Review

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Rebeca Castro-Flores [1], Soila M. Gaxiola [1,*], Efren Diaz-Aparicio [2], Miguel A. Rodriguez-Gaxiola [1], Arnulfo Montero-Pardo [1], Idalia Enriquez-Verdugo [1], Nohemi Castro del Campo [1], Milad Bradi [3], Pavel E. Hernandez-Carreño [4], Diana Z. Velazquez [5], Carlos Lizarraga [1], Gerardo Avila-Butron [6], Enrique J. Delgado-Suarez [4], Armando Ramirez-Valdez [7] and Daniel Diaz [8,*]

[1] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, Culiacán Rosales 80246, Sinaloa, México; rebeca.castro@uas.edu.mx (R.C.-F.); m_angel@uas.edu.mx (M.A.R.-G.); arnulfo.montero@uas.edu.mx (A.M.-P.); enver@uas.edu.mx (I.E.-V.); Ncastro@uas.edu.mx (N.C.d.C.); carlosreyes@uas.edu.mx (C.L.)

[2] Centro Nacional de Investigación Disciplinaria Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Cuajimalpa 01219, Ciudad de México, México; efredia@yahoo.com

[3] Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin 3419915315, Iran; badri22.milad@gmail.com

[4] Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Coyoacán 04510, Ciudad de México, México; pavel.hernandez.mvz@uas.edu.mx (P.E.H.-C.); ejds@fmvz.unam.mx (E.J.D.-S.)

[5] Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados, Gustavo A. Madero 07360, Ciudad de México, Mexico; diana.velazquezv@cinvestav.mx

[6] Facultad de Ciencias de la Salud, Universidad Juárez del Estado de Durango, Zona Centro 34113, Durango, México; mcgerardobutron@gmail.com

[7] Doctorado en Ciencias Agropecuarias, Universidad Autónoma Metropolitana, Unidad Xochimilco, Coyoacán 04960, Ciudad de México, México; 2212801773@alumnos.xoc.uam.mx

[8] Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, Ciudad de México, México; ddiaz@ciencias.unam.mx

* Correspondence: soilagaxiola@uas.edu.mx (S.M.G.); ddiaz@ciencias.unam.mx (D.D.)

Abstract: A systematic review and meta-analysis were conducted to estimate the prevalence of chlamydiosis in small ruminants. The study included 133 publications that reported the presence of *Chlamydia abortus* in 109 and 74 studies in sheep and goats from 45 countries. The prevalence of chlamydiosis was estimated at global, regional, and national levels using meta-analyses of proportions. Meta-regression and cumulative meta-analyses were also performed. Globally, the prevalence of chlamydiosis in sheep and goats was estimated at 20.1% (95% CI: 17.8-22.4) and 14.4% (11.6-17.4), respectively. For sheep, chlamydiosis prevalence was heterogeneous at regional and national levels. South Asia had the highest prevalence (30.6%) and East Asia and Pacific the lowest (14.0%). Nationally, chlamydiosis was more prevalent in Romania, Hungary, and Germany (range 53.3-87.0%) compared to Costa Rica, Australia, and Zimbabwe (4.7-5.2%). Among goats, chlamydiosis prevalence was similar across regions (10.4-19.7%), but varied significantly among countries, 0.0% in Romania and Australia and 91.7% in Bosnia and Herzegovina. In both species, sample size, number of diagnostic techniques, and latitude were significant covariates. Prevalence varied widely in the first decades of publications before stabilizing. Our study found a high prevalence of chlamydiosis in small ruminants, with the pathogen being distributed globally but heterogeneously across countries.

Keywords: enzootic abortion of small ruminants; pathogenic bacteria; chlamydiosis; reproductive failure; zoonosis

1. Introduction

Chlamydia abortus is an obligate intracellular bacterium that causes enzootic abortion in small ruminants [1]. Chlamydiosis is a global animal health issue that affects female sheep, goats, and cattle, resulting in abortion during the final third of gestation [2] as well as early pregnancy losses and the birth of weak lambs [3]. As a result, chlamydiosis causes significant economic losses due to the loss of offspring and milk production. Furthermore, research has shown that male farm animals can carry the bacterium and transmit it to healthy females through sexual contact [4,5].

In small ruminant herds without prior exposure to the disease, the abortion rate is typically low in the first year after the introduction of infected animals [6]. However, in the subsequent 2-3 years, the abortion rate can increase by approximately 30% [7]. Subsequently, the disease results in an annual abortion rate of 5-10%, which may persist for 2-3 years until another epidemic arises, primarily impacting females giving birth for the first time [3]. In addition to these animal health issues, livestock activities during the calving/abortion season can pose a zoonotic risk to humans due to increased contact with bacterial discharges found in placental tissues and vaginal secretions of infected females. *C. abortus* can cause conjunctivitis and pneumonia in humans [7]. Additionally, chlamydiosis can cause complications such as fever and miscarriage in pregnant women [8].

Chlamydiosis has a significant economic impact on small ruminant production worldwide, making the control and eradication of the bacteria a relevant issue. In cattle, abortion has been estimated to cause economic losses ranging from US$500-1000 in the United States and United Kingdom [9]. In some regions of Europe, enzootic abortion in small ruminants causes economic losses estimated at 20 million pounds per year [10]. Recent data suggest that the economic losses due to *C. abortus* outbreaks exceed the cost of preventive management practices [11]. Therefore, it is essential to estimate the prevalence of the disease and examine its distribution globally to make recommendations for controlling the disease in regions with a higher infection rate. In this study, a systematic review and meta-analysis were performed to answer the following question: What is the global, regional, and national prevalence of chlamydiosis in small ruminants? For this purpose, we included primary studies of cross-sectional, retrospective, case series, or longitudinal designs carried out in sheep and goats in which the presence of *C. abortus* was detected. The study results will allow us to summarize the scientific evidence published to date and provide a global overview of the magnitude of chlamydiosis in small ruminant flocks in different regions of the world.

2. Materials and Methods

2.1. Protocol

For this study, we developed an a priori protocol following the PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol) guidelines [12]. The protocol is available at the Open Science Framework website (https://osf.io/p93jf). We also followed the Cochrane guidelines [13] for systematic reviews and meta-analyses and reported our findings according to the updated PRISMA 2020 statement [14].

2.2. Inclusion Criteria

For our study, we used a modification of the PICOS (Population, Intervention, Comparator, Outcome, and Study) approach included in the PRISMA 2020 statement [14]. Briefly, we considered studies that included population, study factor, outcome, and study types. The population included in the studies was defined as sheep or goats without distinction of sex, physiological stage, or zootechnical function. The study factor was the presence of *C. abortus*. A case was defined as any sample from an individual, tissue, or flock that tested positive for *C. abortus* using any diagnostic technique, including molecular, serological, bacteriological, or immunological methods, on any type of sample, such as body fluid or tissue. The investigations included in this study evaluated the prevalence of chlamydiosis, expressed as a percentage of positives out of the total evaluated, either at the individual or flock level. Only peer-reviewed primary studies, including cross-sectional, cohort, case study, case series study, longitudinal, or retrospective studies published in English, Spanish, or Portuguese were considered. No temporal or regional restrictions were applied. To ensure a consistent methodology among the selected studies, we excluded gray literature (unpublished studies) from the present study [15].

2.3. Information Sources and Search Strategies

One reviewer conducted a comprehensive search for relevant studies using various databases including PubMed, Scopus, Science Direct, Virtual Health Library (VHL), CAB Abstracts, and Web of Science between February 24 and March 2, 2022. Additionally, from October 8-14, 2023, the same reviewer employed other methods such as searching websites, seeking expert recommendations, and cross-referencing to identify any additional records not available through digital database searching, as suggested by the PRISMA 2020 statement.

The search terms for this study were defined as follows: population (goat OR caprine) or (sheep OR ovine); study factor (chlamydia abortus OR chlamydophila abortus OR chlamydial OR chlamydiosis); and outcome (prevalence OR epidemiology OR incidence OR surveillance OR frequency). To maximize the finding of potential studies, the reviewer performed independent searches for each productive species. The search process in each database was refined using the available methodological filters and Boolean operators (AND, OR, and NOT) were used for the search commands. Searches were performed on the title, abstract, and keywords, and were defined as follows: (population) AND (study factor) AND (outcome). The following search commands were used to find studies on sheep. ScienceDirect, Title: (ovine OR sheep) AND (chlamydia abortus OR clamydophila abortus OR chlamydiosis) AND (prevalence OR epidemiology OR incidence); CAB abstracts, All fields: ((goat OR caprine)) AND ((chlamydia abortus OR chlamydophila abortus OR chlamydial OR chlamydiosis)) AND ((prevalence OR epidemiology OR incidence OR surveillance OR frequency)). While in Scopus the search command included: TITLE-ABS-KEY ((chlamydia OR Chlamydophila OR abortus) AND (ovine OR sheep) AND (prevalence OR epidemiology OR incidence OR frequency)). In Supplementary Table S1 are summarized the search commands used in all databases with respective number of records found for each species.

2.4. Study Selection Process

The records were collected from each electronic database by one reviewer and subsequently compiled into a library using EndNote 21 (Thomson Reuters, USA). The same reviewer then removed duplicates both automatically and manually before screening the studies based on titles and abstracts. The records were retrieved in full text before the eligibility stage. Two independent reviewers used a questionnaire based on the eligibility criteria to select the final studies. To test the questionnaire, 10% of randomly selected studies were piloted. The questionnaire was then applied to all selected records. Before resolving discrepancies among the reviewers, a moderate level of agreement was found in the selection of studies (Kappa = 0.686, T = 9.501; $p < 0.000$). In cases where there were differences in article selection, a third reviewer was consulted to reach a consensus. The identification and selection of studies, except for duplicate removal and initial screening, were performed by a single reviewer using the same process described for the records found through database searching.

2.5. Information Collection Process and Extracted Data

Data from the selected publications were extracted by a single reviewer using a predefined format based on the eligibility criteria. The criteria were pilot tested on a random selection of 10% of the studies in the database. The data extracted from the study included information on the author and year of publication, the country and World Health Organization (WHO) region where the study was conducted, the characteristics of the evaluated population (breed, age, sex, and clinic status), study design, sample type, diagnostic technique, and prevalence (percentage of *C. abortus*-positive samples divided by the total number of samples included in the study). It is important to note that certain publications included results from both species and reported the prevalence of chlamydiosis at both the individual and flock levels. In these cases, we extracted information for each species and sampling level separately to reduce estimation error [16]. Therefore, some publications included results from two distinct studies (one for each species). The authors were not contacted to obtain or confirm incomplete information. All extracted information is presented in Supplementary Dataset 1 and 2 for sheep and goats, respectively.

2.6. Risk of Bias Assessment

To evaluate the potential for bias in individual studies, we assessed whether each of the following criteria exhibited a risk of bias (Yes or No): 1) confirmation of positive samples to *C. abortus* by 2 or more diagnostic techniques, 2) reports a brief definition of the population studied (including at least two characteristics such as sex, breed, or age) to allow extrapolation of the study results to a population with similar characteristics, 3) description of the clinic status of the population assessed, and 4) includes a description of the type of samples assessed for the presence of *C. abortus*. The study results are presented individually and as a percentage summary for the entire set of studies reviewed. The outcomes are categorized as either meeting the defined criterion and considered free of bias, or not meeting the criterion and having a risk of bias in the evaluated field.

2.7. Summary of Evidence and Statistical Analysis of Data

Our study utilized the proportion (number of positives divided by the total number evaluated) as the primary summary measure. Therefore, we summarized the proportion of chlamydiosis in each small ruminant species using a meta-analysis of proportions without distinguishing the results according to sampling level (individual or flock). This was due to only five studies exclusively reporting data at the flock level. Similarly, we did not include characteristics of the sampled population such as sex, age, or breed, as they were inconsistently reported across studies. To obtain a pooled estimate of individual studies, we used a meta-analysis of proportions based on the Freeman-Tukey double arcsine transformation (FTT) [17]. For individual studies, we calculated the exact 95% confidence interval using the Clopper-Pearson method, and for grouped and overall estimates, we used the inverse of the FTT [18]. We chose this method because FTT stabilizes variance when proportions are close to 0 or 1 and does not require zero-cell adjustment (continuity correction) like raw proportion does in the case of zero prevalence detection [19].

A random-effects (D-L) model was chosen beforehand due to the anticipated heterogeneity of studies [20]. Subgroup meta-analyses were conducted to combine the estimates, first at the regional level, according to the seven regions defined by the World Health Organization, and then at the national level. As previously described [21,22], we used Cochran's Q homogeneity test to assess between-study heterogeneity. We assumed the null hypothesis that the effect sizes across the K independent studies were equal (H_0: $\theta_1 = \theta_2 = \cdots = \theta_K = \theta$) and used the recommended significance level of $p = 0.1$ [18]. However, since the Q statistic does not measure the magnitude of heterogeneity, we used the following measures: Tau^2 (heterogeneity parameter), H^2 ($H^2 = [Q/K\text{-}1]$), and I^2 ($I^2 = [\ Q\text{-}(K\text{-}1)/Q]*100$). A Tau^2 value greater than 1 indicates substantial heterogeneity, while the I^2 statistic determines the proportion of variation in effect estimates due to heterogeneity in true effects rather than sampling error [23]. For national-level meta-analyses, we do not pr3ovide a summary of heterogeneity measures because some countries only had one study. Additionally, we conducted a z-test to determine if the studies produced a significant overall effect size, assuming a null hypothesis of zero effect (H_0: $\theta = 0$). To examine the heterogeneity visually, Galbraith plots were constructed. These plots show the standardized effect size plotted against the precision of each individual study using a regression line through the origin that has the overall effect size as its slope [18].

To investigate potential causes of heterogeneity, we conducted a meta-regression analysis using a random-effects D-L model [24]. We created separate univariate models with continuous moderators (study-level covariates) including sample size (calculated as quintiles for each species), year of publication, latitude of study location, and number of diagnostic techniques used. Table summaries and bubble plots are used to present the results for each model, while funnel plots are constructed to explore publication bias and detect asymmetry. Additionally, Egger's regression test is performed to detect small-study effects [25]. To identify potential trends in overall effect sizes, we conducted a

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cumulative meta-analysis per species, using the year of publication to determine any point in time when a change in prevalence may have occurred [18]. Additionally, we used a leave-one-out meta-analysis to detect any studies in which 'exaggerated results' may have distorted the overall effect size (prevalence of chlamydiosis). These two analyses perform multiple meta-analyses. The first adds one study at a time, while the second excludes one study at a time to detect its influence on the overall estimate [18].

All meta-analyses, secondary analyses, and forest plots were conducted using the "Meta-analysis tool" from the Statistics menu in Stata 18 (StataCorp, USA). The commands and setup for these analyses and graphs are provided in Supplementary Table S2. To aid in the geographical interpretation of chlamydiosis estimates, individual and merged maps of *C. abortus* prevalence per country were constructed for each species. Bubble maps from the free online tool Datawrapper (https://app.datawrapper.de accessed 10/12/2023) were utilized. All additional graphs and maps were created using Excel 16.7 (Microsoft, USA) and Prism 10 (GraphPad Inc., USA). A p value < 0.05 was considered significant unless otherwise stated.

3. Results

3.1. Study Selection

A total of 2,857 records were found through electronic database searches, with ScienceDirect contributing 43.4%, followed by Scopus and CAB abstracts at 21.31% and 14.8%, respectively. After eliminating 1,585 duplicates, 1,272 records were evaluated during the screening process. After applying the inclusion criteria to the title and abstract, 163 publications were selected as they met the defined characteristics for inclusion. These publications were retrieved in full text, although only 152 out of the 163 selected studies were obtained. After applying the eligibility format to the 152 full texts, 73 publications were excluded for not meeting the inclusion criteria due to the following reasons: Of the studies reviewed, 53.4% did not include the defined population, 34.2% did not correspond to the defined study type, 9.6% did not present the prevalence of *C. abortus*, and 2.7% were in a language other than English. A complete list of excluded studies and their primary reasons for exclusion can be found in Supplementary Table S3. In total, 79 publications were included through database searching. Additionally, we found 60 additional records through alternative methods, such as websites (30.0%), expert recommendations (6.7%), and citation searches (63.3%). We obtained the full text of these 60 records, and after applying the inclusion criteria, 54 of them were included in the narrative synthesis.

Based on the PRISMA 2020 flow diagram in Figure 1, the systematic review and meta-analysis included a total of 133 studies. Of these, 109 individual studies reported the prevalence of *C. abortus* in sheep, while 74 individual studies assessed the presence of the pathogen in goats. The complete list of the 133 included studies can be found in Supplementary Table S4.

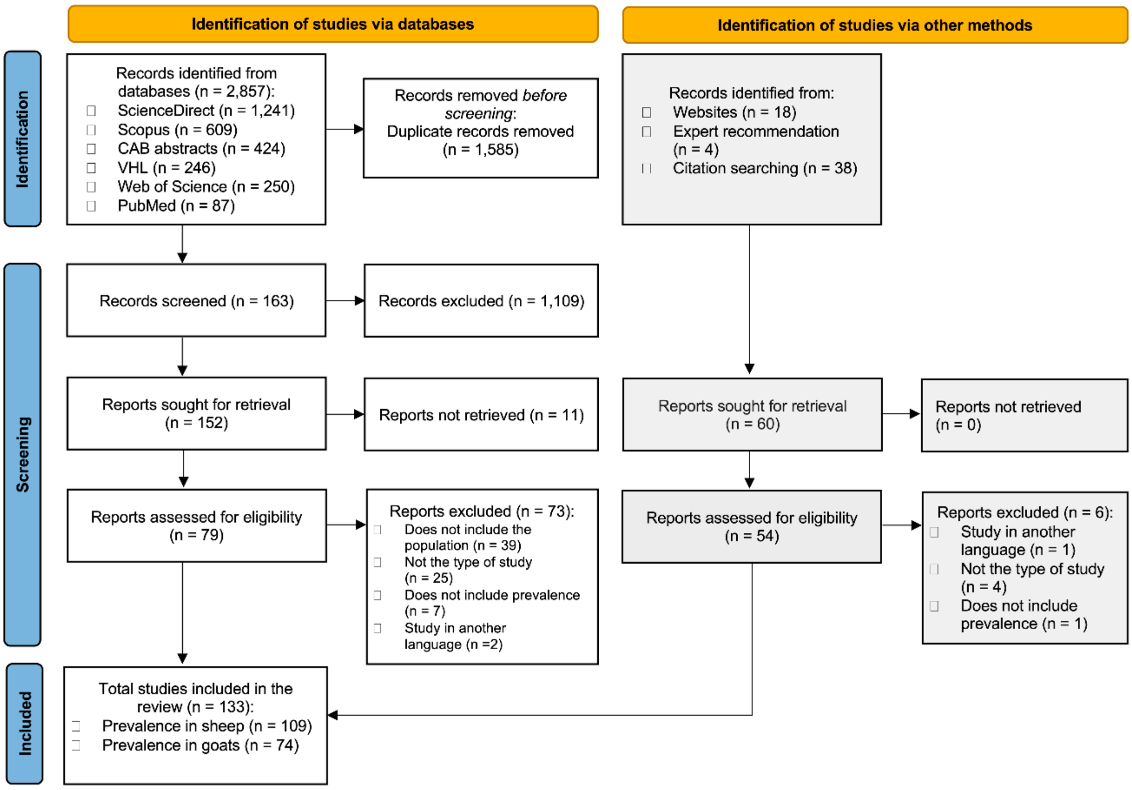

Figure 1. PRISMA 2020 flow diagram for new systematic reviews that include search of database and other sources. The diagram describes the process of identification, selection, and eligibility of studies included in the meta-analysis. Some publications included independent studies in sheep and goats, so the total sum of studies in both species is greater than that of the articles included in the narrative synthesis.

3.2. General Characteristics of the Studies

Based on the publication dates, the 133 studies included in our systematic review and meta-analysis were published between 1989 and 2023. More than half of these investigations (51.1%) were published from 2015 onwards, indicating the growing importance of chlamydiosis research in small ruminants in recent years (Figure 2A). Regarding the study design, 96 out of 133 publications (72.2%) were cross-sectional epidemiological studies, 32 out of 133 (24.0%) were case report/case series studies, and the remaining five studies had a longitudinal design. The majority of the studies (123 out of 133) were published in English, while five were published in Spanish and another five in Portuguese. Regarding the productive species assessed in each study, 59 studies exclusively evaluated the presence of *C. abortus* in sheep. A total of 24 studies included goats as the main species assessed, and another 50 studies independently examined the presence of the bacterium in both types of small ruminants (Figure 2B).

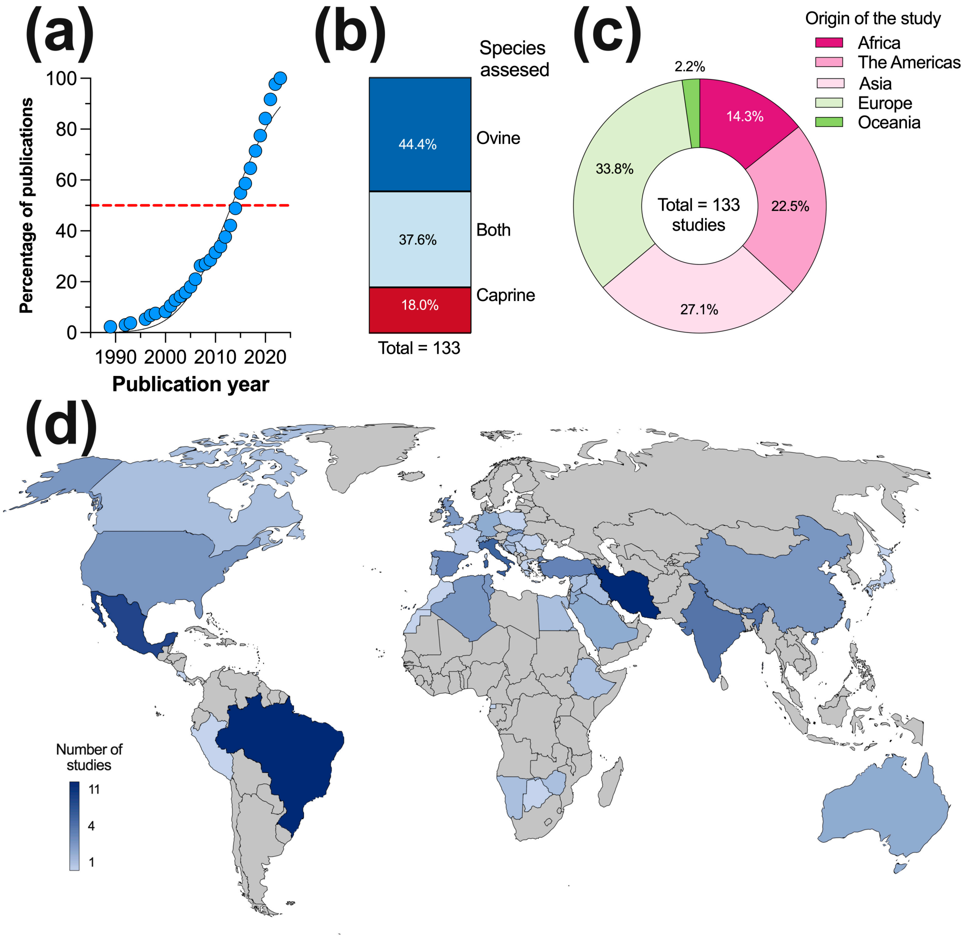

Figure 2. (a) Cumulative relative frequency distribution by year from 1989-2023 of the 133 studies included in the systematic review and meta-analysis, (b) percentage of studies according to the species evaluated, (c) percentage of publication by continent, and (d) geographical distribution of the number of studies at the national level.

Figure 2C shows a global distribution of research on the prevalence of chlamydiosis in small ruminants. A total of 133 publications were analyzed, with studies conducted in countries across all five continents. The majority of publications were from Europe, Asia, and the Americas, which accounted for 83.4% of the total publications (45, 36, and 30 studies, respectively). The results of the studies were reported from 45 countries, including Brazil and Iran (11 studies each), Mexico (9 studies), Italy (7 studies), India (6 studies), and Spain and Turkey (5 studies each). These seven nations contributed to 40.6% of the total publications (Figure 2D).

3.3. Risk of Bias of Individual Studies

As shown in Figure 3, the risk of bias assessment results revealed that 36 out of 133 studies (27.1%) confirmed *C. abortus*-positive samples using two or more diagnostic tests, indicating that they were free of bias. Conversely, 97 out of 133 studies reported using only one technique to detect the pathogen, which qualified them as having a risk of bias in this criterion. Regarding the definition of the population evaluated in the studies, 28.6% of the studies (38/133) reported at least two details about the population, such as sex, age, or breed, and showed no risk of bias. However, the remaining 95 studies did not include detailed information about the population they evaluated, presenting a risk of bias. Regarding the clinical status of the assessed population, 89 out of 133 studies (66.9%) were rated as having no risk of bias, while the remaining 44 studies were rated as having a risk of bias due to the lack of clinical description of the samples tested. Additionally, all studies were rated as having no risk of bias for appropriately describing the type of sample assessed. Please refer to Supplementary Dataset 3 for the risk of bias assessment for each criterion per study.

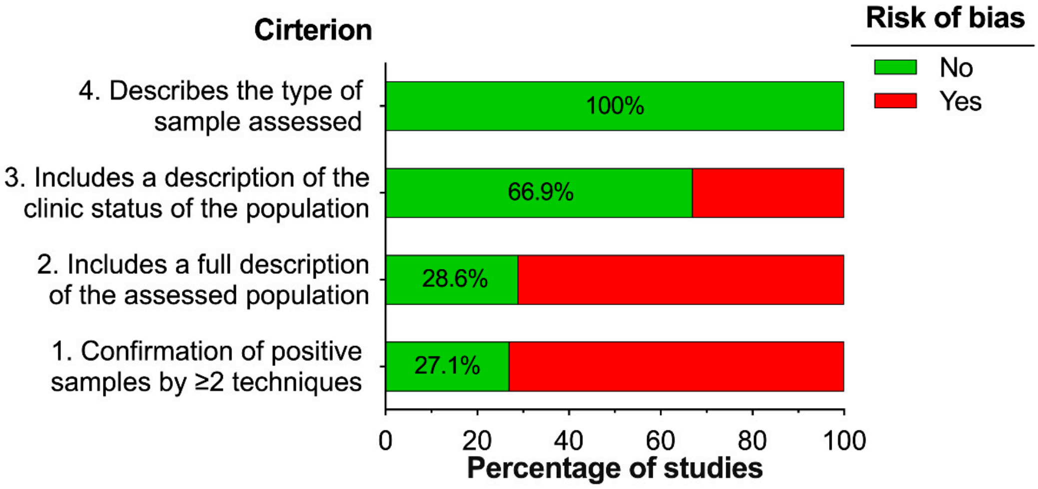

Figure 3. Summary of risk of bias assessment for individual studies. Yes, meets the defined criterion and is considered free of bias; No, does not meet the criterion and there is a risk of bias in the criterion evaluated.

3.4. Individual Characteristics of the Studies Evaluating Sheep

The characteristics of the 109 studies that reported the prevalence of chlamydiosis in sheep are summarized in Supplementary Table S5. Specific information regarding the population analyzed was not presented in most studies: 19 out of 109 studies presented the breed evaluated, 21 out of 109 studies reported an age range of the sheep, and 56 out of 109 studies reported the sex of the animals (37 females, 2 males, and 17 reported animals of both sexes). Out of the 109 studies included in the publications, 14 reported a history of abortion or reproductive failure in the sheep. Additionally, 21 publications evaluated the presence of *C. abortus* in healthy animals or compared healthy animals to those with reproductive issues. Of the 109 studies reviewed, 33 included females with a history of abortion, along with their aborted fetuses and placenta. Two additional studies examined sheep with keratoconjunctivitis for the presence of the pathogen. 39 studies did not provide any information on the clinical characteristics of the population analyzed.

As summarized in Supplementary Dataset 1, 68 out of 109 studies used serum as the main type of sample. A total of 26 studies reported results on fetal tissue, placenta, or vaginal swabs from aborted ewes, whereas 10 studies included reproductive tissue or vaginal swabs to detect chlamydiosis. Two studies used eye swabs, one study reported results found in milk, one study used plasma, and finally, one study assayed semen samples for detecting *C. abortus*. Out of the 109 studies evaluated, 81 reported using a single diagnostic technique to detect the pathogen in the samples. The remaining studies reported using 2, 3, or 4 techniques. Out of the 109 studies analyzed, 58 used ELISA and 40 used PCR for molecular detection of Chlamydia. Additionally, 30 studies reported the prevalence of chlamydiosis using complement fixation test, immunoprecipitation, hemagglutination, or fluorescence assay test, 13 studies used culture or immunofluorescence, and 6 studies used immunohistochemistry on tissues or cells (Supplementary Dataset 1). Please note that some studies reported up to 4 techniques, which is why the total number of techniques exceeds the original 109 studies.

3.5. Global, Regional, and National Prevalence of *C. abortus* in Sheep

The 109 studies that evaluated sheep included a total of 81,788 samples, of which 11,235 tested positive for *C. abortus* (Table 1). The meta-analysis results showed a global estimate of 20.1% for chlamydiosis (95% CI: 17.8 - 22.4) with a significant overall effect size (z = 28.8, p = 0.00). There was significant heterogeneity between studies (Q = X^2 = 6323.7, d.f. = 108; p = 0.00, Supplementary Figure S1). Although the Tau² statistic did not indicate significant heterogeneity (0.08), the I^2 (98.2%) and $H2$ (58.5) statistics suggest that a significant proportion of the variation is attributable to variability across studies.

Among the seven WHO regions, the highest pooled prevalence values were presented by four studies conducted in South Asia (30.6%, 7.1 – 61.1) and 41 studies from Europe and Central Asia (24.8%, 20.2 – 29.6). In contrast, *C. abortus* was less prevalent in 15 studies from Latin America and the Caribbean and seven studies conducted in East Asia and Pacific regions, where the estimates were 15.5% and 14.0%, respectively. The pooled estimates of the three remaining WHO regions were lower than the global prevalence due to their chlamydiosis prevalence ranging between 16.2% and 18.2% (Table 1). The studies from Europe and Central Asia had the highest sample size evaluated (47,107, 57.6%), while the studies from Sub-Saharan Africa and South each provided 2,333 and 1,020 samples, respectively, accounting for 2.8% and 1.2% of the total sample size.

Table 1. Global, regional, and national prevalence of *C. abortus* in sheep and goats.

WHO Region	Country	**Sheep**			**Goats**		
		Studies	Positive/Total	Prevalence (95% CI)	Studies	Positive/Total	Prevalence (95%
Global		109	11,235 /	20.1 (17.8 –	74	2,729 /	14.4 (11.6
Sub-Saharan Africa		5	531 / 2333	17.1 (9.8 –	7	670 / 5341	15.4 (7.5
	Botswana	-	-	-	1	57 / 1799	3.2 (2.4 –
	Equatorial Guinea	1	199 / 1002	19.9 (17.4 –	-	-	-
	Ethiopia	2	52 / 346	16.0 (2.5 –	2	71 / 383	24.8 (2.4
	Namibia	1	277 / 922	30.0 (27.1 –	2	86 / 1076	15.6 (2.9
	Zimbabwe	1	3 / 63	4.7 (0.6 – 11.7)	2	157 / 898	15.5 (5.6
North America		5	230 / 2617	16.2 (7.6 –	2	97 / 471	19.7 (9.8
	Canada	2	52 / 609	18.4 (8.1 –	1	67 / 260	25.8 (20.6
	USA	3	127 / 2008	14.9 (3.8 –	1	30 / 211	14.2 (9.8
Latin America and the Caribbean		15	1358 / 10,174	15.5 (12.1. – 19.3)	14	398 / 3690	12.3 (7.3 – 18.3)
	Brazil	8	403 / 2716	14.0 (10.1 –	6	212 / 2656	8.0 (5.3 –
	Costa Rica	1	19 / 359	5.2 (3.2 – 7.9)	-	-	-
	Grenada	1	24 / 355	6.8 (4.4 – 9.6)	1	10 / 209	4.8 (2.2 –
	Mexico	4	710 / 5806	26.4 (13.7 –	6	175 / 736	24.1 (8.6
	Peru	1	202 / 938	21.5 (19.0 –	-	-	-
	Trinidad and Tobago	-	-	-	1	1 / 89	1.1 (0.0 –
South Asia		4	134 / 1020	30.6 (7.1 –	5	101 / 1235	10.4 (0.09
	India	4	134 / 1020	30.6 (7.1 –	5	101 / 1235	10.4 (0.09
East Asia and Pacific		7	937 / 4425	14.0 (7.4 – 22.2)	5	306 / 2174	13.6 (5.3 – 24.4)
	Australia	3	135 / 987	4.9 (0.0 – 25.2)	1	0 / 5	0.0 (0.0 –
	China	3	769 / 3171	24.7 (14.5 –	1	77 / 911	8.5 (6.7 –
	Japan	1	33 / 267	12.4 (8.7 –	-	-	-
	Taiwan	-	-	-	3	229 / 1258	19.0 (7.1
Europe and Central Asia		41	5757 / 47,107	24.8 (20.2 – 29.6)	22	518 / 5221	17.6 (10.6 – 25.7)

Belgium	1	15 / 95	15.8 (9.1 –	1	1 / 9	11.1 (0.0
Bosnia-Herzegovia	1	77 / 178	43.3 (36.0 –	1	11 / 12	91.7 (67.6
Croatia	2	47 / 353	14.1 (8.2 –	2	13 / 236	6.1 (1.1 –
France	1	116 / 430	27.0 (22.9 –	-	-	-
Germany	3	334 / 1833	53.3 (12.1 –	-	-	-
Greece	1	106 / 464	22.8 (19.1 –	-	-	-
Hungary	2	157 / 305	60.1 (31.6 –	2	17 / 81	35.3 (0.2
Italy	6	664 / 13,178	8.4 (3.7 – 14.7)	5	116 / 1874	11.2 (0.2
Poland	-	-	-	1	2 / 918	0.2 (0.0 –
Portugal	2	38 / 88	43.2 (32.8 –	2	41 / 110	36.6 (24.6
Romania	1	40 / 46	87.0 (75.5 –	1	0 / 10	0.0 (0.0 –
Serbia	1	33 / 552	6.0 (4.1 – 8.1)	-	-	-
Slovakia	4	2614 /	18.5 (7.7 –	2	109 / 1261	14.4 (2.1
Spain	4	950 / 4280	36.4 (8.7 –	2	198 / 673	29.4 (23.0
Switzerland	3	179 / 884	26.2 (15.6 –	1	9 / 15	60.0 (33.8
Turkey	5	106 / 746	12.8 (8.8 –	2	1 / 22	2.8 (0.0 –
United Kingdom	4	281 / 1662	21.1 (2.1 –	-	-	-
Middle East and North Africa	32	2288 / 14.112	18.2 (14.5 – 22.2)	19	639 / 4564	14.3 (9.6 – 19.7)
Algeria	4	220 / 934	25.7 (15.2 –	-	-	-
Egypt	2	117 / 836	13.9 (11.7 –	1	17 / 123	13.8 (8.2
Iran	10	453 / 1230	15.2 (9.4 –	9	317 / 1901	13.8 (7.6
Iraq	2	59 / 210	16.0 (0.0 –	-	-	-
Jordan	3	480 / 2062	44.5 (14.9 –	2	98 / 747	32.8 (0.0
Morocco	1	55 / 202	27.2 (21.3 –	1	16 / 106	15.1 (8.8
Palestine	1	385 / 2608	14.8 (13.4 –	-	-	-
Saudi Arabia	3	222 / 2771	5.4 (0.6 – 14.6)	3	173 / 1500	10.3 (0.2
Syria	2	101 / 978	10.3 (8.5 –	1	9 / 142	6.3 (2.8 –
Tunisia	4	196 / 1244	25.5 (6.1 –	2	9 / 45	35.8 (0.0

Supplementary Figure S2 shows that the heterogeneity parameter (Tau^2) ranged from 0.03 to 0.3, indicating no substantial heterogeneity across WHO region sub-groups in the meta-analysis. However, the heterogeneity measures I^2 (range, 94.5 to 98.9%) and H^2 (range, 18.4 to 99.2) indicated a high proportion of variation due to heterogeneity. In all cases, there was a significant overall effect size for each region (range z = 3.3 to 17.3, p = 0.00). The model indicated a significant test of group differences, revealing heterogeneity between WHO regions (Q_b = 12.5, d.f. = 6; p = 0.05). This suggests that the prevalence of *C. abortus* varies significantly between regions.

The prevalence of chlamydiosis in sheep not only varied regionally but also showed high heterogeneity at the national level. According to Table 1 and Supplementary Figure S3, the 109 studies that reported results from sheep were conducted in 41 countries. Among these countries, Romania (87.0%, 75.5 – 95.4), Hungary (60.1%, 31.6 – 85.3), and Germany (53.3%, 12.1 – 91.9) had the highest prevalence estimates of *C. abortus* in sheep. It is important to note that these estimates are subject to regional and national variations. Costa Rica, Australia, and Zimbabwe, from different regions, exhibited the lowest prevalence of chlamydiosis (range, 4.7% to 5.2%). Six other countries located in Europe and Central Asia region (Bosnia and Herzegovina, Portugal, Spain, France, Switzerland, and Greece) showed above-average estimations (global prevalence, 20.1%) that ranged from 22.8% to 43.2%. However, within the same region, another six countries showed estimates (range, 6.0% to 18.5%) below the global average. Similarly, among the 10 countries mapped from the Middle East and North Africa region, Jordan, Tunisia, and Algeria had above-average estimates (ranging from 25.5% to 44.5%), while the remaining six countries had below-average chlamydiosis prevalence. Pooled estimates from Canada and the United States of America in the North America region were consistently below the global average, as were Brazil and Grenada from the same continent. To aid in visualizing these geographical differences, we present a bubble heat map of the national pooled estimates of chlamydiosis in sheep in Figure 4A.

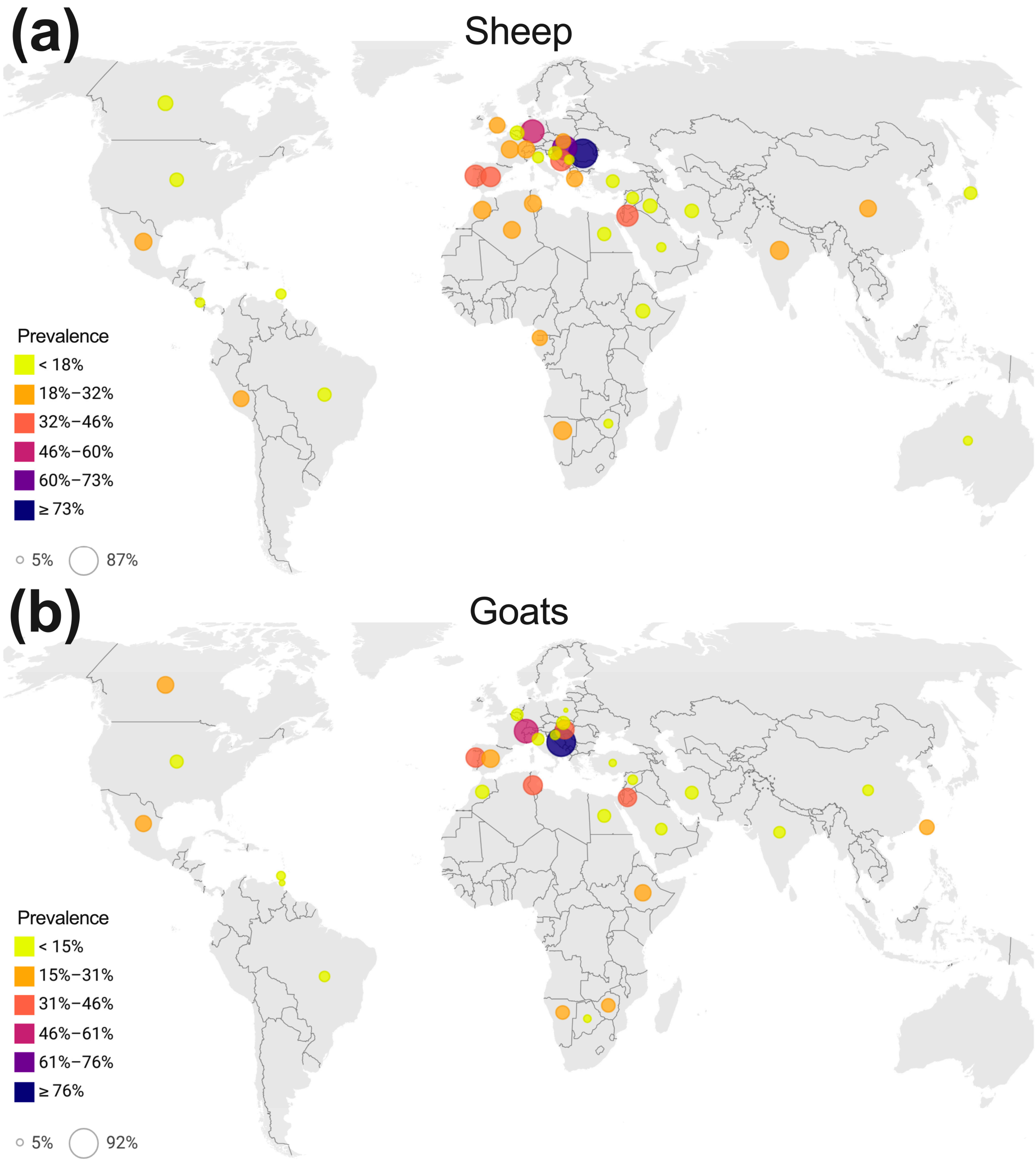

Figure 4. Bubble heatmap of the national prevalence of *C. abortus* estimated using random-effects meta-analyses of (a) 109 individual studies in sheep involving 81,788 samples from 41 countries and (b) 74 individual studies in goats involving 22,696 samples from 33 countries.

3.5.1. Additional Analysis of the Prevalence Estimations of *C. abortus* in Sheep

Based on the Galbraith plot in Supplementary Figure S4A, there was significant variability among the 109 individual studies reporting the prevalence of chlamydiosis in sheep. The majority of data points were dispersed above and below the global prevalence estimation (regression line) and outside the 95% CI region of the line, indicating substantial heterogeneity. Furthermore, the visual examination of the funnel plot presented in Supplementary Figure S4B suggests the presence of asymmetry in the funnel, which may be due to the lack of small-sample studies reporting a prevalence of *C. abortus* close to zero. Accordingly, we conducted Egger's regression to formally test for the presence of small-study effects and found a significant effect (H_0: ß1 = 0; no small-study effects,

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$ß1 = 3.55$, $t = 6.31$, Prob > $|t| = 0.000$) that confirmed that among the 109 studies included, those with small sample sizes reported a higher prevalence than larger studies.

As shown in Supplementary Figure S5, the univariate random-effects meta-regression models were significant ($p < 0.05$) in explaining the between-study heterogeneity. The coefficients for the covariates ranged from -0.119 to 0.165, indicating a contrasting effect on the prevalence. Specifically, the number of positive samples of *C. abortus* in sheep tended to decrease as the publication year and sample size increased (panels 5A and 5D). In contrast, studies conducted at higher latitudes and utilizing more diagnostic techniques tended to report higher prevalence estimates of chlamydiosis (panels 5B and 5C).

The cumulative meta-analysis presented in Supplementary Figure S6 reveals a wide variation in chlamydiosis prevalence estimates in sheep across studies published between 1989 and 2007. However, from 2008 to 2016, the prevalence estimates were less variable before stabilizing in 2017 with a prevalence range of 20-22% and narrowed 95% CIs, indicating higher precision in the estimates. Additionally, Supplementary Figure S7 displays the forest plot from the leave-one-out meta-analysis. The results indicate that the overall effect sizes, when excluding one study at a time, were similar to the pooled global prevalence (represented by the vertical red line based on all studies). The 95% confidence intervals also intersected this global estimate, suggesting the absence of studies that significantly influence the overall estimate.

3.6. Individual Characteristics of the Studies That Evaluated Goats

The characteristics of the 74 studies reporting the prevalence of chlamydiosis in goats are summarized in Supplementary Table S5. Similar to sheep, there is limited information on the population characteristics of the evaluated goats, with only 40, 14, and 12 studies including information on sex, breed, or age, respectively. Of the studies that reported on sex, 26 focused on females, 13 included both sexes, and one study focused on males. Out of the 74 studies analyzed, 20 reported results on aborted females, placenta, or aborted fetuses, 17 presented healthy animals or compared healthy females with those with a history of abortion, 15 included goats with reproductive failure or a history of abortion, and one study presented results on goats with eye problems. The remaining 21 studies did not specify the clinical characteristics of the animals evaluated. According to Supplementary Dataset 2, 51 out of 74 studies reported using a single diagnostic technique to detect chlamydiosis in goats. The remaining studies declared using 2, 3, or 4 techniques. ELISA was the most frequently used technique for diagnosing chlamydiosis in goats, reported in 37 studies. PCR was the second most commonly used technique, reported in 34 studies for molecular detection. Immunological techniques were used in 16 reports, while culture for bacteriological isolation of the pathogen was used in 12 studies. Immunohistochemistry or immunofluorescence was used to detect *C. abortus* in four studies. Some studies reported up to four diagnose techniques; therefore, the sum of the studies by technique is expected to overpass the original 74 studies included for goats.

Out of the 74 studies, 43 evaluated the presence of Chlamydia in serum. Following this, 17 studies reported the prevalence of the pathogen in fetal tissue, placenta, and vaginal swabs of aborted females. A total of 11 studies included samples of reproductive tissue or vaginal swabs. Only one study included eye swabs, while another analyzed semen. Additionally, one study reported the prevalence of *C. abortus* in milk.

3.7. Global, Regional, and National Prevalence of C. abortus *in Goats*

A total of 2,729 samples tested positive for *C. abortus* out of the 22,696 samples tested in 74 goat studies (Table 1). The global prevalence of chlamydiosis in goats was estimated to be 14.4% (95% CI: 11.6 - 17.4), with significant heterogeneity between studies ($Q = X^2 = 1404.4$, d.f. = 73, $p = 0.00$) and an overall significant effect size (z =16.0, $p = 0.00$; Supplementary Figure S8). As indicated by a value of $I^2 = 96.6\%$ and $H^2 = 29.7$, both measures of heterogeneity suggest the presence of a significant amount of variation attributable to heterogeneity across the studies.

In contrast to sheep, regional estimates for goats were less variable between regions and the global estimate (14.4%). Studies conducted in Sub-Saharan Africa, Europe, and Central Asia, and

North America reported the highest prevalence rates (15.4%, 17.6%, and 19.7%, respectively). In contrast, studies conducted in the South Asia region reported a lower prevalence rate (10.4%, 0.09 – 26.6). The other three regions had prevalence rates similar to the global estimate, ranging from 12.3% to 14.3% (Table 1). Regionally, the studies from Sub-Saharan Africa, Europe, and Central Asia, and the Middle East and North Africa provided 15,126 samples, accounting for 66.6% of the total number of goat samples. Across the WHO region, Tau^2 values ranged from 0.04 to 0.2, indicating no significant heterogeneity among these sub-groups (Supplementary Figure S9). However, I^2 values (ranging from 89.7% to 97.4%) and *H*2 values (ranging from 9.7 to 76.9) indicated moderate to high variation due to heterogeneity between studies. Furthermore, there was a significant overall effect size for each WHO region (with a range of z = 2.5 to 9.1, $p = 0.00$). The model did not reveal any significant differences between the prevalence of chlamydiosis in goats across WHO regions (Q_b = 3.0, d.f. = 6; $p = 0.81$). This indicates that the prevalence of chlamydiosis in goats was similar across all regions.

Table 1 and Supplementary Figure S10 summarize the national estimates of *C. abortus* prevalence in goats. The prevalence varied widely, ranging from 0.0% in Romania and Australia to 91.7% in Bosnia and Herzegovina. The 74 goat studies we mapped included results from 33 countries. Four out of the top six countries with the highest prevalence were from the Europe and Central Asia region, namely Bosnia and Herzegovina, Switzerland, Portugal, and Hungary. Tunisia and Jordan ranked fourth and sixth, respectively, with a prevalence of 35.8% and 32.8% outside of these countries in the Middle East and North Africa. In the region of Europe and Central Asia, Croatia, Turkey, Poland, and Romania had a low prevalence of the bacterium in goats, ranging from 0.0% to 6.1%, which was comparable to estimates from Australia (0.0%), Trinidad and Tobago (1.1%), Botswana (3.2%), and Grenada (4.8%). Egypt, Iran, and Morocco from the Middle East and North Africa region, as well as Namibia and Zimbabwe from Sub-Saharan Africa, and the United States of America from North America, had estimates ranging from 13.7% to 15.1%, which falls within the global range of 14.1% (11.6 to 17.4). Mexico, Ethiopia, Canada, and Spain had above-average estimates, with prevalence values ranging from 24.1% to 29.4%. To aid in visual interpretation of the geographical disparities, we present a bubble heat map in Figure 4B that shows the national pooled estimates of chlamydiosis in goats.

3.7.1. Additional Analysis of the Prevalence Estimations of *C. abortus* in Goats

The Galbraith plot in Supplementary Figure S11A confirms substantial heterogeneity across the 74 studies included in the meta-analysis to estimate the global prevalence of chlamydiosis in goats. Although some studies fall within the 95% CI of the regression line representing the overall global estimation, there is a broad distribution of data points above or below the interval throughout the red line. Furthermore, similar to sheep, we observed an asymmetry in the distribution of individual effect size estimates in the lower left section of the funnel plot illustrated in Supplementary Figure S11B. This outcome could be attributed to the absence of small-sample studies with low values of chlamydiosis prevalence in goats. Furthermore, Egger's regression test confirmed the presence of small-study effects, as indicated by the significant coefficient value (H_0: ß1 = 0; no small-study effects, ß1 = 1.5, $t = 3.6$, Prob > $|t|$ = 0.00). This suggests that studies with smaller sample sizes reported a higher prevalence than larger studies.

The summary of results from the univariate random-effects meta-regression models presented in Supplementary Figure S12 showed that only the latitude where a study was conducted, the number of techniques used to diagnose *C. abortus* in goats, and the quintile of the sample size assessed were significant ($p < 0.00$) as study-level covariates to explain the heterogeneity found across the estimates. The coefficients for latitude and number of techniques were positive (0.004 and 0.183, respectively), indicating that studies conducted at higher latitudes and with larger sample sizes tend to report higher prevalence values (panel 12B and 12C). In contrast, the quintile of the sample size had a negative coefficient of -0.119, indicating that as the quintile of the sample size increased, the prevalence of chlamydiosis in goats tended to decrease (panel 12D).

Based on the cumulative meta-analysis presented in Supplementary Figure S13, the prevalence estimates of chlamydiosis in goats exhibited significant variability from 1989 to 2005 before stabilizing at 14-15% between 2006 and 2017. The current prevalence rate is 14%, with narrowed 95% confidence intervals. The leave-one-out meta-analysis ultimately showed no studies with potential influence on the overall estimate (vertical red line based on all the studies), as determined by the distribution around the vertical red line of the overall effect sizes excluding one study at a time.

4. Discussion

Abortions in small ruminants caused by C. abortus are a significant problem in livestock production systems due to the resulting productive losses. Chlamydiosis not only causes abortion but also leads to various pathologies with clinical, productive, and reproductive implications in infected animals [26]. Therefore, it poses a threat to the livestock industry. This systematic review and meta-analysis found a higher prevalence of chlamydiosis in sheep (20.1%) compared to goats (14.4%) across 109 and 74 studies, respectively. Significant heterogeneity was observed among WHO regions in the prevalence of *C. abortus* only in sheep. Studies from South Asia (30.6%) and Europe and Central Asia (24.8%) reported higher prevalence rates of chlamydiosis. In contrast, the presence of *C. abortus* in sheep was estimated to be lowest in Latin America and the Caribbean, as well as in East Asia and the Pacific regions (15.5% and 14.0%, respectively). Regarding goats, meta-analysis revealed that although there was slight regional variation, no substantial heterogeneity was found across regions. However, chlamydiosis was more prevalent in goats from Sub-Saharan Africa and Europe and Central Asia (15.4% and 17.6%, respectively) compared to South Asia (10.4%). The results confirm the high prevalence of this pathogen in small ruminants from Asia and Europe [2,27]. Differences in epidemiology between species across regions were also demonstrated.

The estimates were derived from 133 publications across 45 different continents, including a large sample size for sheep (81,788) and goats (22,696). This systematic review and meta-analysis confirms the worldwide distribution of *C. abortus* [28] and highlights significant national differences. Chlamydiosis was found to be highly prevalent in sheep from European countries such as Romania, Hungary, and Germany, with prevalence ranging from 53.3% to 87.0%. In contrast, low prevalence values were found in Zimbabwe and Australia (4.7% and 4.9%). Notably, the prevalence of chlamydiosis in goats showed a distinct pattern. Romania and Australia had zero prevalence, while Bosnia and Herzegovina had the highest estimation, with a 91.7% prevalence of chlamydiosis. Both species have a high prevalence of the pathogen in countries from Europe and Central Asia. In Northern Europe, this pathogen is the primary cause of infectious abortions. In the United Kingdom and Spain, 44.0-56.0% of small ruminant abortions were caused by this disease [29]. Similarly, countries from the Middle East and North Africa, such as Jordan, Tunisia, Algeria, and Egypt, have a high incidence of *C. abortus* in their flocks of sheep and goats. Chlamydiosis infection is a significant cause of abortion in sheep and goats in Jordan and Saudi Arabia [29]. Serological reports from Egypt indicate that up to 68.0% of aborted ewes tested positive for the pathogen [30].

Disparate estimates were observed among countries in the Americas. Sheep prevalence was below average in Costa Rica, Canada, the United States of America, Brazil, and Grenada, while Mexico and Peru showed the highest prevalence in the region. On the other hand, Canada, the United States of America, and Mexico had the highest prevalence of chlamydiosis in goats, while South American countries had below-average estimates. A recent review from South America reported the presence of *C. abortus* in sheep and goats in Argentina, Chile, Brazil, and Peru. The authors presented a broad variation in the prevalence of the bacterium across countries [31]. However, we were unable to include the other countries due to the lack of peer-reviewed scientific articles and because gray literature was not included in our systematic review and meta-analysis.

The heterogeneity among countries is consistent with previous reports that have shown variation in the global seroprevalence of chlamydiosis [5]. Our study also supports the suggestion that the infection is not endemic or is relatively low in Oceania [8]. Turin, *et al.* [32] presented a visual summary of global seroprevalence rates in different animal species, including sheep and goats. However, the authors provide a summary of values for each species and country without utilizing statistical techniques, such as those used here, to estimate and validate the prevalence of *C. abortus* in small ruminants. To complement the significant effort made by these authors in Figure 5, we present a bubble map of the merged prevalence estimates for both species. This geographical representation facilitates comprehension of the distribution pattern of *C. abortus* in these two productive species and enables a direct comparison of its prevalence both between and within countries. However, Turin, Surini, Wheelhouse and Rocchi [32] suggest that this distribution may underestimate the true prevalence of *C. abortus* due to the limited global data available and the variable specificity of available tests for detecting this pathogen.

Meta-regression analysis was conducted to evaluate potential causes of heterogeneity. The study found that sample size, latitude of the study location, and the number of diagnostic techniques were significant study-level covariates in both species. However, the constructed models' determination coefficients explained little to none of the heterogeneity observed between studies ($R^2 = 0.0\%$ to 7.9%). Therefore, the heterogeneity found may be associated with differences in the production systems included in the studies, individual animal characteristics, reproductive management, chlamydiosis control and prevention strategies, and study design. Further studies are necessary to investigate the specific causes of such discrepancies. The observed heterogeneity in prevalence has direct implications for the design of strategies aimed at controlling and eradicating the infection, as well as for the epidemiological surveillance of chlamydiosis.

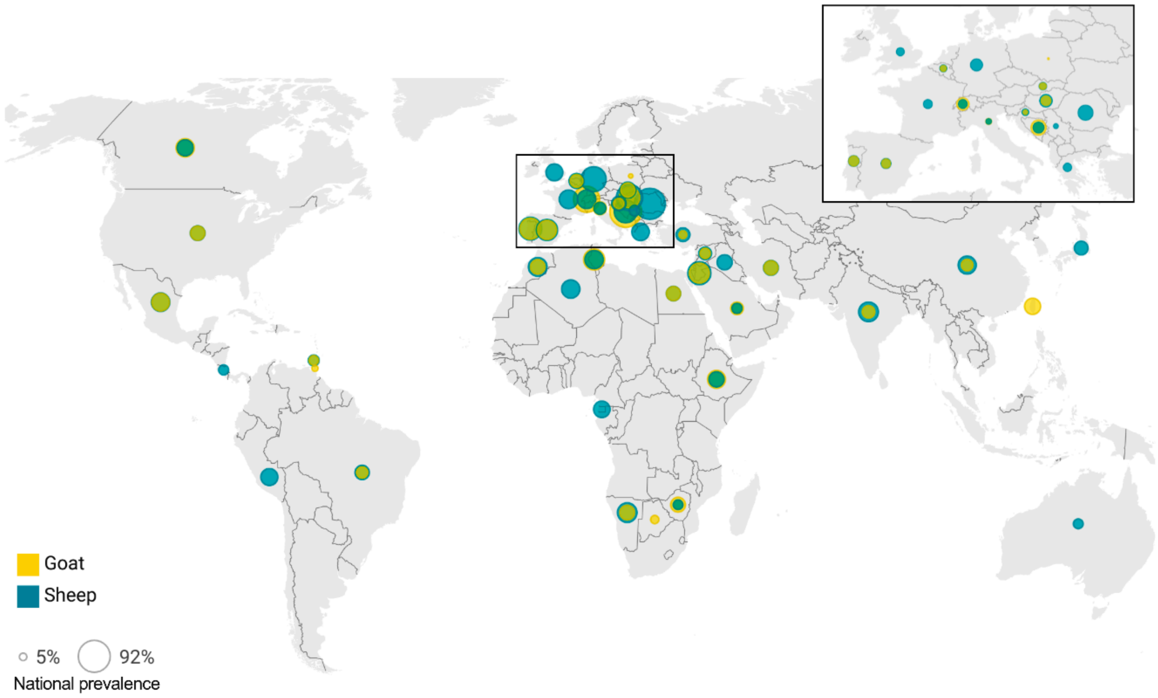

Figure 5. Merge bubble map of the national prevalence of *C. abortus* in sheep and goats estimated with random-effects meta-analysis of 109 individual studies in sheep involving 81,788 samples from 41 countries and 74 individual studies in goats involving 22,696 samples from 33 countries.

This is the first systematic review and meta-analysis to present global, regional, and national estimates of chlamydiosis prevalence in sheep and goats. No secondary systematic study has been published to produce such estimates, which are necessary to understand the magnitude of the infection and the distribution of this pathogen in small ruminants worldwide. Our study systematically searched and collected the most relevant and recent literature on the topic. We used meta-analysis and secondary analysis techniques to provide a reliable summary of all available evidence on the subject. Our estimates can guide evidence-based decisions by identifying countries and regions where chlamydiosis is more prevalent. In addition, our estimations can serve as a reference point for future updates and comparisons of changes in chlamydiosis prevalence resulting from control and prevention programs aimed at reducing the burden of this pathogenic bacterium.

The quality of evidence in our study was rated as moderate using the GRADE system [33]. This rating was mainly due to the inclusion of only observational studies, a high risk of bias in three of the four defined criteria, inconsistencies in the studies, and publication bias and small-study effects. However, due to the inclusion of a large number of studies and the use of secondary analysis to evaluate various aspects during the meta-analysis, our summary of evidence and the estimates presented are reliable enough to be considered by policymakers and stakeholders regarding the prevalence of chlamydiosis in sheep and goats.

Chlamydiosis-induced abortion typically occurs during late gestation and is often linked to a pre-existing persistent infection. Identifying persistently infected females using traditional diagnostic methods before abortion is challenging due to the unknown persistence of the bacteria [34]. Chlamydia-positive sheep or goats may be introduced into a healthy flock without detection, making it difficult to implement effective control measures before the reproductive season [35]. Despite the well-known pathogenic role of Chlamydia species and their ability to cause infections in various animal species, routine testing for these microorganisms is not conducted [3]. Therefore, it is vital to implement a rapid and effective diagnostic method and ensure correct collection of clinical information. This requires cooperation between the farmer and the veterinarian [36]. However, serological diagnosis of *C. abortus* can be challenging in some cases, mainly due to low specificity or cross-reactivity with other species of Chlamydia [37]. To gain more knowledge about the epidemiology of *C. abortus* and implement adequate control strategies, specific diagnostic tests are necessary [38].

Current evidence suggests that vaccines with live attenuated microorganisms against Chlamydia have low efficacy and have been associated with Chlamydia outbreaks in vaccinated flocks [32,39]. Therefore, there is an urgent need to produce a safer, stable, and more effective vaccine against this pathogenic bacterium to significantly reduce the number of infectious animals at parturition [40]. Furthermore, the prevention of chlamydiosis can be hindered by the extended use of antibiotics, such as tetracyclines, which are commonly used for both prophylaxis and treatment. This is due to the potential for the development of antibiotic-resistant microorganisms [41]. For around 60 years, the treatment of certain Chlamydia species has centered on tetracycline-supplemented feed [42]. It is commonly recommended to use a single dose to avoid resistance. However, routine use of these antibiotics can only suppress Chlamydia excretion, and there is no guarantee of pathogen eradication after treatment [3,8].

To control or prevent chlamydiosis, farm management strategies may include maintaining a closed flock, implementing biosecurity measures, quarantine, vaccination, and preventive antibiotic treatment. However, long-term antibiotic treatment should be avoided due to the risk of resistance generation [43]. Additional measures may involve the proper disposal of abortion products and all reproductive material from infected or suspected cases. This is because they are a source of high bacterial loads, which can ultimately pose a risk to humans and naive animals [8]. As concluded by Robertson, Handel and Sargison [11], the economic losses due to chlamydial abortion outweigh the cost of routine preventive management. Therefore, they recommend investing in vaccination, biosecurity, and acquiring animals and replacements only from disease-free sources.

Due to the diversity of ecosystems and the heterogeneity of territories where small ruminants are produced, there are different productive systems, such as the extensive or free-grazing production system. This system results in lower costs for producers [44]. However, during grazing, animals infected with Chlamydia can disseminate the bacteria into the waste produced, such as the fetus in the case of abortion and vaginal discharges, contaminating the pasture that healthy animals could consume [7,45]. Distinct types of flock management may influence the magnitude of environmental contamination and the spread of the pathogen. In intensive systems, where more animals are kept in smaller enclosures, chlamydiosis prevalence is higher due to a more concentrated contamination of the space [32]. It is important to note that *C. abortus* can survive in the environment for days to months, even in unfavorable conditions, mainly due to a spore-like cell wall that confers resilience [46]. The bacterium's resistance increases the likelihood of infecting multiple animal species, both farmed and wild, and subsequently spreading the infection to humans. Research indicates that contact with wild animals is a risk factor for chlamydial infection in both domestic animals and humans [47]. The informal trade of animals among producers is another significant factor in the spread of the disease. Infected animals can contaminate healthy ones during transportation, highlighting the insufficiency of quarantine measures when receiving new animals [44].

After animals are infected, treatment is important, but it does not guarantee complete elimination of the pathogen in the host [3,8]. Therefore, prevention of infection should be the primary focus. It is also important to raise awareness among producers and authorities about the impact of this pathogen on animal and public health, and to join efforts to prevent its spread and infection worldwide. Failure to implement proper sanitary measures can lead to the spread of bacteria to healthy animals in contact with asymptomatic animals that continue to expel and disseminate the bacteria in the corral. Therefore, timely diagnosis is crucial in separating positive animals from negative ones. Additionally, it is important to take care of personnel who may be exposed, such as veterinarians, producers, livestock workers (including children living on the ranch), and laboratorians. This is because they often do not use protective equipment or sanitary measures when handling infected animals. While *C. abortus* is not highly contagious in humans, the infection can have disastrous consequences for pregnant women who come into close contact with infected sheep and goats [7].

Zoonotic infection with *C. abortus* is considered an occupational hazard. Therefore, extreme safety and protection of veterinarians and field personnel involved in farrowing activities and in the handling of abortion product materials is necessary due to the global presence of this bacterium. Zoonotic diseases, such as toxoplasmosis or chlamydiosis, may be infrequent in the population or underdiagnosed and underreported, making their frequency unknown [48]. Evidence suggests a variable prevalence of antibodies against *C. abortus* in humans [49]. Furthermore, *C. abortus* infection in pregnant women is a serious concern, requiring prompt treatment to prevent miscarriage and maternal death [6,7].

5. Limitations

The investigation revealed some limitations: 1) The meta-analysis included studies from 45 countries, but it may not provide a complete picture of infection globally. 2) A limited number of publications were contributed by some countries, which could lead to an over- or underestimation of prevalence at the national level. #) There was a significant disparity in the sample size evaluated between regions and countries, which affects the error estimate of prevalence. 4) Most of the studies do not report the characteristics of the sheep and goat population they evaluated, which could limit the extrapolation of results to other flocks with similar characteristics.

6. Conclusions

Our systematic review and meta-analysis revealed that the prevalence of *C. abortus* was higher in sheep than in goats. We found reports of the pathogen in 45 countries across five continents and seven WHO regions, confirming its global distribution. Prevalence estimates for sheep varied significantly between regions, while values for goats were similar. National estimates indicate a broad variation in the prevalence of chlamydiosis among productive species across different countries. The data confirms that Europe, Asia, and Africa have a higher prevalence of the pathogen bacterium, while Australia has a low prevalence of *C. abortus* in sheep. In some countries, the prevalence of chlamydiosis was consistently above or below the global average for both sheep and goats. In contrast, some other countries showed the highest prevalence in sheep and the lowest values for goats. Therefore, these results suggest a complex epidemiological pattern that varies broadly both between countries and species. Our meta-analysis revealed small-study effects due to the lack of small studies reporting low prevalence values of chlamydiosis. Although significant covariates were found (sample size, latitude, and number of diagnostic techniques), the amount of explained heterogeneity was relatively low. Therefore, further studies are needed to explore the potential causes of the substantial between-study variability. Finally, it was observed that the prevalence of *C. abortus* varied with time in both species, particularly from 1989-2017, becoming more stable and lower in recent years. The meta-analysis shows a high prevalence of *C. abortus* in small ruminants. Therefore, it is necessary to maintain epidemiological surveillance of this pathogen not only in the countries included in the study but also in other nations that lack current data.

6. Future Directions

It is necessary to design and develop regional and national surveillance programs for the presence of Chlamydia in sheep and goats, both at the flock and individual levels. This is important not only for these animals but also for wildlife and other species, as the pathogen is not host-specific. Furthermore, additional studies are needed to assess the efficacy and safety of vaccines. Based on these results, vaccination campaigns should be implemented to prevent infection. A fundamental task is related to the pharmacological treatment of the disease with antibiotics. It is urgent to generate evidence regarding their efficacy and mid- and long-term effects on the generation of Chlamydia strains with antibiotic resistance. Additionally, it is necessary to assess the potential health risk to both humans and animals linked to the potential dispersion of such strains. This information will help determine which pharmacological treatments are effective and recommended for controlling and eradicating the pathogen from animals and productive systems. Additionally, it is important to conduct studies on the presence and spread of Chlamydia in the environment, particularly to understand how long the bacteria can survive and under what conditions after being expelled from infected animals. This information will be highly valuable in understanding the capacity for reinfection or infection of healthy animals, as well as for establishing and increasing anti-chlamydia protocols in productive systems and installations.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Supplementary Table S1. Search methods: detailed search strategy. Supplementary Table S2. STATA 18 commands used to produce the meta-analyses and all secondary analyses and graphs. Supplementary Table S3. Excluded trials found through database searching and the primary reason for exclusion. Supplementary Table S4. List of the 133 studies included in the systematic review and meta-analysis. Supplementary Table S5. Main characteristics of the 133 studies included in the systematic review that included the summary for both species. Supplementary Figure S1. Forest plot of the meta-analysis to estimate the prevalence of *C. abortus* in sheep at the global level. Supplementary Figure S2. Forest plot of the meta-analysis to estimate the prevalence of *C. abortus* in sheep at the WHO region level. Supplementary Figure S3. Forest plot of the meta-analysis to estimate the prevalence of *C. abortus* in sheep at the national level. Supplementary Figure S4. A) Galbraith plot and B) funnel plot for 109 that reported the prevalence of *C. abortus* in sheep. Supplementary Figure S5. Univariate meta-regression analysis for quantitative moderators for sheep. Supplementary Figure S6. Cumulative meta-analysis for sheep by year. Supplementary Figure S7. Leave-one-out meta-analysis for sheep. Supplementary Figure S8. Forest plot of the meta-analysis to estimate the prevalence of *C. abortus* in goats at the global level. Supplementary Figure S9. Forest plot of the meta-analysis to estimate the prevalence of *C. abortus* in goats at the WHO region level. Supplementary Figure S10. Forest plot of the meta-analysis to estimate the prevalence of *C. abortus* in goats at the national level. Supplementary Figure S11. A) Galbraith plot and B) funnel plot of 74 studies that reported the prevalence of *C. abortus* in goats. Supplementary Figure S12. Univariate meta-regression analysis for quantitative moderators for goats. Supplementary Figure S13. Cumulative meta-analysis for goats by year. Supplementary Figure S14. Leave-one-out meta-analysis for goats.

Author Contributions: We used the CRediT taxonomy to define coauthorship. Conceptualization, supervision, and project administration, R.C.-F., S.M.G., and D.D.; methodology, data curation, and visualization R.C.-F. and D.D.; software and formal analysis, P.E.H-C. and D.D.; investigation, R.C.-F., E.D.-A., M.A.R.-G., A.M.-P., I.E.-V., N.C.-C.; writing—original draft preparation, R.F.-C. and D.D.; writing—review and editing, M.B., P.E.H.-C., D.Z.V., C.L., G.A.-B., E.J.D.-S., and A.R.-V. All authors have read and agreed to the published version of the manuscript.

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