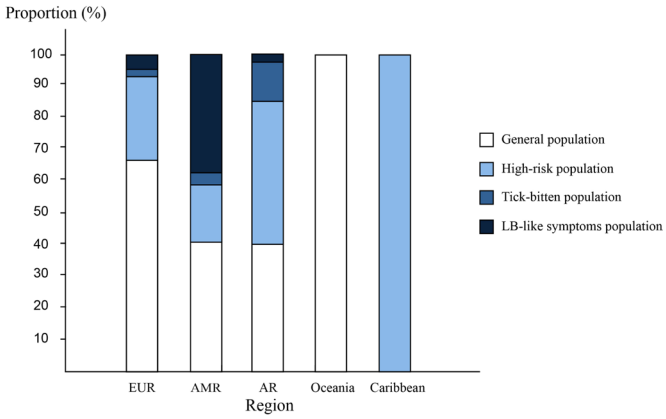

Figure 3 Distribution of included samples by population category and WHO region. AMR, Region of the Americas; AR, Asian Region; EUR, European Region; LB, Lyme borreliosis.

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).[116 117]

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.

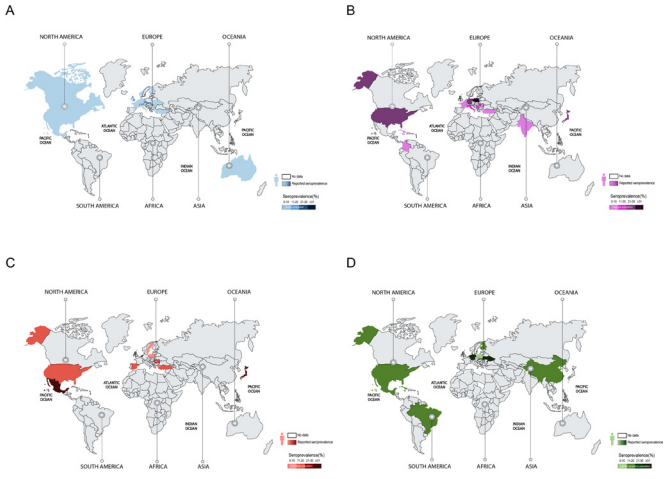

Figure 4 Estimated western blotting (WB)-based *Borrelia burgdorferi sensu lato* (*Bb*) seroprevalence in different groups of human populations in reported countries. Different colours represent different groups of people and their disease severity, and grey areas represent countries reporting no *Bb* seroprevalence in humans. (A) General population. (B) High-risk population. (C) Tick-bitten population. (D) Lyme borreliosis (LB)-like symptoms population.

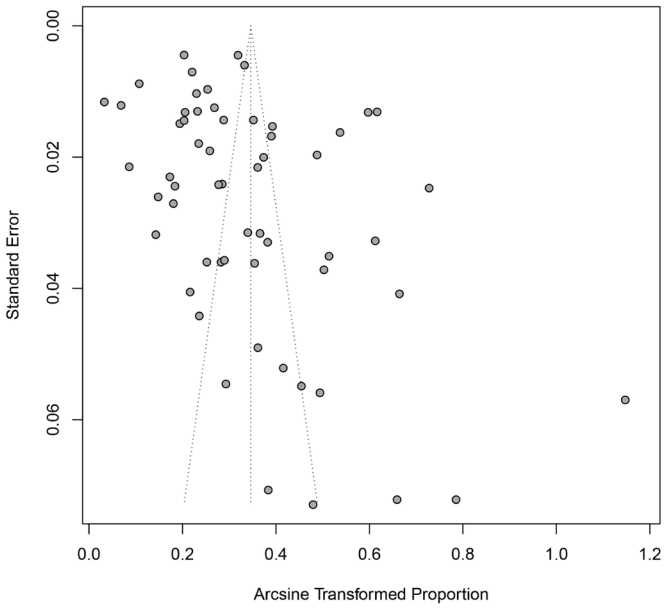

Figure 5 Publication bias result of funnel plot.

We acknowledge the limitations of this study. First, due to the limited data, few studies were conducted with longitudinal follow-up, and it was not possible to systematically assess whether *Bb* antibody positivity has any long-term effect on the risk of developing LB or the risk of recurrence. Second, the heterogeneity of the included studies was high, and most of the reports lacked important information, such as exact definitions of high-risk groups, the inclusion of subjects with suspicious LB symptoms and no indication regarding whether random sampling was used, which may lead to heterogeneity. We did not identify specific sources of heterogeneity via subgroup, sensitivity or meta-regression analyses; thus, ongoing monitoring is needed to generate more data to address sources of heterogeneity and obtain more globally representative estimates. Third, our extraction of potential risk factors may be incomplete because not all studies reported demographic characteristics, which may have resulted in small sample sizes for some subgroups analysed, making estimates for those subgroups inaccurate. Fourth, secondary WB results for LB detection varied depending on the primary immunological method used, serum concentration, antigen type (eg, OspC-I, C6VlsE, circulating immune complex), antigen concentration, antibody type (eg, IgG, IgM), secondary antibody concentration and subjective judgement ability.

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). 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. LB is a widely distributed infectious disease, but it has not received much attention worldwide. One of the major public health challenges regarding LB 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 LB and identify risk factors for the disease, which could inform the development of public health response policies and LB control programmes.

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