2021, Scienceline Publication
World's Veterinary Journal
World Vet J, 11(1): 01-15, March 25, 2021
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2021.wvj1

Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies

D. Katterine Bonilla-Aldana,[1,2,3,4] Keidenis Quintero-Rada[3], Juan Pablo Montoya-Posada[3], Diego Soler-Tovar[4,5], Paola Barato[4,6], Kovy Arteaga-Livias[7,8], Lysien I. Zambrano[4,9], Álvaro A. Faccini-Martínez[2,4,10], and Alfonso J. Rodriguez-Morales[2,4,7,11]*

[1]*Semillero de Investigación en Zoonosis (SIZOO), Grupo de Investigación BIOECOS, Fundación Universitaria Autónoma de las Américas, Pereira, Risaralda, Colombia*

[2]*Committee on Tropical Medicine, Zoonoses and Travel Medicine, Asociacion Colombiana de Infectologia, Colombia*

[3]*Faculty of Veterinary Medicine and Zootechnics, Fundación Universitaria Autónoma de las Américas, Pereira, Risaralda, Colombia*

[4]*Red Colombiana de Enfermedades Transmitidas por Garrapatas en Pequeños Animales (RECEPA) – Colombian Network of Tick-Borne Diseases in Small Animals (RECEPA), Pereira, Risaralda, Colombia*

[5]*Epidemiology and Public Health Group, School of Agricultural Sciences, Universidad de La Salle, Bogota, DC, Colombia*

[6]*Corporación Patología Veterinaria (Corpavet), MolecularVet SAS, Bogotá, Colombia*

[7]*Master in Clinical Epidemiology and Biostatistics, Universidad Cientifica del Sur, Lima, Peru*

[8]*Faculty of Medicine, Universidad Nacional Hermilio Valdizán, Huánuco, Peru*

[9]*Departments of Physiological and Morphological Sciences, School of Medical, Sciences, Universidad Nacional Autónoma de Honduras (UNAH), Tegucigalpa, Honduras*

[10]*Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA*

[11]*Grupo de Investigación Biomedicina, Faculty of Medicine, Fundación Universitaria Autónoma de las Américas, Pereira, Risaralda, Colombia*

*Corresponding authors' Email: arodriguezm@utp.edu.co; ORCID: 0000-0001-9773-2192

SYSTEMATIC REVIEW
pii: S232245682100001-11
Received: 17 Dec 2021
Accepted: 02 Feb 2021

ABSTRACT

While some *Ehrlichia* species, such as *E. ruminantium* and *E. minasensis*, are not popular even among veterinarians, they can infect cattle. The current study aimed to review studies on *Ehrlichia* spp. to evaluate its worldwide molecular prevalence, given the lack of information about bovine ehrlichiosis and the lack of previous systematic reviews and meta-analyses on this subject. In order to determine the molecular prevalence of *Ehrlichia* spp. in cattle, a systematic review of the literature was conducted in three databases. A meta-analysis with a random-effects model was performed to calculate the pooled prevalence with 95% confidence intervals (95% CI) and measures of heterogeneity were reported. Subgroup analyses were performed in terms of *Ehrlichia* species, country, and regions. The literature search yielded 1051 papers until August 1, 2019, with 71 studies entirely eligible for review. The pooled molecular prevalence for *Ehrlichia* at the individual level (N = 6232) was 2.3% (95% CI: 1.7-2.9%) with the highest value of 82.4%. Studies identified the highest pooled molecular prevalence of 6.6% (95% CI: 0.6-12.7%) for *E. canis*, followed by *E. ruminantium* (n = 4695, 75.33%) 52 studies, with 1.7% (95% CI: 1.1-2.3%) and *E. chaffeensis* with 1.5% (95% CI: 0.0-0.3%). Moreover, the obtained result was indicative of only one study addressing *E. minasensis*. As the findings suggested, heartwater (*E. ruminantium* infection) is a notifiable disease of domestic and wild ruminants, recorded by the World Organization for Animal Health. There is a possible risk of endemic heartwater in the Americas due to the climatic features. Furthermore, *E. minasensis*, *E. chaffeensis*, and *E. canis* were observed in cattle although the two last species could be a molecular misidentification with regard to their phylogenetic relationships with *E. minasensis*.

Keywords: Bacteria, Bovine, *Ehrlichia*, Systematic review, Tick-borne

INTRODUCTION

Ehrlichia species, belonging to the family Anaplasmataceae, can infect cattle (Anifowose et al., 2020; Fargnoli et al., 2020) although some of which, including *E. ruminantium* and *E. minasensis*, are not well-known species even among veterinarians (Hector et al., 2019). Bovine ehrlichiosis is manifested by fever without a pattern, ears drooping, turning, and lymphadenitis. In some studies, high mortality has been reported within a few hours in the peri-acute form of the disease, within 36-48 hours in the acute form of the disease, which is usually associated with subclinical infection in the occasional report of severe forms (Stewart, 1992).

Given the lack of previous systematic reviews and meta-analysis about bovine ehrlichiosis, the current study aimed to collect studies addressing *Ehrlichia* spp. to assess its molecular prevalence worldwide with regard to the available public health reports and observational studies. Moreover, the present review was set to address the prevalence of ehrlichiosis in terms of species, countries, and continents.

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

MATERIAL AND METHODS

Protocol and registration

The employed protocol followed the recommendations established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

Eligibility criteria

This systematic review was conducted on published peer-reviewed articles that reported *Ehrlichia* species in bovines. Diagnostic methods included only molecular methods because there is a lack of good studies using an appropriate serological test. Serological tests for ehrlichiosis have a limited sensibility and specificity since it is difficult to appropriately discriminate the species involved in the infection. There was no restriction regarding the language of the article and all publications dated from January 1, 1950 to August 1, 2019 were included. Therefore, all reviews, opinion articles, and letters not offering original data as well as studies reporting cases with incomplete information were excluded.

Information sources and search strategy

The relevant articles were searched in three databases, including Medline/PubMed, Scopus, and Web of Science. The search terms included "ehrlichiosis," "*Ehrlichia*", "bovine", "cattle", "*Cowdria*," and "*Anaplasmataceae*", using multiple combinations of the main Boolean operators (AND, OR). The search process ended by August 1, 2019. The obtained results were articles in English, Spanish, and Portuguese. Four different researchers independently evaluated the search results in order to reduce the risk of bias in the interpretations.

Study selection

Initial search strategy results were screened by the title and abstract. The full texts of relevant articles were examined for inclusion and exclusion criteria (Figure 1). When an article reported duplicate information, reports were combined in order to obtain complete data. Observational studies that reported *Ehrlichia* species detection using different diagnostic methods were included for quantitative synthesis (meta-analysis).

Data collection process and data items

Data extraction forms, including information on the type of publication, country, year, date of publication, *Ehrlichia* species detection, and diagnostic method, were filled independently by four researchers. The fifth investigator checked the article list and data extractions to guarantee no duplicate articles or duplicate information and also resolved discrepancies about the included studies.

Assessment of methodological quality and risk of bias

The critical appraisal tool of the Quality Appraisal of Case Series Studies Checklist of the Institute of Health Economics (IHE) was used in the present study to assess the quality of cross-sectional studies (AXIS, IHE, 2014; Downes et al., 2016). Publication bias was assessed using a funnel-plot. A random-effects model was used to calculate the pooled prevalence and 95% CI has shown varying degrees of data heterogeneity and the inherent heterogeneity in any systematic review of studies from the published literature. Egger's test was also performed for publication bias.

Statistical approach

Unit discordance for variables was resolved by converting all units to a standard measurement for each variable. Percentages and means ± standard deviation (SDs) were calculated to describe the distributions of categorical and continuous variables, respectively. The baseline data were analyzed using the Stata version 14.0, licensed for Universidad Tecnológica de Pereira in Colombia. The meta-analyses were performed using Stata, and the software OpenMeta[Analyst] (Wallace et al., 2012), JASP (Version 0.12.2)®, and Comprehensive Meta-Analysis ve.3.3® licensed for Universidad Tecnológica de Pereira. Pooled prevalences and their 95% confidence intervals (95% CIs) were used to summarize the weighted effect size for each study grouping variable using a binary random-effects model (which takes into consideration sample sizes of individual studies) except for median age, where a continuous random-effect model was applied (DerSimonian-Laird procedure, Viechtbauer, 2010; Kontopantelis and Reeves, 2012). Measures of heterogeneity, including Cochran's Q statistic, I^2 index, and tau-squared test, were estimated and reported. Subgroup analyses and meta-analyses were also performed for some variables of interest.

RESULTS

Study selection and characteristics

A total of 1051 articles were retrieved using the defined search strategy. After screening the abstracts and titles, 120 articles were selected for full-text assessment. Of these, 49 were excluded due to the lack of information on laboratory diagnosis, and 71 were finally included for final qualitative synthesis and meta-analysis (Figure 1). Table S1 shows the main characteristics of the included studies. The present review included 71 studies that were published between January 1, 1950 and August 1, 2019, most of which were from China (23.1%), Zambia (19.2%), Namibia (6.4%), Cameroon (5.1%), Tanzania (5.1%), and Benin (5.1%), among others (Table S1), including a total of 6,232 animals assessed by molecular methods. All the studies were cross-sectional ones (Table S1). The meta-analyses

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

included the analysis of 10 variables (Table 1). Publication bias was assessed with a funnel plot for standard error, with no evidence of bias (Figure 2), but the Egger test suggested possible publication bias (z = 4.440; p < 0.001). Kendall's tau test was reported as 0.087 (p = 0.207).

Main findings

The median number of individuals per study was 55, with positive rates ranging from 0.14 to 82.4% (Table 1). The pooled molecular prevalence for *Ehrlichia* was 2.3% (95% CI: 1.7-2.9%, $\tau^2 = 0.001$, $I^2 = 81.944$, Q = 387.685, p < 0.001) with the highest value of 82.4% for China (2016, Table 1, Figure 3). Studies identified the highest pooled molecular prevalence of 6.6% for *E. canis* (95% CI: 0.6-12.7%, $I^2 = 90.74$, Q = 43.208, p<0.001), followed by *E. ruminantium* (n = 4,695 [75.33%] 52 studies) with 1.7% (95% CI: 1.1-2.3%, $I^2 = 77.29$, Q = 224.569; p < 0.001) and *E. chaffeensis* with 1.5% (95% CI: 0.0-0.3%, $I^2 = 60.96$, Q = 12.806, p = 0.025). Regarding *E. minasensis*, only one study was included (3%, Table 1, Figure 4). In China, with 18 included studies, the prevalence was 1.8% (95% CI: 0.7-3.0%, $I^2 = 87.54$, Q = 136.50, p<0.001, Figure 5). In this regard, Asia (18 studies) and Africa (48 studies) contributed the most with the prevalence of 1.8% (95% CI: 1.1-2.4%). The molecular prevalence rate was reported as 13.2% (95% CI: 0.6-27.0%) in 5 studies conducted in Americas (Table 1, Figure 6). According to the diagnostic techniques, the higher prevalence was reached with DNA sequencing by 13.2% (95% CI: 0.0-27.0%, Figure 7) from which 12 studies were conducted on *Bos taurus* (0.6%, 95% CI: 0.1-1.4%) and 4 on *Bos indicus* (11.9%, 95% CI: 2.4-21.3%, Figure 8, Table 1).

Table 1. Meta-analysis outcomes (random-effects model)

Variable	Number of Studies	Pool Prevalence (%)	95% CI*	n	Q†	I^2 ‡	t^2 §	p value
All	71	2.3	1.7-2.9	6,232	387.685	81.944	0.001	<0.001
E. canis	5	6.6	0.6-12.7	299	43.208	90.74	n/c	<0.001
E. ruminantium	52	1.7	1.1-2.3	4,695	224.569	77.29	n/c	<0.001
E. chaffeensis	6	1.5	0.0-0.3	396	12.806	60.96	n/c	0.025
China	18	1.8	0.7-3.0	2,035	136.450	87.54	n/c	<0.001
Zambia	15	2.4	0.8-4.1	897	60.167	76.73	n/c	<0.001
Africa	48	1.8	1.1-2.4	3,812	153.997	69.48	n/c	<0.001
Asia	18	1.8	0.7-3.0	2,035	136.450	87.54	n/c	<0.001
Bos indicus	5	11.9	2.4-21.3	442	49.371	91.9	n/c	<0.001
Bos taurus	13	0.6	0.1-1.4	2,348	80.467	85.09	n/c	<0.001

*95% CI = 95% confidence interval, **Q**†: Cochran's Q statistic for heterogeneity, ‡ I^2: Index for the degree of heterogeneity, §: Tau-squared measure of heterogeneity, n/c: Not calculated.

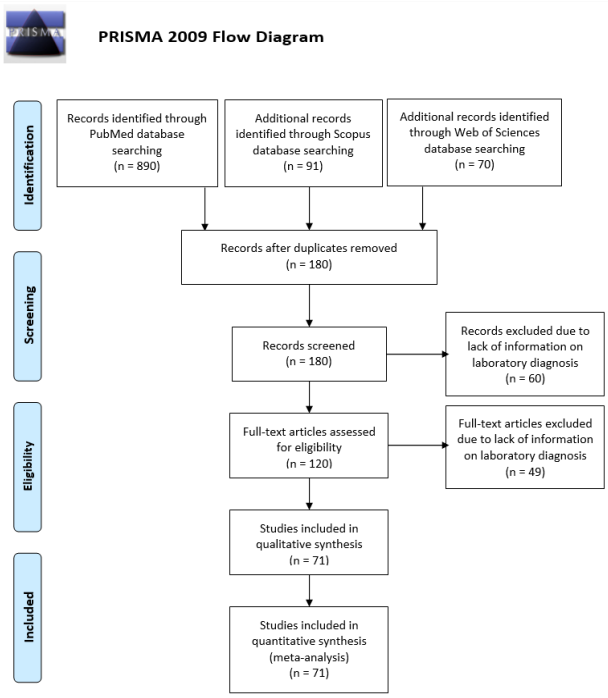

Figure 1. Study selection and characteristics

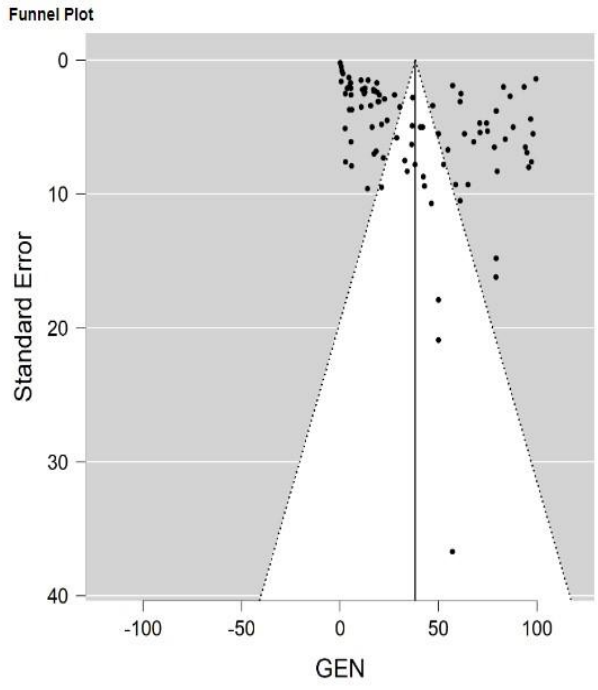

Figure 2. Funnel-plot for the standard error to assess for publication bias.

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

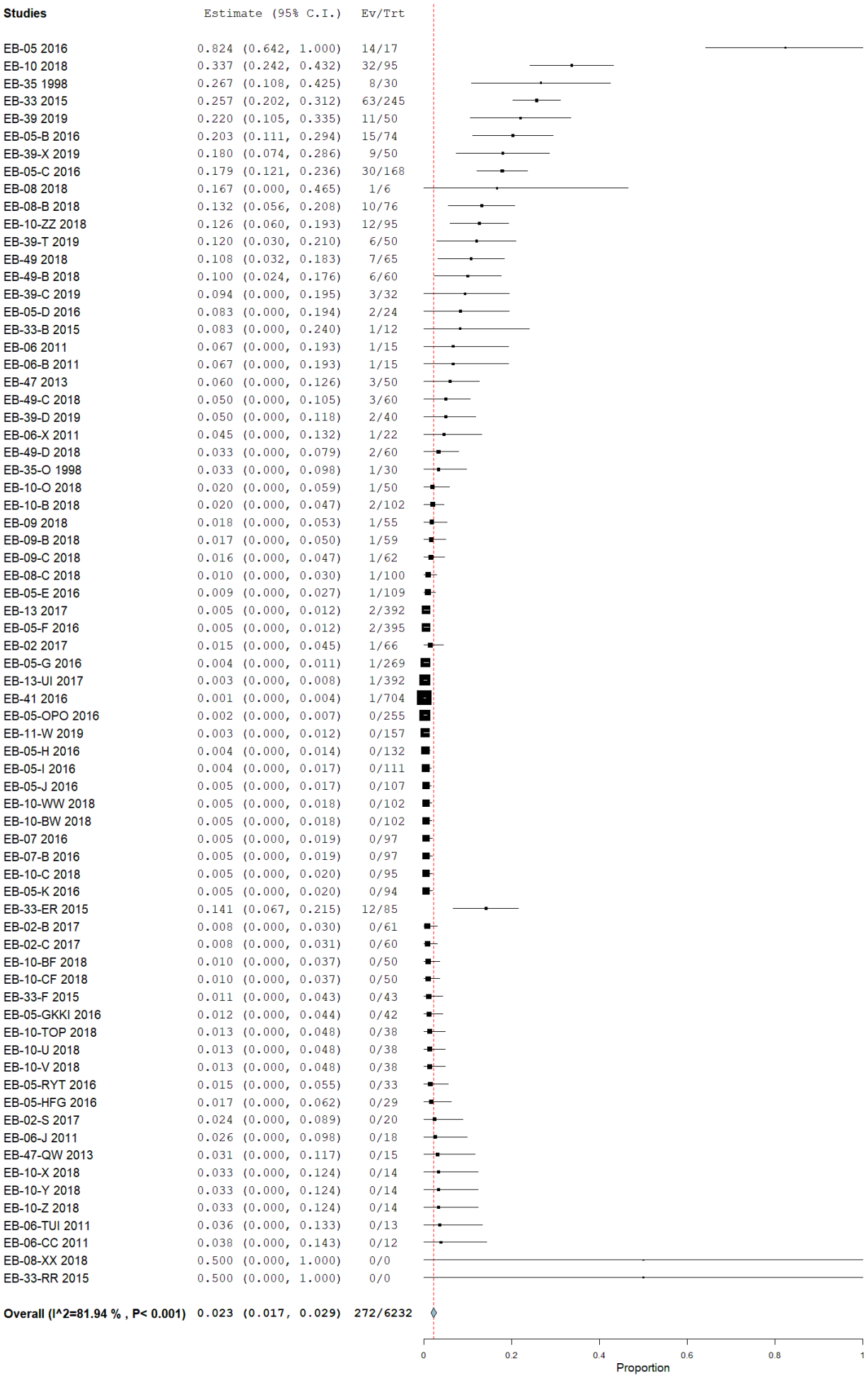

Figure 3. Pool prevalence forest plot of bovine ehrlichiosis

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

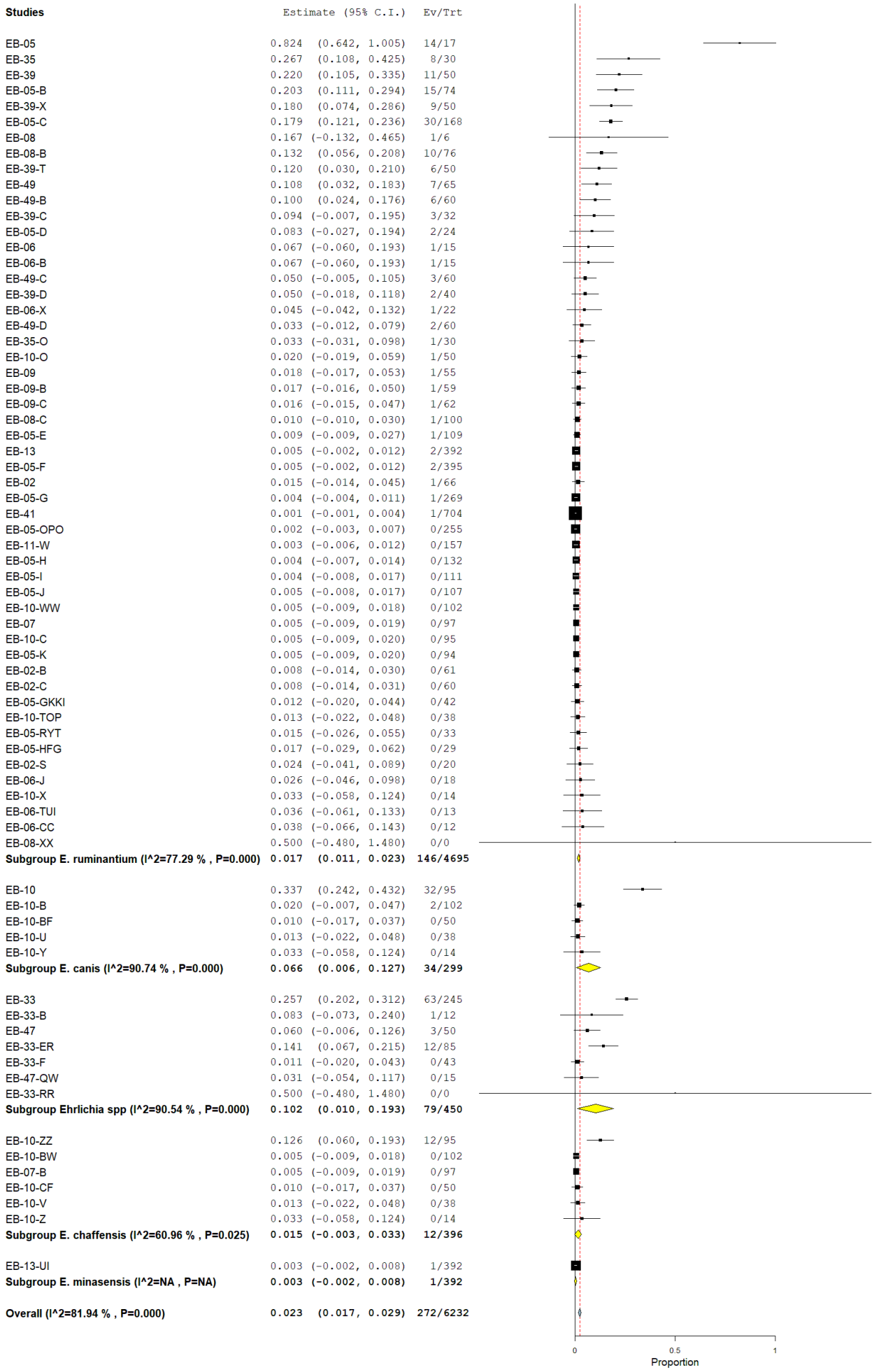

Figure 4. Pool prevalence forest plot of bovine ehrlichiosis based on *Ehrlichia* species

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

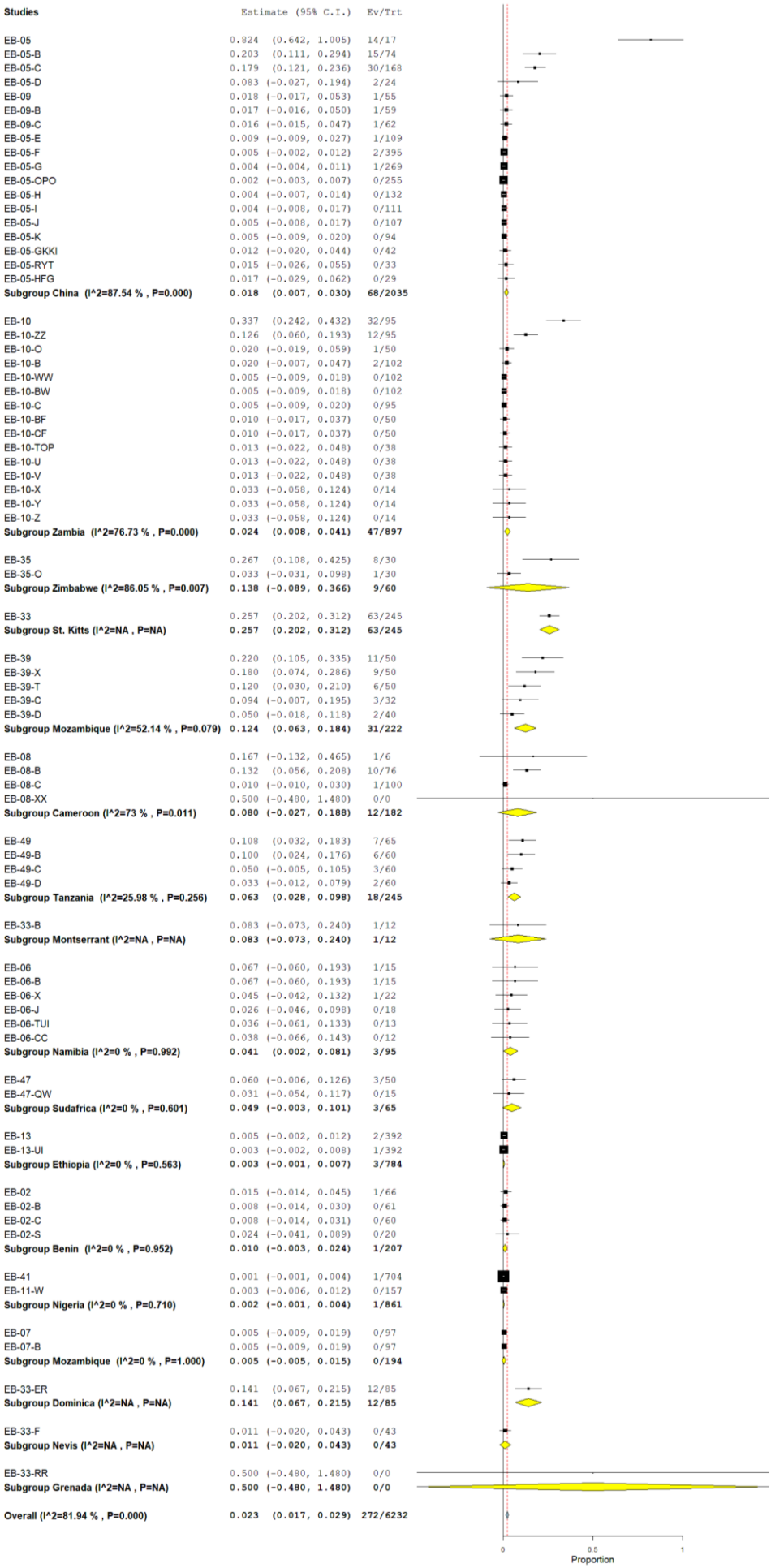

Figure 5. Pool prevalence forest plot of bovine ehrlichiosis based on countries

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

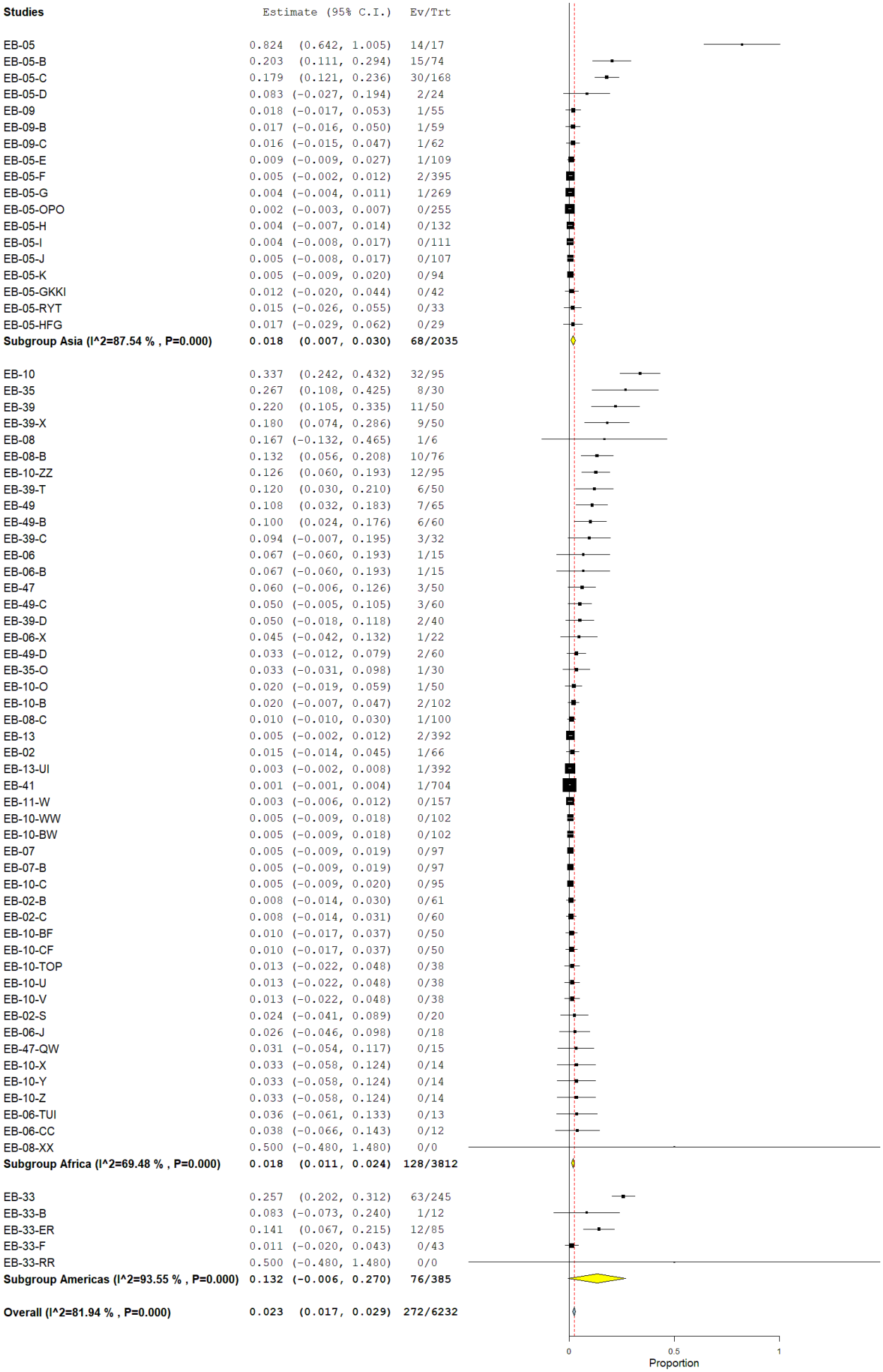

Figure 6. Pool prevalence forest plot of bovine ehrlichiosis based on continents

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

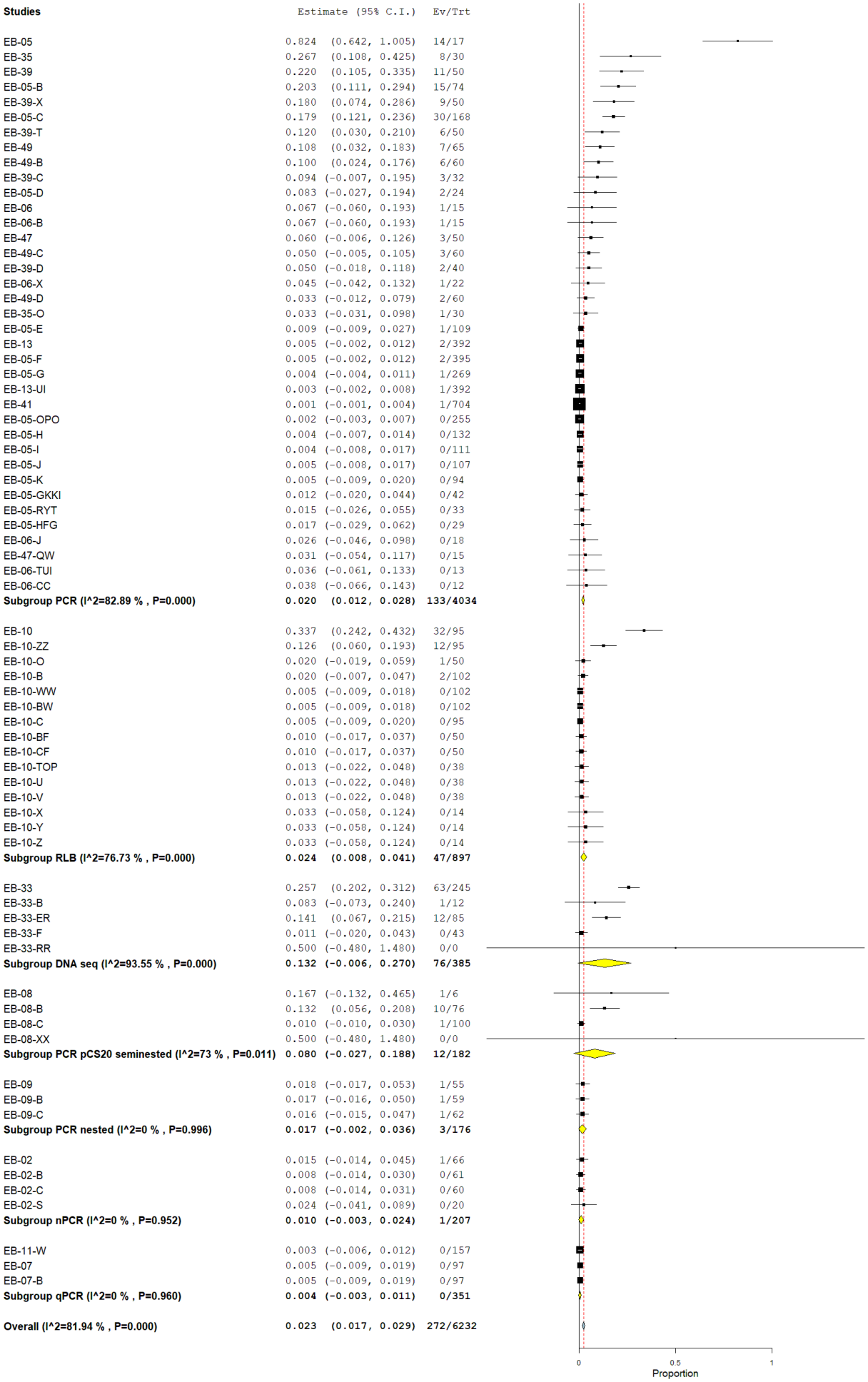

Figure 7. Pool prevalence forest plot of bovine ehrlichiosis based on the employed technique

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

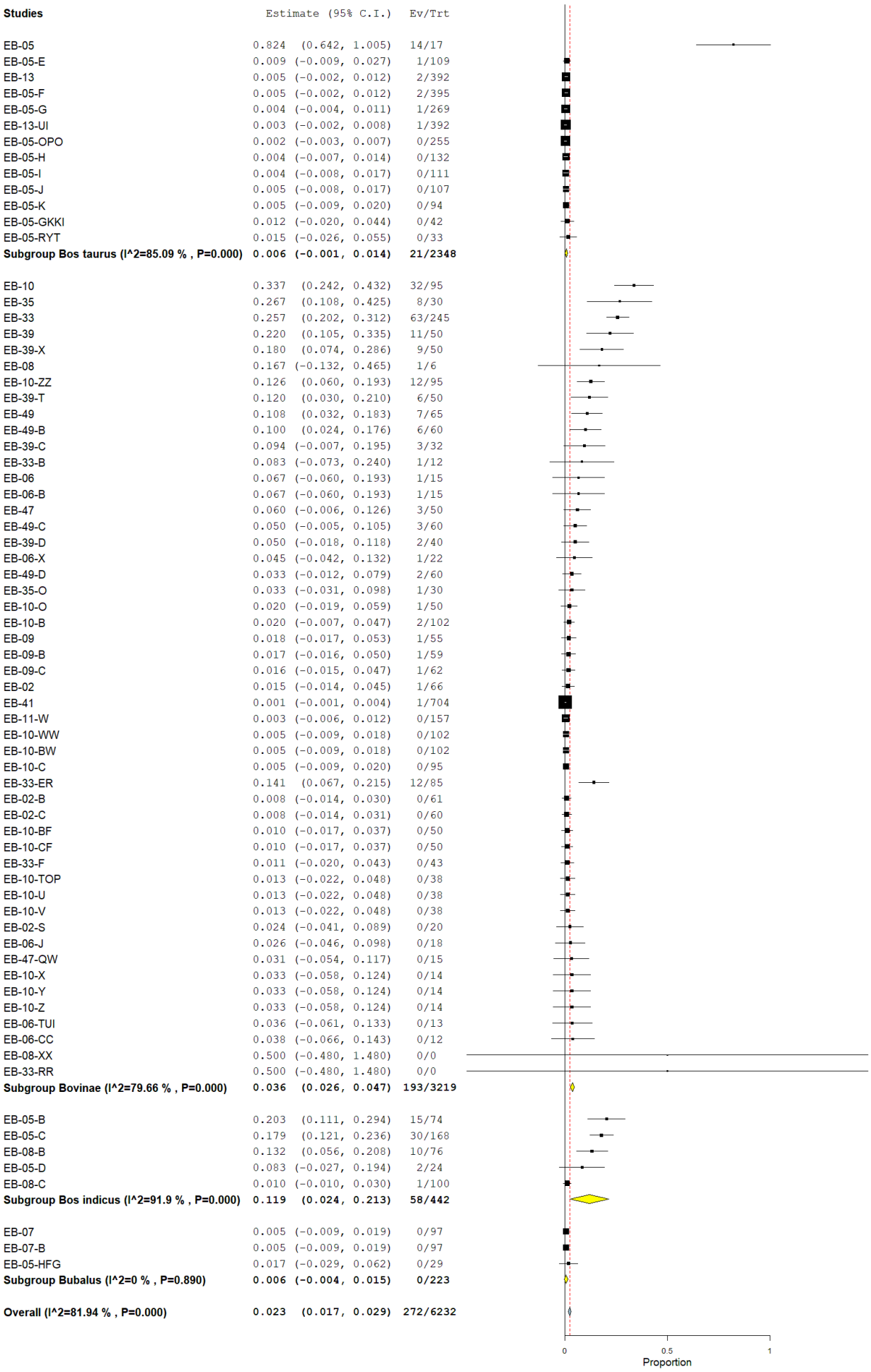

Figure 8. Pool prevalence forest plot of bovine ehrlichiosis based on animal species

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

DISCUSSION

Heartwater (caused by *E. ruminantium* infection) is a notifiable disease listed by the World Organization for Animal Health (OIE) (Allsopp, 2015). *Ehrlichia ruminantium*, is a Gram-negative bacterium, belonging to the order Rickettsiales and the family Anaplasmataceae which is an obligately intracellular organism. Heartwater or cowdriosis is a tick-borne disease transmitted by species in the genus *Amblyomma*, and occurs in wild and domestic ruminants, primarily in Africa, and in some parts of the Caribbean (Allsopp, 2015). The disease was recognized in South Africa in the 19th Century and determined to be tick-borne in 1900 while the organism was identified in 1925 and first cultured *in vitro* in 1985 (Allsopp, 2015).

Some authors suggest that the risk that endemic heartwater could pose in the Americas is relevant possible given the climate and the presence of some tick species, including *A. maculatum* as it is a good experimental vector for *E. ruminantium* (Vachiery et al., 2013). The existence of heartwater on three islands of the Central Lesser Antilles and the presence of an efficient vector originating from Africa, *Amblyomma variegatum*, on most of the islands of this region can present a serious threat for livestock on the American mainland (Barre et al., 1987).

In addition to *E. ruminantium*, *E. minasensis* (Aguiar et al., 2019), *E. chaffeensis* (Zhang et al., 2015), and *E. canis* (Seo et al., 2020) have been reported in cattle (Gajadhar et al., 2010; Aguiar et al., 2014; Moura de Aguiar et al., 2019). Nevertheless, considering the strong phylogenetic relationships of *E. canis* and *E. chaffeensis* with *E. minasensis*, plus the wide distribution of the latter species in the Americas, Europe, Asia, and Africa, it is possible that the molecular detection of *E. canis*/*E. chaffeensis* in bovines are false positives or an inadequate molecular identification (cross detection due to unspecificity of the primers), being really *E. minasensis*, as common primers used in the PCR are unable to discriminate such species (Thomson et al., 2018; Cabezas-Cruz et al., 2019). Considering the above-mentioned points, the only species of *Ehrlichia* that naturally infect cattle are *E. ruminantium* and *E. minasensis* (Gajadhar et al., 2010; Vachiery et al., 2013; Aguiar et al., 2014; Moura de Aguiar et al., 2019), which would affect the obtained results on *E. ruminantium*. The molecular prevalence for *E. ruminantium* would be higher than for *E. canis*. Although the most similar species to *E. minasensis* is *E. canis*, *E. chaffeensis* is also quite close phylogenetically (Cabezas-Cruz et al., 2016), which may also affect the results. Recently, some reports, not included in the current analyses as they did not correspond to the prevalence studies, indicated the circulation of *E. minasensis* in Canada and Brazil in cattle (Gajadhar et al., 2010; Aguiar et al., 2014).

As indicated in this meta-analysis, the prevalence of *Ehrlichia* is low in bovine (2.3%), higher for *E. canis*, which naturally infect dogs and other mammals without any significant difference from other species (Table 1). As expected, the higher prevalence was in an African country, Zambia (2.4%) although there was no difference between Africa and Asia (1.8%) regarding the worldwide prevalence (Table 1).

Unexpectedly, the prevalence was significantly higher in *Bos indicus* (11.9%, 95% CI: 2.4-21.3%) than *Bos taurus* (0.6%, 95% CI: 0.1-1.4%, Table 1) although *B. taurus* is more susceptible to ticks than *B. indicus*. It is known that tick resistance in cattle varies from more tick-susceptible *Bos taurus taurus* (*B. t. taurus*) to more tick-resistant *B. t. indicus* breeds, between bovine crosses as well as within a single cattle breed. Most of the studies have indicated that resistance is acquired through exposure to ticks (Roberts, 1968; George et al., 1985; Wambura et al., 1998; Robbertse et al., 2017).

Considering the large populations of cattle in different regions of the world, especially in those countries where bovine ehrlichiosis is not usually considered, they may be potentially affected by *E. ruminantium*. Accordingly, there is a need to conducte more studies on this specific pathogen. In this setting is also worthy to say that *E. minasensis* should be considered as a probable emerging etiology in bovine ehrlichiosis based on the obtained results of natural infection in cattle residing in Canada, Brazil, and Ethiopia (Gajadhar et al., 2010; Aguiar et al., 2014), in addition to the detection in ticks collected from cattle in countries, such as Pakistan (Rehman et al., 2019), China (Li et al., 2019), Malaysia (Koh et al., 2018), South Africa (Iweriebor et al., 2017), and Corsica (France, Cicculli et al., 2019).

CONCLUSION

As the findings indicated, heartwater, *E. ruminantium* infection, is a notifiable disease of domestic and wild ruminants, listed by the World Organization for Animal Health (OIE) although still needs to be meticulously investigated in different continents and countries. Its specific diagnosis is complex, as the serological and molecular diagnostic tests are insufficient to achieve a correct species identification which highlights the importance of genomic surveillance and phylogenetic analyses. There is a possible risk of endemic heartwater in the Americas due to the climate and the apparent increase in multiple infectious and vector-borne diseases, including ehrlichiosis. Furthermore, *E. minasensis*, *E. chaffeensis*, and *E. canis* have been reported in cattle although the two last species could be a molecular misidentification given their phylogenetic relationships with *E. minasensis*.

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

DECLARATIONS

This study was previously presented in part at the XVII Colombian Congress of Parasitology and Tropical Medicine, Cali, Colombia, December 4-6, 2019 (Poster D96) and at the VI Symposium of Research of the Fundacion Universitaria Autonoma de las Americas, Pereira, Colombia, October 30, 2019 (Oral Presentation, Preliminary Main Results).

Authors' contributions

DKBA conceived the idea of the study. KQR, JPMP, collected data. AJRM and DKBA analyzed data. AJRM wrote the first draft. DKBA, KQR, JPMP, DST, PB, KAL, LIZ, and AAFM wrote and revised the subsequent drafts. All authors approved the final submitted version and the data analysis.

Funding

From the Dirección de Investigación Científica, Humanística y Tecnológica (2-05-01-01), National Autonomous University of Honduras, Tegucigalpa, MDC, Honduras, Central America.

Competing interests

All authors declare no competing interests to be reported.

REFERENCES

Aguiar DM, Araujo JP, Nakazato L, Bard E, and Cabezas-Cruz A (2019). Complete genome sequence of an ehrlichia minasensis strain isolated from cattle. Microbiology Resource Announcements, 8(15): e00161-19. DOI: https://www.doi.org/10.1128/MRA.00161-19

Aguiar DM, Ziliani TF, Zhang X, Melo AL, Braga IA, Witter R, Freitas LC, Rondelli AL, Luis MA, Sorte EC et al. (2014). A novel ehrlichia genotype strain distinguished by the trp36 gene naturally infects cattle in brazil and causes clinical manifestations associated with ehrlichiosis. Ticks and Tick-borne Diseases, 5(5): 537-544. DOI: https://www.doi.org/10.1016/j.ttbdis.2014.03.010

Allsopp BA (2015). Heartwater--ehrlichia ruminantium infection. Revue Scientifique et Technique, 34(2): 557-568. DOI: https://www.doi.org/10.20506/rst.34.2.2379

Anifowose OI, Takeet MI, Talabi AO, and Otesile EB (2020). Molecular detection of ehrlichia ruminantium in engorged ablyomma variegatum and cattle in ogun state, nigeria. Journal of Parasitic Diseases, 44(2): 403-410. DOI: https://www.doi.org/10.1007/s12639-020-01218-4

Barre N, Uilenberg G, Morel PC, and Camus E (1987). Danger of introducing heartwater onto the american mainland: Potential role of indigenous and exotic amblyomma ticks. Onderstepoort Journal of Veterinary Research, 54(3): 405-417. Available at: https://pubmed.ncbi.nlm.nih.gov/3329328/

Cabezas-Cruz A, Zweygarth E, and Aguiar DM (2019). Ehrlichia minasensis, an old demon with a new name. Ticks and Tick-borne Diseases, 10(4): 828-829. DOI: https://www.doi.org/10.1016/j.ttbdis.2019.03.018

Cabezas-Cruz A, Zweygarth E, Vancova M, Broniszewska M, Grubhoffer L, Passos LMF, Ribeiro MFB, Alberdi P, and de la Fuente J (2016). Ehrlichia minasensis sp. Nov., isolated from the tick rhipicephalus microplus. International Journal of Systematic and Evolutionary Microbiology, 66(3): 1426-1430. DOI: https://www.doi.org/10.1099/ijsem.0.000895

Cicculli V, Masse S, Capai L, de Lamballerie X, Charrel R, and Falchi A (2019). First detection of ehrlichia minasensis in hyalomma marginatum ticks collected from cattle in corsica, france. Veterinary Medicine and Science, 5(2): 243-248. DOI: https://www.doi.org/10.1002/vms3.140

Downes MJ, Brennan ML, Williams HC, and Dean RS (2016). Development of a critical appraisal tool to assess the quality of cross-sectional studies (axis). BMJ Open, 6(12): e011458. DOI: https://www.doi.org/10.1136/bmjopen-2016-011458

Fargnoli L, Fernandez C, and Monje LD (2020). Novel ehrlichia strain infecting cattle tick amblyomma neumanni, argentina, 2018. Emerging Infectious Diseases Journal, 26(5): 1027-1030. DOI: https://www.doi.org/10.3201/eid2605.190940

Gajadhar AA, Lobanov V, Scandrett WB, Campbell J, and Al-Adhami B (2010). A novel ehrlichia genotype detected in naturally infected cattle in North America. Veterinary Parasitology, 173(3-4): 324-329. DOI: https://www.doi.org/10.1016/j.vetpar.2010.06.034

George JE, Osburn RL and Wikel SK (1985). Acquisition and expression of resistance by bos indicus and bos indicus x bos taurus calves to amblyomma americanum infestation. Journal of Parasitology, 71(2): 174-182. Available at https://pubmed.ncbi.nlm.nih.gov/3998956/

Hector E, Elelu N, Ferrolho J, Couto J, Sanches G, Antunes S, Domingos A, and Eisler M (2019). Pcr detection of ehrlichia ruminantium and babesia bigemina in cattle from kwara state, nigeria: Unexpected absence of infection. Parasitology Research, 118(3): 1025-1029. DOI: https://www.doi.org/10.1007/s00436-019-06204-1

Institute of Health Economics (IHE) (2014). Quality appraisal of case series studies checklist. Available at http://www.ihe.ca/research-programs/rmd/cssqac/cssqac-about

Iweriebor BC, Mmbaga EJ, Adegborioye A, Igwaran A, Obi LC, and Okoh AI (2017). Genetic profiling for *Anaplasma* and *Ehrlichia* species in ticks collected in the eastern cape province of south africa. BMC Microbiology, 17(1): Article number: 45. DOI: https://www.doi.org/10.1186/s12866-017-0955-0

Koh FX, Kho KL, Kisomi MG, Wong LP, Bulgiba A, Tan PE, Lim YAL, Nizam QNH, Panchadcharam C, and Tay ST (2018). *Ehrlichia* and *Anaplasma* infections: Serological evidence and tick surveillance in peninsular malaysia. Journal of Medical Entomology, 55(2): 269-276. DOI: https://www.doi.org/10.1093/jme/tjx204

Kontopantelis E, and Reeves D (2012). Performance of statistical methods for meta-analysis when true study effects are non-normally distributed: A comparison between dersimonian-laird and restricted maximum likelihood. Statistical Methods in Medical Research, 21(6): 657-659. DOI: https://www.doi.org/10.1177/0962280211413451

Li J, Liu X, Mu J, Yu X, Fei Y, Chang J, Bi Y, Zhou Y, Ding Z, and Yin R (2019). Emergence of a novel *Ehrlichia minasensis* strain, harboring the major immunogenic glycoprotein trp36 with unique tandem repeat and c-terminal region sequences, in *Haemaphysalis hystricis* ticks removed from free-ranging sheep in Hainan province, China. Microorganisms 7(9): Article number: 369. DOI: https://www.doi.org/10.3390/microorganisms7090369

Moher D, Liberati A, Tetzlaff J, Altman DG, and Group P (2009). Preferred reporting items for systematic reviews and meta-analyses: The prisma statement. PLOS Medicine, 6(7): e1000097. DOI: https://www.doi.org/10.1371/journal.pmed.1000097

Moura de Aguiar D, Pessoa Araujo Junior J, Nakazato L, Bard E, Aguilar-Bultet L, Vorimore F, Leonidovich Popov V, Moleta Colodel E, and Cabezas-Cruz A (2019). Isolation and characterization of a novel pathogenic strain of *Ehrlichia minasensis*. Microorganisms, 7(11): Article number: 528. DOI: https://www.doi.org/10.3390/microorganisms7110528

Rehman A, Conraths FJ, Sauter-Louis C, Krucken J, and Nijhof AM (2019). Epidemiology of tick-borne pathogens in the semi-arid and the arid agro-ecological zones of punjab province, Pakistan. Transboundary and Emerging Diseases, 66(1): 526-536. DOI: https://www.doi.org/10.1111/tbed.13059

Robbertse L, Richards SA, and Maritz-Olivier C (2017). Bovine immune factors underlying tick resistance: Integration and future directions. Frontiers in Cellular and Infection Microbiology, 7: Article number: 522. DOI: https://www.doi.org/10.3389/fcimb.2017.00522

Roberts JA (1968). Resistance of cattle to the tick boophilus microplus (canestrini). I. Development of ticks on bos taurus. Journal of Parasitology, 54(4): 663-666. Available at: https://www.jstor.org/stable/3277017?seq=1

Seo MG, Kwon OD, and Kwak D (2020). Genotypic analysis of piroplasms and associated pathogens from ticks infesting cattle in korea. Microorganisms, 8(5): Article number:728. DOI: https://www.doi.org/10.3390/microorganisms8050728

Stewart CG (1992). Bovine ehrlichiosis. Tick vector biology: Medical and veterinary aspects. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 101-107. Available at: https://www.springer.com/gp/book/9783642766459

Thomson K, Yaaran T, Belshaw A, Curson L, Tisi L, Maurice S, and Kiddle G (2018). A new taqman method for the reliable diagnosis of ehrlichia spp. In canine whole blood. Parasites & Vectors, 11(1): Article number: 350. DOI: https://www.doi.org/10.1186/s13071-018-2914-5

Vachiery N, Marcelino I, Martinez D, and Lefrancois T (2013). Opportunities in diagnostic and vaccine approaches to mitigate potential heartwater spreading and impact on the american mainland. Developmental Biology (Basel), 135: 191-200. DOI: https://www.doi.org/10.1159/000190050

Viechtbauer W (2010). Conducting meta-analyses in r with the metafor package. Journal of statistical software, 36(3): 1-48. DOI: https://www.doi.org/10.18637/jss.v036.i03

Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, and Schmid CH (2012). Closing the gap between methodologists and end-users: R as a computational back-end. Journal of statistical software, 49(5): 1-15. DOI: https://www.doi.org/10.18637/jss.v049.i05

Wambura PN, Gwakisa PS, Silayo RS, and Rugaimukamu EA (1998). Breed-associated resistance to tick infestation in *Bos indicus* and their crosses with *Bos taurus*. Veterinary Parasitology, 77(1): 63-70. DOI: https://www.doi.org/10.1016/s0304-4017(97)00229-x

Zhang J, Kelly P, Guo W, Xu C, Wei L, Jongejan F, Loftis A, and Wang C (2015). Development of a generic *Ehrlichia* fret-qpcr and investigation of ehrlichioses in domestic ruminants on five caribbean islands. Parasites & Vectors, 8: Article number: 506. DOI: https://www.doi.org/10.1186/s13071-015-1118-5

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

Supplementary Materials

Table S1. Characteristics of the included studies on bovine ehrlichiosis

Number/Code	Year-Publication	Years-Study	*Ehrlichia*	State	Country	Region	Lab Technique	Species	N	n(+)	%
EB-05	2016	2007 - 2013	*E. ruminantium*	Wuhu, Anhui	China	Asia	PCR	*Bos taurus*	17	14	82.4
EB-10	2018	2010	*E. canis*	Kowa	Zambia	Africa	RLB	*Bovinae*	95	32	33.68
EB-35	1998	1998	*E. ruminantium*		Zimbabwe	Africa	PCR	*Bovinae*	30	8	26.7
EB-33	2015	2014	*Ehrlichia* spp.	St. Kitts	St. Kitts	Americas	DNA seq	*Bovinae*	245	63	25.7
EB-39	2019	2019	*E. ruminantium*	Boane	Mozambique	Africa	PCR	*Bovinae*	50	11	22
EB-05-B	2016	2007 - 2013	*E. ruminantium*	Haikou, Hainan	China	Asia	PCR	*Bos indicus*	74	15	20.3
EB-39-X	2019	2019	*E. ruminantium*	Magude	Mozambique	Africa	PCR	*Bovinae*	50	9	18
EB-05-C	2016	2007 - 2013	*E. ruminantium*	Kunming, Yunnan	China	Asia	PCR	*Bos indicus*	168	30	17.9
EB-08	2018	2010	*E. ruminantium*	SDR	Cameroon	Africa	PCR pCS20 seminested	*Bovinae*	6	1	16.7
EB-08-B	2018	2010	*E. ruminantium*	SDR	Cameroon	Africa	PCR pCS20 seminested	*Bos indicus*	76	10	13.9
EB-10-ZZ	2018	2010	*E. chaffeensis*	Kowa	Zambia	Africa	RLB	*Bovinae*	95	12	12.63
EB-39-T	2019	2019	*E. ruminantium*	Matutuine	Mozambique	Africa	PCR	*Bovinae*	50	6	12
EB-49	2018	2018	*E. ruminantium*	Mkoani	Tanzania	Africa	PCR	*Bovinae*	65	7	10.77
EB-49-B	2018	2018	*E. ruminantium*	Wete	Tanzania	Africa	PCR	*Bovinae*	60	6	10
EB-39-C	2019	2019	*E. ruminantium*	Namaacha	Mozambique	Africa	PCR	*Bovinae*	32	3	9.38
EB-05-D	2016	2007 - 2013	*E. ruminantium*	Putian, Fujian	China	Asia	PCR	*Bos indicus*	24	2	8.3
EB-33-B	2015	2014	*Ehrlichia* spp.	Montserrant	Montserrant	Americas	DNA seq	*Bovinae*	12	1	8.3
EB-06	2011	2009	*E. ruminantium*	Bwabwata-Mahango	Namibia	Africa	PCR	*Bovinae*	15	1	6.67
EB-06-B	2011	2009	*E. ruminantium*	Bwabwata-Buffalo	Namibia	Africa	PCR	*Bovinae*	15	1	6.67
EB-47	2013	2013	*Ehrlichia* spp.		Sudafrica	Africa	PCR	*Bovinae*	50	3	6
EB-49-C	2018	2018	*E. ruminantium*	Chake	Tanzania	Africa	PCR	*Bovinae*	60	3	5
EB-39-D	2019	2019	*E. ruminantium*	Moamba	Mozambique	Africa	PCR	*Bovinae*	40	2	5
EB-06-X	2011	2009	*E. ruminantium*	Mamili	Namibia	Africa	PCR	*Bovinae*	22	1	4.55
EB-49-D	2018	2018	*E. ruminantium*	Micheweni	Tanzania	Africa	PCR	*Bovinae*	60	2	3.33
EB-35-O	1998	1998	*E. ruminantium*		Zimbabwe	Africa	PCR	*Bovinae*	30	1	3.3
EB-10-O	2018	2010	*E. ruminantium*	Kapamba	Zambia	Africa	RLB	*Bovinae*	50	1	2
EB-10-B	2018	2010	*E. canis*	Chifulo	Zambia	Africa	RLB	*Bovinae*	102	2	1.96

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

Number/Code	Year-Publication	Years-Study	*Ehrlichia*	State	Country	Region	Lab Technique	Species	N	n(+)	%
EB-09	2018	2017	*E. ruminantium*	kashgar	China	Asia	PCR nested	*Bovinae*	55	1	1.8
EB-09-B	2018	2017	*E. ruminantium*	Yecheng	China	Asia	PCR nested	*Bovinae*	59	1	1.7
EB-09-C	2018	2017	*E. ruminantium*	Hotan	China	Asia	PCR nested	*Bovinae*	62	1	1.6
EB-08-C	2018	2010	*E. ruminantium*	UFR	Cameroon	Africa	PCR pCS20 seminested	*Bos indicus*	100	1	1
EB-05-E	2016	2007 - 2013	*E. ruminantium*	Bengbu, Anhui	China	Asia	PCR	*Bos taurus*	109	1	0.9
EB-13	2017	2013	*E. ruminantium*	Illubabor	Ethiopia	Africa	PCR	*Bos taurus*	392	2	0.51
EB-05-F	2016	2007 - 2013	*E. ruminantium*	Yancheng, Jiangsu	China	Asia	PCR	*Bos taurus*	395	2	0.5
EB-02	2017	2015	*E. ruminantium*	Gogounou	Benin	Africa	nPCR	*Bovinae*	66	1	0.5
EB-05-G	2016	2007 - 2013	*E. ruminantium*	Yangzjou, Jiangsu	China	Asia	PCR	*Bos taurus*	269	1	0.4
EB-13-UI	2017	2013	*E. minasensis*	Illubabor	Ethiopia	Africa	PCR	*Bos taurus*	392	1	0.26
EB-41	2016	2008	*E. ruminantium*	Plateau	Nigeria	Africa	PCR	*Bovinae*	704	1	0.14
EB-05-OPO	2016	2007 - 2013	*E. ruminantium*	Shanghai, Shanghai	China	Asia	PCR	*Bos taurus*	255	0	0
EB-11-W	2019	2019	*E. ruminantium*	Kwara	Nigeria	Africa	qPCR	*Bovinae*	157	0	0
EB-05-H	2016	2007 - 2013	*E. ruminantium*	Chifeng, Inner Mongolia	China	Asia	PCR	*Bos taurus*	132	0	0
EB-05-I	2016	2007 - 2013	*E. ruminantium*	Qiqihar, Heilongjjang	China	Asia	PCR	*Bos taurus*	111	0	0
EB-05-J	2016	2007 - 2013	*E. ruminantium*	Sanyuan, Beijing	China	Asia	PCR	*Bos taurus*	107	0	0
EB-10-WW	2018	2010	*E. ruminantium*	Chifulo	Zambia	Africa	RLB	*Bovinae*	102	0	0
EB-10-BW	2018	2010	*E. chaffeensis*	Chifulo	Zambia	Africa	RLB	*Bovinae*	102	0	0
EB-07	2016	2011	*E. ruminantium*	Reserve de Marromeu	Mozambique	Africa	qPCR	*Bubalus*	97	0	0
EB-07-B	2016	2011	*E. chaffeensis*	Reserve de Marromeu	Mozambique	Africa	qPCR	*Bubalus*	97	0	0
EB-10-C	2018	2010	*E. ruminantium*	Kowa	Zambia	Africa	RLB	*Bovinae*	95	0	0
EB-05-K	2016	2007 - 2013	*E. ruminantium*	Tianjin, Tianjin	China	Asia	PCR	*Bos taurus*	94	0	0
EB-33-ER	2015	2014	*Ehrlichia* spp.	Dominica	Dominica	Americas	DNA seq	*Bovinae*	85	12	14.1
EB-02-B	2017	2015	*E. ruminantium*	Tchaourou	Benin	Africa	nPCR	*Bovinae*	61	0	0
EB-02-C	2017	2015	*E. ruminantium*	Nikki	Benin	Africa	nPCR	*Bovinae*	60	0	0
EB-10-BF	2018	2010	*E. canis*	Kapamba	Zambia	Africa	RLB	*Bovinae*	50	0	0
EB-10-CF	2018	2010	*E. chaffeensis*	Kapamba	Zambia	Africa	RLB	*Bovinae*	50	0	0
EB-33-F	2015	2014	*Ehrlichia* spp.	Nevis	Nevis	Americas	DNA seq	*Bovinae*	43	0	0
EB-05-GKKI	2016	2007 - 2013	*E. ruminantium*	Jining, Shandong	China	Asia	PCR	*Bos taurus*	42	0	0
EB-10-TOP	2018	2010	*E. ruminantium*	Chisanga	Zambia	Africa	RLB	*Bovinae*	38	0	0
EB-10-U	2018	2010	*E. canis*	Chisanga	Zambia	Africa	RLB	*Bovinae*	38	0	0

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.

Number/Code	Year-Publication	Years-Study	*Ehrlichia*	State	Country	Region	Lab Technique	Species	N	n(+)	%
EB-10-V	2018	2010	*E. chaffeensis*	Chisanga	Zambia	Africa	RLB	*Bovinae*	38	0	0
EB-05-RYT	2016	2007 - 2013	*E. ruminantium*	Bionzhou, Shandong	China	Asia	PCR	*Bos taurus*	33	0	0
EB-05-HFG	2016	2007 - 2013	*E. ruminantium*	Yancheng, Jiangsu	China	Asia	PCR	*Bubalus*	29	0	0
EB-02-S	2017	2015	*E. ruminantium*	Kpinnou	Benin	Africa	nPCR	*Bovinae*	20	0	0
EB-06-J	2011	2009	*E. ruminantium*	Bwabwata West	Namibia	Africa	PCR	*Bovinae*	18	0	0
EB-47-QW	2013	2013	*Ehrlichia* spp.		Sudafrica	Africa	PCR	*Bovinae*	15	0	0
EB-10-X	2018	2010	*E. ruminantium*	Mungwi central	Zambia	Africa	RLB	*Bovinae*	14	0	0
EB-10-Y	2018	2010	*E. canis*	Mungwi central	Zambia	Africa	RLB	*Bovinae*	14	0	0
EB-10-Z	2018	2010	*E. chaffeensis*	Mungwi central	Zambia	Africa	RLB	*Bovinae*	14	0	0
EB-06-TUI	2011	2009	*E. ruminantium*	Eastern flood plains	Namibia	Africa	PCR	*Bovinae*	13	0	0
EB-06-CC	2011	2009	*E. ruminantium*	Mudumu	Namibia	Africa	PCR	*Bovinae*	12	0	0

To cite this paper: Bonilla-Aldana DK, Quintero-Rada K, Montoya-Posada JP, Soler-Tovar D, Barato P, Arteaga-Livias K, Zambrano LI, Faccini-Martínez ÁA and Rodriguez-Morales AJ (2021). Bovine Ehrlichiosis Prevalence: A Systematic Review and Meta-Analysis of Molecular Studies. *World Vet. J.*, 11 (1): 01-15.