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Author for correspondence:
ThankGod E. Onyiche,
E-mail: et.onyiche@unimaid.edu.ng

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Equine piroplasmosis: an insight into global exposure of equids from 1990 to 2019 by systematic review and meta-analysis

ThankGod E. Onyiche[1,2,3], Moeti O. Taioe[4], Nthatisi I. Molefe[1], Abdullahi A. Biu[2], Joshua Luka[2], Isaac J. Omeh[5], Naoaki Yokoyama[3] and Oriel Thekisoe[1]

[1]Unit for Environmental Sciences and Management, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2520, South Africa; [2]Department of Veterinary Parasitology and Entomology, University of Maiduguri, P. M. B. 1069, Maiduguri 600230, Nigeria; [3]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan; [4]National Zoological Gardens of South Africa, South African National Biodiversity Institute, PO Box 754, Pretoria 0001, South Africa and [5]Department of Veterinary Physiology and Biochemistry, University of Maiduguri, P. M. B. 1069, Maiduguri 600230, Nigeria

Abstract

Equine piroplasmosis (EP) is a tick-borne disease of economic importance, relevant in the international movement of equids. The causative agents are at least two apicomplexan protozoan parasites *Babesia caballi* and *Theileria equi*. To date, there is no study that estimates global and regional exposure of equids to EP. We therefore conducted a systematic review and meta-analysis to estimate the pooled prevalence and heterogeneity of EP using random-effects model. Six electronic databases were searched for publications on EP and assessed according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. A total of 66 eligible studies published between 1990 and 2019 and representing 24 041 equids were included. The overall pooled prevalence estimates (PPEs) of *B. caballi* was 22.3% (95% CI 21.7–22.8), while the overall PPE for *T. equi* was 29.4% (95% CI 28.7–30.0). The overall pooled prevalence due to co-infection with both parasites was 11.8% (95% CI 11.32–12.32). Also, subgroup analysis according to sex, age, diagnostic technique, equid species, region and publication years showed a substantial degree of heterogeneity across studies computed for both *B. caballi* and *T. equi* infections in equids. Awareness of the current status of EP globally will alert the relevant authorities and stakeholders where necessary on the need for better preventive and control strategies against the disease.

Introduction

Equine piroplasmosis (EP) is a disease of equids caused by at least two haemoparasites, *Babesia caballi* and *Theileria equi* transmitted by Ixodid ticks. Clinical manifestations of the disease in infected animals include lethargy, anorexia, fever, jaundice, haemolysis and petechia haemorrhages in mucous membranes (Scoles and Ueti, 2015).

EP is responsible for economic losses in the equid industry. *Babesia caballi* and *T. equi* are in the group of apicomplexan parasites collectively called piroplasms (Levine, 1985). *Babesia caballi* lack a pre-erythrocytic cycle and *T. equi* have no documented transovarial transmission (Homer *et al.*, 2000). *Babesia caballi* is considered less virulent than *T. equi* because the latter acute phase infects leucocytes before erythrocytes and the infection is long-lasting (Ramsay *et al.*, 2013). Surviving animals remain chronic carriers with low levels of parasitaemia and serve as reservoirs for ticks (Wise *et al.*, 2013; Scoles and Ueti, 2015).

EP is widespread in subtropical and tropical regions of the world (Uilenberg, 2006). It is endemic in several parts of Africa, Asia, America and Europe where competent tick vectors are present (Rothschild, 2013; Onyiche *et al.*, 2019). International movement of chronically infected animals has played some role in the epidemiology of this disease necessitating proper screening of animals prior to movement (Ayala-Valdovinos *et al.*, 2014).

Due to non-specific clinical signs associated with EP, diagnosis is often challenging. Furthermore, the sensitivity of different diagnostic tests such as microscopy, serology and PCR is another issue in diagnosis (Mans *et al.*, 2015). These issues have led to many different types of epidemiological prevalence studies from different areas. This is also complicated by several factors such as presence and abundance of competent vectors, management practices, host activity and effectiveness of control programmes for ticks (reviewed by Onyiche *et al.*, 2019).

To date, there has been no systematic review to ascertain the current global status of EP. Therefore, we conducted a systematic review and meta-analysis to determine the global exposure and evaluated risk factors potentially associated with their occurrence.

Materials and methods

Search strategy and selection criteria

The study was carried out in accordance with the methodology recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Fig. 1) (Moher *et al.*, 2015). We searched the primary literature of published articles from 1 January 1990 to 25 February 2019 in English databases of Scopus, Science Direct, PubMed, Web of Science, Embase and Springer link. Keywords used for the systemic search were 'Equine Piroplasmosis', 'Prevalence', 'Seroprevalence' '*Babesia caballi*', '*Theileria equi*', 'Tick-borne', 'Equids', 'Horses', 'Donkeys', 'Equines' and 'Mules'. Keywords were used individually and in combination with 'OR' and/or 'AND' operators. The titles of the articles were scanned, and relevant articles were downloaded. In addition, the reference list of the searched articles was also screened for relevant studies.

Inclusion and exclusion criteria

After a review of titles and abstracts, the selected studies were screened further by a detailed review of the full text. Articles that were included in the study had to fulfil all the following criteria: (1) original research articles without geographical limitation (global); (2) the full texts were available; (3) the publication was in English; (4) conducted between 1 January 1990 and 25 February 2019; (5) study design was cross-sectional/prevalence study; (6) the diagnostic method was clearly stated; (7) the geographical location of the study was clearly stated; (8) the species of equid was clearly stated; (9) the number of positive cases and sample size were provided; (10) the species of the piroplasms was clearly identified; (11) the study screened for both *B. caballi* and *T. equi*; and (12) the sample size was at least a minimum of 50 equids. Any study that did not fulfil the criteria stated was excluded. Eligibility and inclusion as well as data extraction were carried out by two trained investigators working independently. At the end of the search and screening, the investigators met and compared findings. No attempt was made to contact the authors of the original manuscripts for any additional information or retrieval of unpublished studies.

Subgroup analyses

We performed several subgroup analyses to study the independent effects of infection of *B. caballi* and *T. equi* on several risk factors including age, sex, publication years, diagnostic methods, species of equid and continent and/or region. In the estimation of the overall pooled prevalence, we used the individual infection rates reported for all the eligible studies that were arrived at using either microscopy, molecular or serological technique. Where more than one technique was used, we used the data for serological technique (IFAT/ELISA/ICT) ahead of molecular test and microscopy. Due to a small number of sample size for mules, this was excluded from the subgroup analysis. We combined data from both North and South America as a single subgroup called the Americas.

Data extraction and analysis

From the eligible studies, data extracted included first author surname; publication year; sample size; number of positives; country of study; diagnostic method; the species of piroplasms and age; sex and species of equids. Data collected were entered into spreadsheets. Graph-Pad Prism version 5.0 was used for preliminary analysis. Meta-analysis was conducted with Comprehensive Meta-Analysis Version 3.0. For each of the eligible studies, the prevalence was calculated as a percentage by expressing positive cases to sample size. Pooled prevalence and their 95% confidence interval (CI) were determined using MedCalc® statistical software. The prevalence's estimates as well as the *P* value and 95% CI were obtained using a random-effects model (Hedges and Vevea, 1998). Cochran's heterogeneity (Q) within studies as well as percentage variation in prevalence (I^2) was evaluated using the Cochran's Q-test. Heterogeneity was described as low, moderate or high depending on if I^2 was $\leqslant$25, 50 or $\geqslant$75%, respectively (Higgins and Thompson, 2002). Publication bias was evaluated using the Egger's regression intercept (Egger *et al.*, 1997). The effect size and corresponding CI for each subgroup was calculated and expressed on forest plots. Furthermore, to determine the source of heterogeneity within subgroups, e.g. sex, age, geographical regions, diagnostic technique and years of study, meta-regression analyses were performed.

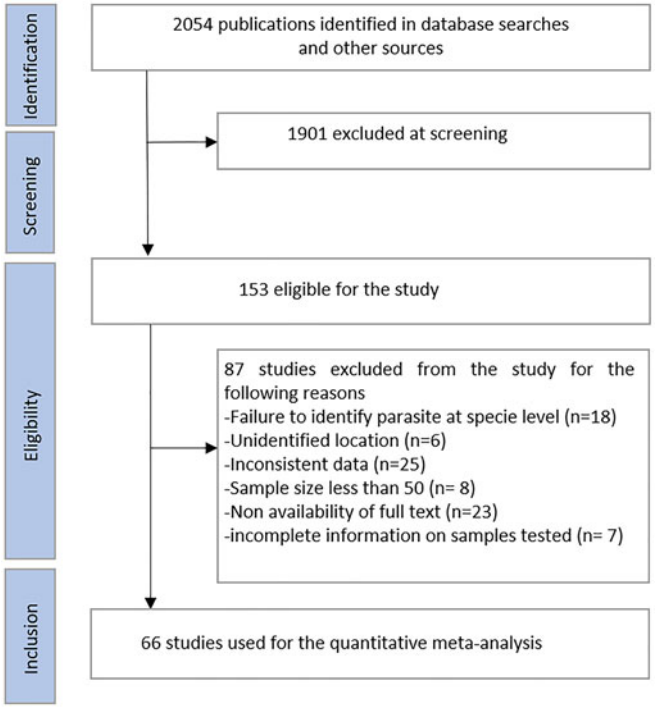

Fig. 1. Flow diagram showing the selection process of eligible studies according to the PRISMA guidelines.

Results

Search results and eligible studies

Following a search on the six databases, about 2054 relevant published materials were identified and retrieved. Following the review of their titles, abstracts and duplicates, a total of 1901 studies were excluded. The remaining 153 studies were further screened for eligibility. Studies ($n = 87$) were excluded for failure to identify the parasite to species level ($n = 18$); unidentified locations ($n = 6$); inconsistent data ($n = 25$); studies with sample size below 50 ($n = 8$); non-availability of full text ($n = 23$); and incomplete information on the number of samples tested ($n = 7$). A total of 66 studies (Table 1) were eligible and subsequently used for the meta-analysis (Fig. 1).

The study parameters included the species of piroplasm; single or mixed infection, equine sex; equine species; equine age; diagnostic technique employed, region(s) of the world and year of publication. All eligible studies were conducted between 1 January 1990 and 25 February 2019. All studies included peer-

Table 1. List and characteristics of 66 eligible studies included in the meta-analysis

Study reference	Country	Host type	Sample size	*B. caballi* No. of positives; prevalence %	*T. equi* No. of positives; prevalence %	Mixed infection No. of positives; prevalence %
Gizachew *et al.* (2013)	Ethiopia	Donkey	395	52; 13.2	220; 55.7	39; 9.9
Gummow *et al.* (1996)	South Africa	Horses	176	58; 32.9	95; 53.9	–
Hawkins *et al.* (2015)	Kenya	Donkey	71	0; 0	67; 30.9	–
Mahmoud *et al.* (2016)	Egypt	Horse/donkey	139	31; 22.3	37; 26.6	–
Motloang *et al.* (2008)	South Africa	Horses	138	51; 36.9	97; 70.3	–
Oduori *et al.* (2015)	Kenya	Donkeys	314	0; 0	255; 81.2	–
Ros-García *et al.* (2013)	Tunisia	Horses	104	1; 0.9	11; 10.6	1; 0.9
Salim *et al.* (2008)	Sudan	Horses	158	7; 4.4	80; 50.6	–
Salim *et al.* (2013)	Sudan	Horses/donkey	308	0; 0	121; 39.3	–
Sanusi *et al.* (2014)	Nigeria	Horses	400	60; 15.0	8; 2.0	4; 1.0
Turaki *et al.* (2014)	Nigeria	Horses	240	6; 2.5	94; 39.2	–
Xu *et al.* (2003)	China	Horses	111	36; 32.4	38; 34.2	–
Wang *et al.* (2014)	China	Horses	1990	1018; 51.2	229; 11.5	–
Sumbria *et al.* (2016)	India	Horses/donkeys/ mules	180	113; 62.8	133; 73.9	–
Ybañez *et al.* (2018)	Philippines	Horses	105	2; 1.9	23; 21.9	–
Seo *et al.* (2011)	Korea	Horses	184	0; 0	2; 1.1	–
Rüegg *et al.* (2007)	Mongolia	Horses	499	328; 65.7	393; 78.8	–
Nugraha *et al.* (2018)	Indonesia	Horses	235	15; 6.4	5; 2.1	–
Sevinc *et al.* (2008)	Turkey	Horses	481	4; 0.8	78; 16.2	7; 1.5
Munkhjargal *et al.* (2013)	Mongolia	Horses	250	129; 51.6	49; 19.6	26; 10.4
Kizilarslan *et al.* (2015)	Turkey	Horses	203	4; 1.9	6; 2.9	–
Karatepe *et al.* (2009)	Turkey	Horses	125	12; 9.6	16; 12.8	5; 4.0
Kamyingkird *et al.* (2016)	Thailand	Horses/mules	240	12; 5.0	21.0; 8.8	0; 0.0
Ikadai *et al.* (2002)	Japan	Horses	2019	109; 5.4	44; 2.2	–
Hussain *et al.* (2014)	India	Horses/donkey/ mules	430	93; 21.6	177; 41.2	44; 10.2
Akkan *et al.* (2003)	Turkey	Horses	110	5; 4.6	71; 64.6	1; 0.9
Al-Obaidi *et al.* (2016)	Malaysia	Horses	306	193; 63.07	157; 51.3	105; 34.3
Acici *et al.* (2008)	Turkey	Horses/donkey/ mules	153	53; 34.6	33; 21.6	8; 5.2
Guven *et al.* (2017)	Turkey	Horses	125	0; 0	11; 8.8	–
Cruz-Flores *et al.* (2010)	Philippines	Horses	104	45; 43.3	6; 5.8	12; 11.5
Boldbaatar *et al.* (2005)	Mongolia	Horses	254	102; 40.2	185; 72.8	78,30.7
Chahan *et al.* (2006)	China	Donkey	93	36; 38.7	9; 9.7	2; 2.2
Avarzed *et al.* (1997)	Mongolia	Horses	110	93; 84.5	97; 88.18	–
Sigg *et al.* (2010)	Switzerland	Horses	689	10; 1.5	30; 4.4	10; 1.5
Piantedosi *et al.* (2014)	Italy	Donkey	203	72; 35.5	90; 44.3	46; 22.7
Moretti *et al.* (2010)	Italy	Horses	412	74; 17.9	51; 12.4	157; 38.1
Kouam *et al.* (2010)	Greece	Horses/mules	544	12; 2.2	60; 11.0	9.0; 1.7
Guidi *et al.* (2015)	France	Horses	443	57; 12.9	257; 58.01	36; 8.1
Grandi *et al.* (2011)	Italy	Horses	294	1; 0.3	24; 8.2	0; 0.0
García-Bocanegra *et al.* (2013)	Spain	Horses/donkey/ mules	537	61; 11.4	270; 50.3	45; 8.4

(*Continued*)

Table 1. (*Continued.*)

Study reference	Country	Host type	Sample size	*B. caballi* No. of positives; prevalence %	*T. equi* No. of positives; prevalence %	Mixed infection No. of positives; prevalence %
Gallusová *et al.* (2014)	Romania	Horses	178	8; 4.5	69; 38.8	–
Cortés *et al.* (2017)	Spain	Horses	3100	643; 20.7	1381; 44.6	398; 12.8
Camino *et al.* (2018)	Spain	Horses	536	25; 4.7	117; 21.8	11; 2.1
Camacho *et al.* (2005)	Spain	Horses	60	17; 28.3	24; 40.0	12; 20
Butler *et al.* (2012)	Netherland	Horses	300	9; 3.0	5; 1.7	3; 1.0
Bartolome *et al.* (2016)	Italy	Horses	673	60; 8.9	268; 39.8	–
Abedi *et al.* (2014)	Iran	Horses	100	2; 2.0	48; 48.0	3, 3.0
Abedi *et al.* (2015)	Iran	Donkey	106	0; 0	54; 50.9	–
Abutarbush *et al.* (2012)	Jordan	Horses	253	0; 0	37; 14.6	–
Alanazi *et al.* (2012)	Saudi Arabia	Horses	241	18; 7.5	25; 10.4	7; 2.9
Jaffer *et al.* (2010)	United Arab Emirates	Horses	105	11; 10.5	35; 33.3	13; 12.4
Kakekhani *et al.* (2017)	Iran	Horses	186	0; 0	1; 0.5	–
Qablan *et al.* (2013)	Jordan	Horses/donkey	288	21; 7.3	54; 18.8	–
Malekifard *et al.* (2014)	Iran	Horses	240	14; 5.8	26; 10.8	4; 1.67
Posada-Guzmán *et al.* (2015)	Costa Rica	Horses	285	90; 31.6	115; 40.4	81; 28.4
Cantú-Martínez *et al.* (2012)	Mexico	Horses	248	41; 16.5	85; 34.3	27; 10.9
Díaz-Sánchez *et al.* (2018)	Cuba	Horses	100	25; 25.0	73; 73.0	20; 20.0
Asgarali *et al.* (2007)	Trinidad	Horses	93	64; 68.8	31; 33.3	18; 19.4
Vieira *et al.* (2013)	Brazil	Horses	198	137; 69.2	155; 78.3	99; 50.0
Vieira *et al.* (2018)	Brazil	Horses	90	5; 5.6	17; 18.9	2; 10.5
Rosales *et al.* (2013)	Venezuela	Horses	694	161; 23.2	97; 13.9	90; 12.9
Mujica *et al.* (2011)	Venezuela	Horses	360	254; 70.6	181; 50.3	128; 35.6
Machado *et al.* (2012)	Brazil	Donkey	88	82; 93.2	65; 73.9	59; 67.0
Kerber *et al.* (2009)	Brazil	Horses	582	405; 69.6	155; 26.6	–
Heuchert *et al.* (1999)	Brazil	Horses	740	505; 68.2	211; 28.5	–
Heim *et al.* (2007)	Brazil	Horses	487	443; 90.9	404; 82.9	–

reviewed journal articles and no attempt was made to check dissertations or thesis. Studies were from Africa ($n = 11$), Asia ($n = 20$), Europe ($n = 14$), the Middle East ($n = 8$) and Americas ($n = 13$) (Table 1).

Pooled prevalence estimates

An overall pooled prevalence estimate (PPE) due to EP caused by *B. caballi* was 22.3% (95% CI 21.7–22.8) from the 66 eligible studies that reported 5348 cases in over 24 041 equids screened (Table 2). Individual point estimates were determined for studies reporting the occurrence of *B. caballi* (Fig. 2). Furthermore, a significant difference between study heterogeneity was observed ($Q = 8531.7$, $I^2 = 99.9$, 95% CI 99.2–99.3, $P < 0.0001$). The overall PPE due to *T. equi* was 29.4% (95% CI 28.7–30.0) from the 66 eligible studies with 7074 cases in 24 041 equids screened (Table 3). Individual point estimates were determined for the 66 eligible studies with regards to infection with *T. equi* (Fig. 3). Finally, 43 studies reported mixed infection with an overall PPE of 11.8% (95% CI 11.3–12.3) of the 16 250 equid samples.

According to region

The Americas had the highest prevalence of 47.9% (95% CI 45.8–49.9%, $Q = 1583.3$, $I^2 = 99.3$, $P < 0.0001$) while the lowest prevalence was in the Middle East (4.8%; 95% CI 3.7–5.8%, $Q = 92.1$, $I^2 = 92.4$, $P < 0.0001$) (Table 2 and Fig. 4). Although Asia region had the highest number of eligible studies examined within the period ($n = 22$) as well as the largest number of animals ($n = 8307$; 2124 cases), the prevalence was (25.6%; 24.5–26.6%). Similarly, the prevalence due to *T. equi* was highest in the Americas (46.5%; 95% CI 44.5–48.6%, $Q = 1131.5$, $I^2 = 99.0$, $P < 0.0001$) compared to the Middle East (18.1%; 95% CI 16.1–20.2%, $Q = 211.7$, $I^2 = 96.7\%$, $P < 0.0001$) and Asia (18.1%, 95% CI 17.2–18.9, $Q = 2379.4$, $I^2 = 99.1\%$, $P < 0.0001$) (Table 3).

According to sex

Infection due to both piroplasms was slightly higher in males. For infection due to *B. caballi*, the PPE in male equids was 5.5% (95% CI 4.9–6.0%, $Q = 813.7$, $I^2 = 98.4$, $P < 0.0001$) compared with 4.5% (95% CI 4.0–4.9%, $Q = 549.7$, $I^2 = 97.6$, $P < 0.0001$) in females

Table 2. Pooled prevalence and risk factors associated with *Babesia caballi* infection in equines 1990–2019

		Pooled prevalence estimates			Measure of heterogeneity		
Risk factors	Number of studies	Sample size	No of positives	Prevalence 95% CI (%)	*Q*	I^2 (95% CI)	*Q*–*P*
Overall							
B. caballi	66	24 041	5348	22.3 (21.7–22.8)	8531.7	99.9 (99.2–99.3)	0.0001
Region							
Africa	11	2443	266	10.9 (9.90–12.1)	416.5	97.6 (96.8–98.2)	0.0001
Asia	22	8307	2124	25.6 (24.5–26.6)	2915.7	99.3 (99.2–99.4)	0.0001
Middle East	8	1519	73	4.8 (3.7–5.8)	92.1	92.4 (87.4–95.4)	0.0001
Europe	13	7807	987	12.6 (11.9–13.4)	827.7	98.6 (98.2–98.9)	0.0001
Americas	12	3965	1898	47.9 (45.8–49.9)	1583.3	99.3 (99.2–99.4)	0.0001
Sex							
Male	14	6952	383	5.5 (4.9–6.0)	813.7	98.4 (97.9–98.7)	0.0001
Female	14	6952	312	4.5 (4.0–4.9)	549.7	97.6 (96.9–98.2)	0.0001
Age							
<5	11	2489	342	13.7 (12.4–15.1)	554.7	98.2 (97.6–98.6)	0.0001
>5	11	2489	283	11.4 (10.1–12.6)	420.9	97.6 (96.8–98.2)	0.0001
Diagnostic technique							
Microscopy	23	5129	212	4.1 (3.6–4.7)	459.8	95.2 (93.9–96.3)	0.0001
ELISA	26	11 006	3014	27.4 (26.5–28.3)	4779.3	99.5 (99.4–99.5)	0.0001
IFAT	26	10 230	2344	22.9 (22.0–23.8)	2849.9	99.1 (99.0–99.2)	0.0001
PCR	30	6143	515	8.4 (7.7–9.1)	1039.1	97.2 (96.6–97.7)	0.0001
Species							
Horses	56	21 358	4870	22.8 (21.2–23.4)	77 805	99.3 (99.2–99.4)	0.0001
Donkey	11	2148	333	15.5 (14.4–17.7)	807.4	98.9 (98.6–99.1)	0.0001
Years of study							
1990–1999	3	1026	277	26.9 (23.9–30.0)	64.7	96.9 (93.7–98.5)	0.0001
2000–2009	15	5363	1589	29.6 (28.2–31.0)	3236.6	99.6 (99.5–99.6)	0.0001
2010–2019	48	17 652	3482	19.7 (19.1–20.4)	4976.3	99.1 (98.9–99.2)	0.0001

ELISA, enzyme linked immunosorbent assay; IFAT, Immunofluorescence Antibody Test; PCR, polymerase chain reaction.

(Table 2). A similar observation was also noted in respect to infection with *T. equi*, males had a prevalence of 16.9% (95% CI 15.5–18.5%, $Q = 1003.3$, $I^2 = 98.2$) compared with 16.4% in females (95% CI 15.6–17.3%, $Q = 682.7$, $I^2 = 97.4$, $P < 0.0001$) (Table 3 and Fig. 5).

According to age

For *T. equi* infections, the prevalence was slightly higher for those <5 years (16.9%; 95% CI 15.5–18.5%, $Q = 397.2$, $I^2 = 97.5$, $P < 0.0001$) compared with those >5 years (16.4%; 95% CI 14.9–17.9%, $Q = 229.9$, $I^2 = 95.7$, $P < 0.0001$) (Table 3 and Fig. 6). A similar observation was noted in infection due to *B. caballi*, with prevalence higher in those animals <5 years (13.7%; 95% CI 12.4–15.1%, $Q = 554.7$, $I^2 = 98.2$, $P < 0.0001$) compared with equids >5 years (11.4%; 95% CI 10.1–12.6%, $Q = 420.9$, $I^2 = 97.6$, $P < 0.0001$) (Table 2, S1-Supplementary file).

According to diagnostic technique

The PPE for different *B. caballi* diagnostic methods indicated that ELISA tests were associated with the highest exposure (27.4%; 95% CI 26.5–28.3%, $Q = 4779.3$, $I^2 = 99.5$, $P < 0.0001$) (Table 2, Fig. 7), followed by IFAT (22.9%; 95% CI 22.0–23.8%, $Q = 2849.9$, $I^2 = 99.1$, $P < 0.0001$), PCR (8.4%; 95% CI 7.7–9.1%) and microscopy (4.1%; 95% CI 3.6–4.7%, $Q = 459.8$, $I^2 = 95.2$, $P < 0.0001$) (Table 2). The PPE for *T. equi* using diferent diagnostic methods indicates that IFAT technique was associated with the highest exposure (41.1%; 95% CI 39.9–42.3%, $Q = 3167.4$, $I^2 = 99.2$, $P < 0.0001$) (Table 3, Fig. 8), followed by PCR (31.6%; 95% CI 30.2–32.9%, $Q = 2610.6$, $I^2 = 98.9$, $P < 0.0001$), ELISA (21.9%; 95% CI 21.0–22.7%, $Q = 3037.3$, $I^2 = 99.2$, $P < 0.0001$) and microscopy (8.1%; 95% CI 7.4–8.9%, $Q = 863.0$, $I^2 = 97.5$, $P < 0.0001$) (Table 3).

According to equid species

Infection due to *B. caballi* was higher in horses (*Equus caballus*) (22.8%; 95% CI 21.2–23.4%, $Q = 77\,805$, $I^2 = 99.3$, $P < 0.0001$) as compared to donkeys (*Equus asinus*) (15.5%, 95% CI 14.4–17.7%, $Q = 807.4$, $I^2 = 98.9$, $P < 0.0001$) (Table 2, S2-Supplementary file). Infection due to *T. equi* was higher in donkeys (50.9%; 95% CI 48.1–53.9%, $Q = 379.1$, $I^2 = 97.4$, $P < 0.0001$) as compared to horses (27.8%; 95% CI 27.1–28.5%, $Q = 7478.8$, $I^2 = 99.3$, $P < 0.0001$) (Table 3).

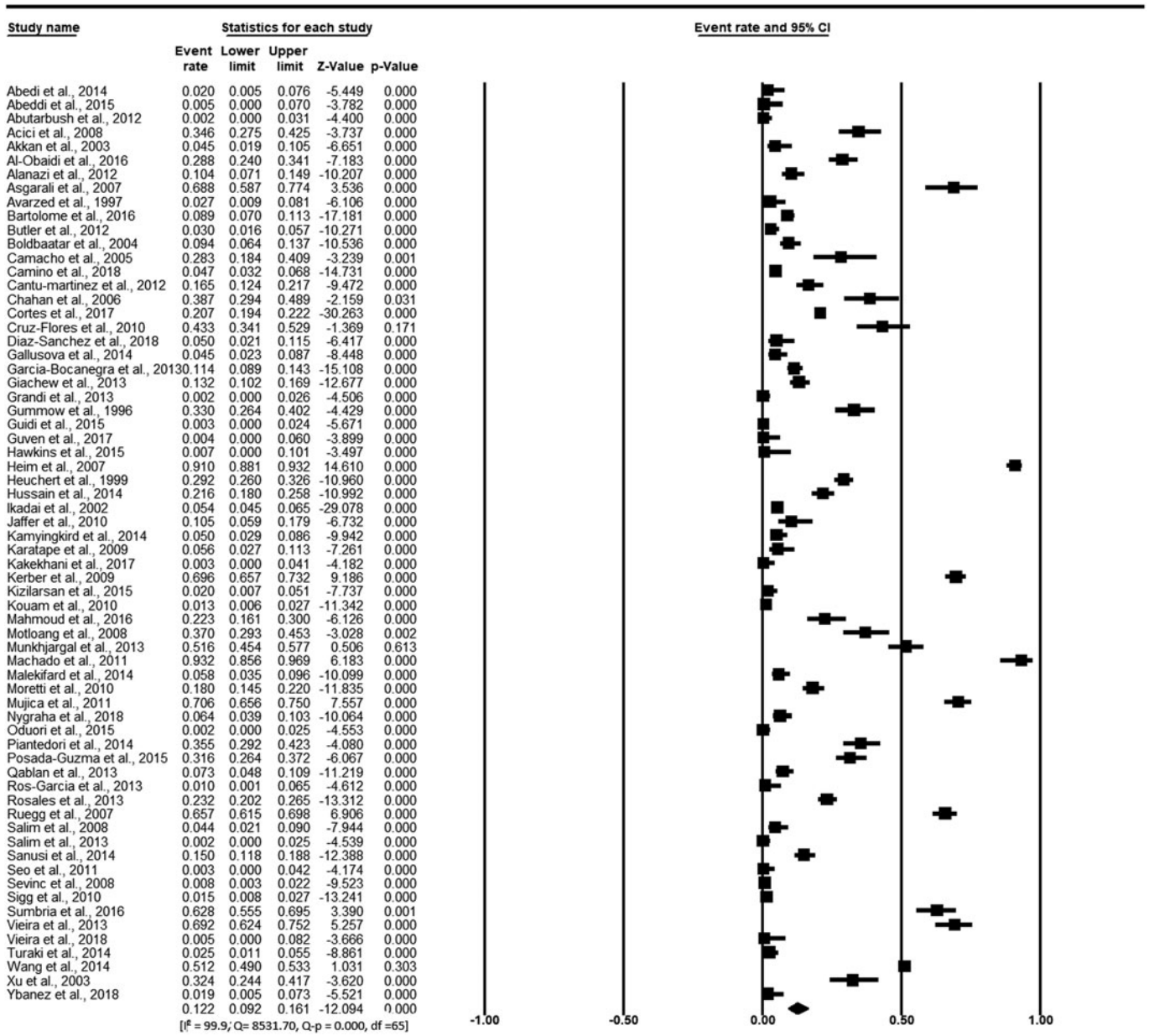

Fig. 2. Forest plot of the prevalence estimates of *Babesia caballi* in equids globally from 1990 and 2019. Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

According to years of study

The time span of 1990–1999 had a higher PPE of *B. caballi* (26.9%; $n = 3$, $Q = 64.7$, $I^2 = 96.9$ $P < 0.0001$) as compared to the period of 2010–2019 (19.7%; $n = 46$, $Q = 4976.3$, $I^2 = 99.1$, $P < 0.0001$) (Table 2; S3-Supplementary file). Similarly, the time span of 1990–1999 had a higher *T. equi* PPE (59.7%; $Q = 202.1$, $I^2 = 99.0$, $P < 0.0001$) as compared to the period of 2010–2019 (29.3%; $Q = 4399.2$, $I^2 = 98.9$, $P < 0.0001$) (Table 3).

Heterogeneity and publication bias

Results from our study showed strong heterogeneity between the selected studies which were largely influenced by the number of studies and diagnostic techniques. No publication bias was observed in subgroup analysis due to diagnostic technique, age, sex and region. Major publication bias was observed only in the overall PPEs due to *B. caballi* [Egger's intercept ($B0$) = −4.79, $P = 0.003$] and *T. equi* [Egger's intercept ($B0$) = −3.67, $P < 0.05$].

Discussion

It is evident that EP is widespread and endemic in various regions of the world. The PPE for *T. equi* infection was 29.4%. This estimate is relatively similar to the prevalence of 30.9% reported in Kenya (Hawkins *et al.*, 2015) and 26.6% in Brazil and Egypt (Kerber *et al.*, 2009; Mahmoud *et al.*, 2016). Higher prevalences have been reported in several studies across different regions of the world (Gummow *et al.*, 1996; García-Bocanegra *et al.*, 2013; Sumbria *et al.*, 2016; Díaz-Sánchez *et al.*, 2018; Onyiche *et al.*, 2020). The PPE due to *B. caballi* was 22.3%, lower than that of *T. equi*. Generally, the prevalence of *T. equi* has been found to be higher than that of *B. caballi* likely due to the fact that *T. equi*-infected animals remain infected for life (Rüegg *et al.*, 2007). Another possible reason for the differences in the PPE between the two pathogens could be due to differences in vector distribution (Salim *et al.*, 2008). Mixed infection of the two piroplasms has been reported in different studies and is unconnected with the presence of the tick vectors responsible for the transmission of both pathogens within the same geographical area infesting their host.

Table 3. Pooled prevalence and risk factors associated with *Theileria equi* infection in equines 1990–2019

		Pooled prevalence estimates			Measure of heterogeneity		
Risk factors	Number of studies	Sample size	No of positives	Prevalence 95% CI (%)	*Q*	I^2 95% CI	*Q–P*
Overall							
T. equi	66	24 041	7074	29.4 (28.7–30.0)	8265.4	99.2 (99.2–99.3)	0.0001
Region							
Africa	11	2443	1085	44.4 (41.9–46.9)	1583.3	99.3 (99.2–99.4)	0.0001
Asia	22	8307	1503	18.1 (17.2–18.9)	2379.4	99.1 (98.9–99.2)	0.0001
Middle East	8	1519	275	18.1 (16.1–20.2)	211.7	96.7 (95.1–97.8)	0.0001
Europe	13	7807	2366	30.3 (29.2–31.5)	1786.2	99.3 (99.2–99.4)	0.0001
Americas	12	3965	1845	46.5 (44.5–48.6)	1131.5	99.0 (98.8–99.2)	0.0001
Sex							
Male	19	8449	1435	16.9 (16.2–17.8)	1003.3	98.2 (97.8–98.5)	0.0001
Female	19	8449	1391	16.5 (15.6–17.3)	682.7	97.4 (96.7–97.9)	0.0001
Age							
<5	11	2489	423	16.9 (15.5–18.5)	397.2	97.5 (96.6–98.1)	0.0001
>5	11	2489	409	16.4 (14.9–17.9)	229.9	95.7 (93.8–96.9)	0.0001
Diagnostic technique							
Microscopy	23	5129	418	8.1 (7.4–8.9)	863.0	97.5 (96.9–97.9)	0.0001
ELISA	26	11 006	2406	21.9 (21.0–22.7)	3037.3	99.2 (99.1–99.3)	0.0001
IFAT	26	10 230	4209	41.1 (39.942.3)	3167.4	99.2 (99.1–99.3)	0.0001
PCR	30	6143	1940	31.6 (30.2–32.9)	2610.6	98.9 (98.7–99.0)	0.0001
Species							
Horses	56	21 358	5932	27.8 (27.1–28.5)	7478.8	99.3 (99.2–99.3)	0.0001
Donkey	11	2148	1095	50.9 (48.1–53.9)	379.1	97.4 (96.4–98.1)	0.0001
Years of study							
1990–1999	3	1026	614	59.8 (55.3–64.4)	202.1	99.0 (98.4–99.4)	0.0001
2000–2009	15	5363	1573	29.3 (27.9–30.7)	2885.5	99.5 (99.4–99.6)	0.0001
2010–2019	48	15 809	4635	29.3 (28.5–30.1)	4399.2	98.9 (98.9–99.1)	0.0001

ELISA, enzyme linked immunosorbent assay; IFAT, Immunofluorescence Antibody Test; PCR, polymerase chain reaction.

Diagnosis of EP can be achieved by either the use of direct or indirect methods (Abedi *et al.*, 2015). The gold standard for piroplasm's diagnosis is microscopy but poor sensitivity during low parasitaemia limits its use (Böse *et al.*, 1995). Microscopy and PCR techniques are considered direct methods as they indicate active infection and serological assays are considered indirect as they detect the presence of antibodies which is an indicator of exposure rather than an indication of infection status (Abedi *et al.*, 2015). We observed that the IFAT method detected higher exposure to *T. equi*, and ELISA detected higher exposure of *B. caballi*. Infection with *B. caballi* is transient and best detected during the acute phase of the infection due to low parasitaemia associated with it. Competitive ELISA (cELISA) based on *rap*-1 demonstrated higher exposure to antibodies of *B. caballi* as observed in the Venezuelan isolates (Rosales *et al.*, 2013). Nonetheless, the *rap*-1 region is believed to be highly polymorphic as demonstrated in some epidemiological studies with no positive samples detected using cELISA in Egypt, South Africa and Israel (Bhoora *et al.*, 2010; Rapoport *et al.*, 2014; Mahmoud *et al.*, 2016). Due to variation in the *rap*-1 gene between geographically diverse isolates with differences in their amino acid sequences, this has led to inconsistency in the commercial *rap*-1 cELISA assays for the detection of *B. caballi* strain (Idoko *et al.*, 2020). Therefore, the commercial cELISA for *B. caballi* is problematic and can lead to a high number of false negatives hence leading to lack of positive samples in some region of the world (Bhoora *et al.*, 2010).

On the other hand, infection with *T. equi* is often lifelong and exposed equids seroconvert after a brief period of infection, usually within 14–16 days. According to the OIE (2005), horses deemed for export must have a negative result to EP when screened using either IFAT or ELISA techniques which remain to be recommended diagnostic methods based on the OIE manual for diagnostic tests and vaccines for terrestrial manual. Therefore, it is not surprising that serological techniques (IFAT and ELISA) were the most efficient in determining the exposure of equids to EP.

Furthermore, microscopy was associated with low prevalence for both pathogens. In several studies, piroplasms were not detected in blood smears but were detected using other techniques such as PCR and serology on same samples that were initially negative (Abutarbush *et al.*, 2012; Munkhjargal *et al.*, 2013). However, the OIE diagnostic manual recommends that microscopic examination to be used in some situations (OIE,

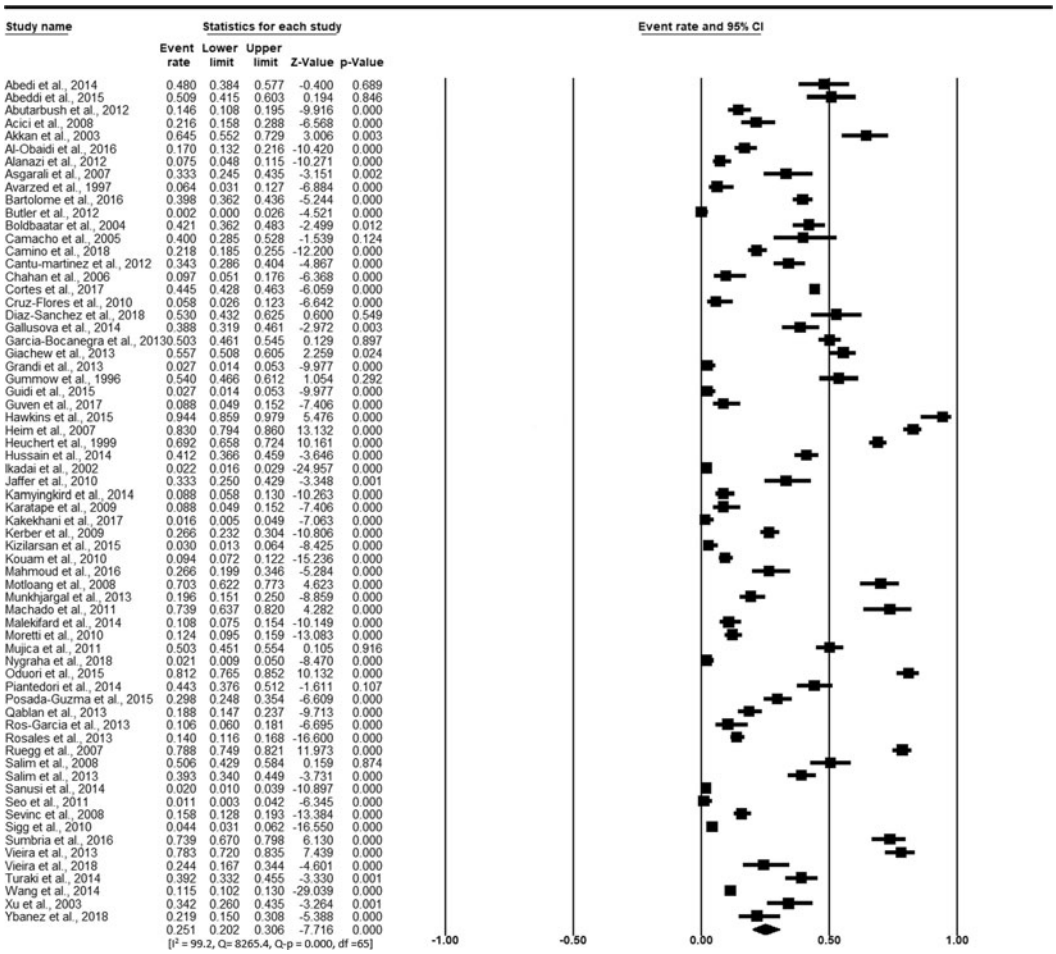

Fig. 3. Forest plot of the prevalence estimates of *Theileria equi* in equids globally from 1990 and 2019. Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

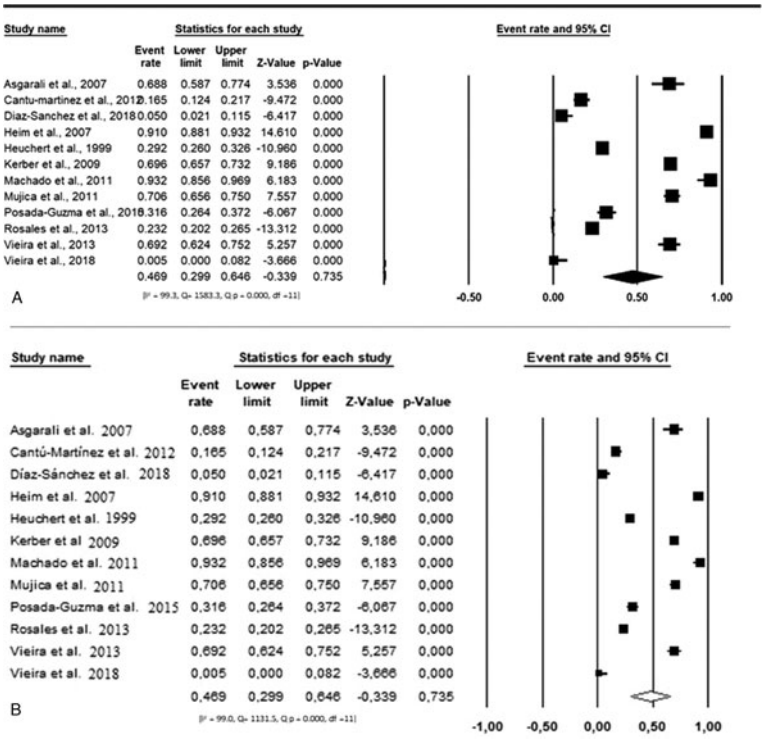

Fig. 4. Forest plot of the prevalence estimates due to equine piroplasms in the Americas. Prevalence due to *T. equi* in the Americas is illustrated in (A) while estimates due to *B. caballi* are shown in (B). Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

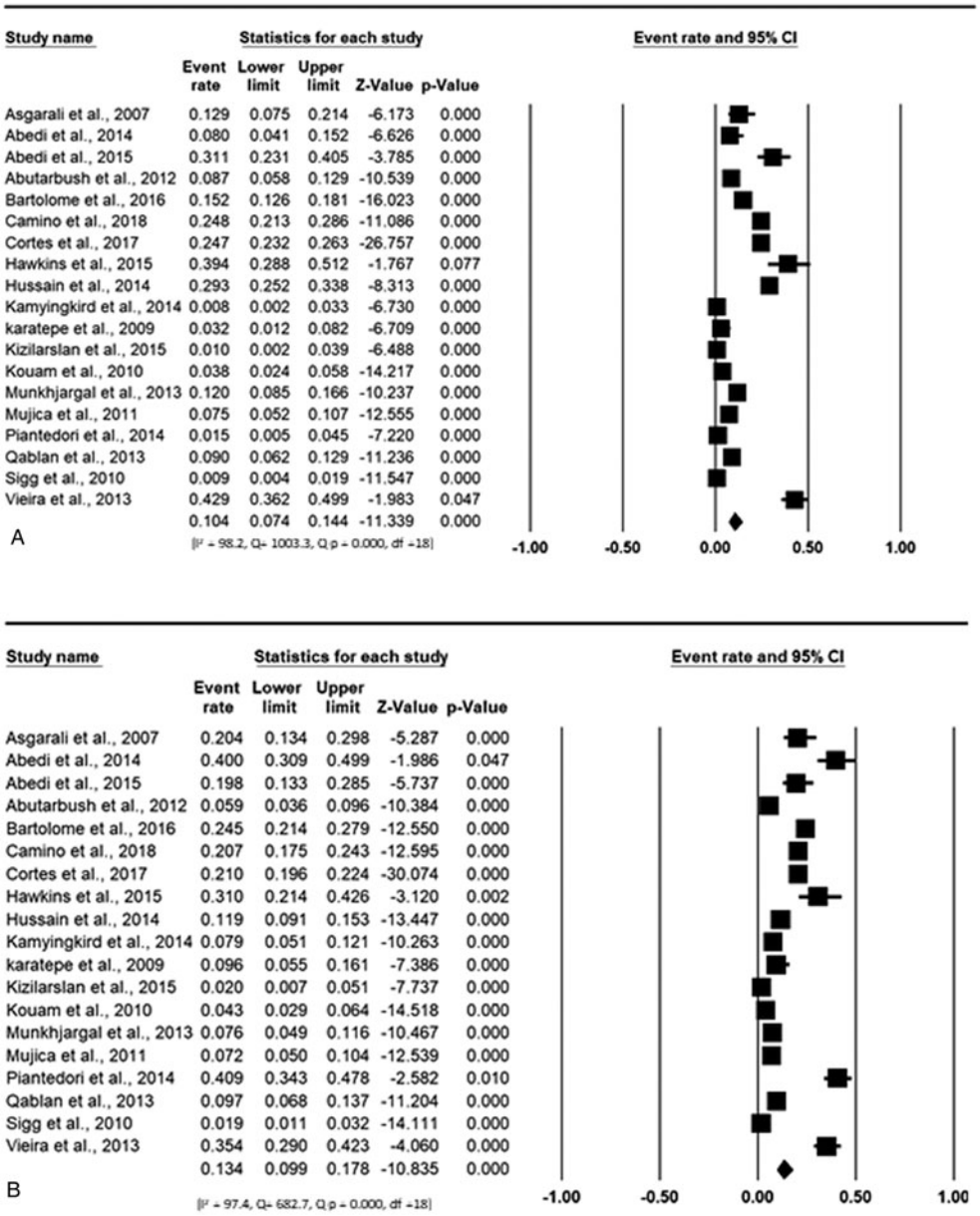

Fig. 5. Forest plot of the prevalence estimates of *Theileria equi* in male equids (A) compared with females (B) from 1990 and 2019. Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

2018). The method continues to be applied in resource-poor countries despite its disadvantages of poor sensitivity during low parasitaemia.

The Americas had the highest PPE for both *B. caballi* and *T. equi* infection while the Middle East had the least estimates for both pathogens. The difference in prevalences among geographical regions may be due to the sensitivity of the various diagnostic tests that have been used in the different epidemiological studies; abundance and occurrence of competent tick vectors; husbandry system; activity of the equids; effectiveness of the control measures instituted at the farm and national levels (Kouam *et al.*, 2010). In some parts of Africa, the prevalence of EP caused by *T. equi* was high (Motloang *et al.*, 2008; Hawkins *et al.*, 2015; Oduori *et al.*, 2015). Also, few epidemiological studies have been conducted in the continent despite a handful of equitation sports and traditional local festival where the use of horses is common. It is therefore expedient that more testing be conducted which is necessary before the institution of treatment and control.

The PPE for both *B. caballi* and *T. equi* indicates that these parasites are more prevalent in males as compared to females. Individual studies have reported contrasting observations (Sigg *et al.*, 2010; Abedi *et al.*, 2014). Nevertheless, the difference between sexes has not been significant in majority of the individual studies. However, males may have higher tick exposure and immune-suppression due to stress arising from strenuous physical activities (Vieira *et al.*, 2013). This may consequently lead to higher infection rates in males. Furthermore, younger equids (<5 years) had a slightly higher PPE for both pathogens compared to the older ones (>5 years). Generally, young horses may reside longer in the fields and consequently, more exposure to tick vectors which increases their likelihood of infection with tick-borne pathogens as compared to adults.

A majority of EP studies focused on horses. The high interest in research-related studies on horses compared to other equids could be due to their high economic value compared with donkeys and other equids (Onyiche *et al.*, 2019). *Theileria equi* PPE was higher in the donkeys as compared to horses. Donkeys are asymptomatic carriers of piroplasms with low parasitaemia and positive antibody titres throughout their lifetime (Balkaya *et al.*, 2010). However, PPE was higher in the infection of horses with *B. caballi*.

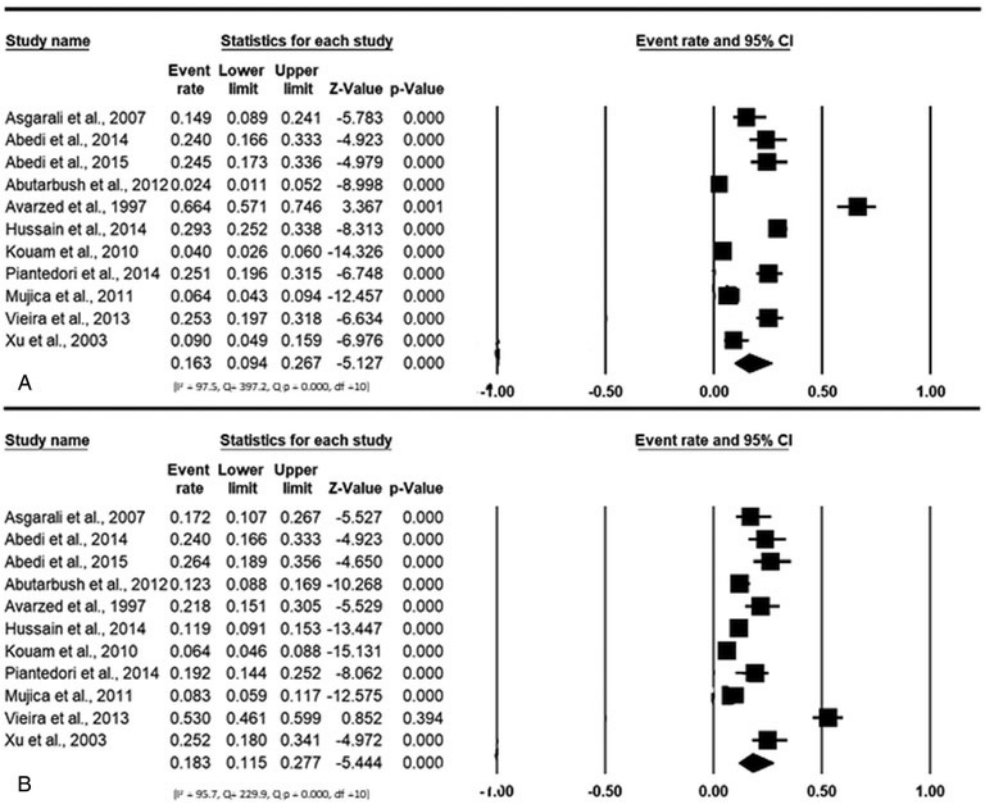

Fig. 6. Forest plot of the prevalence estimates of *Theileria equi* in equids <5 years old (A) compared with those above 5 years old (B) from 1990 and 2019. Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

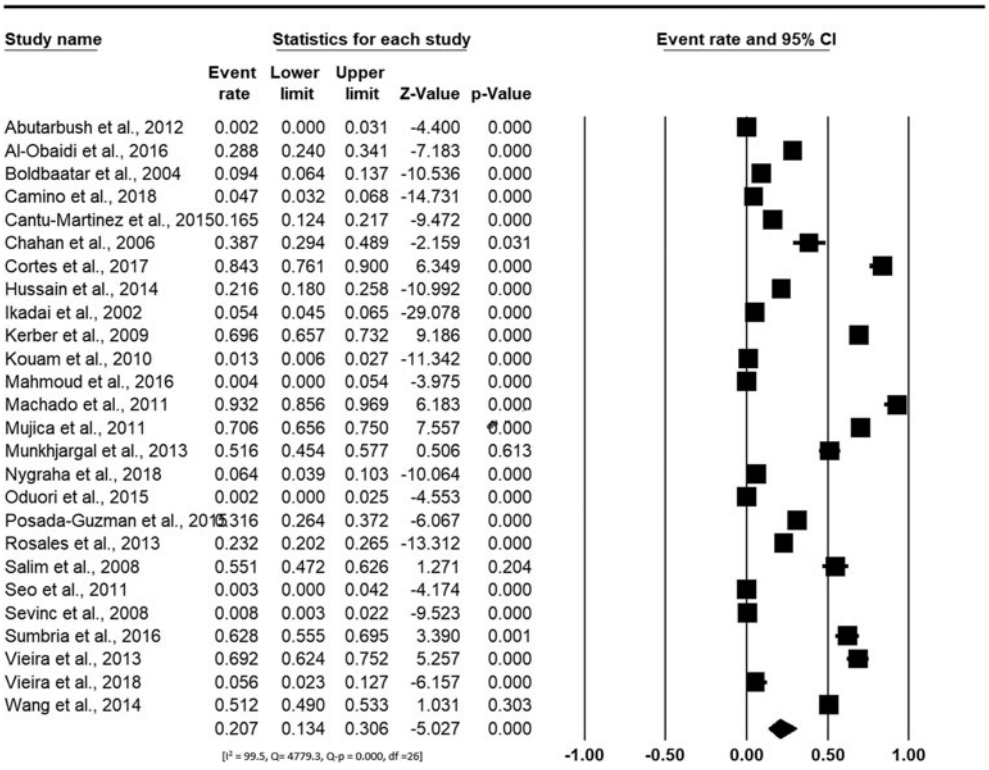

Fig. 7. Forest plot of the prevalence estimates of *Babesia caballi* using ELISA as a diagnostic technique in equids from 1990 and 2019. Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

In the period spanning 2010–2019, the global PPE due to *T. equi* has remained stable at 29.3% down from the earlier 59.8% during the period 1990–1999. Similarly, the global PPE due to *B. caballi* has decreased from 26.9% between the period spanning 1990 and 1999 to 19.7% covering the period 2010–2019. The decrease in the prevalence could be attributed to a better

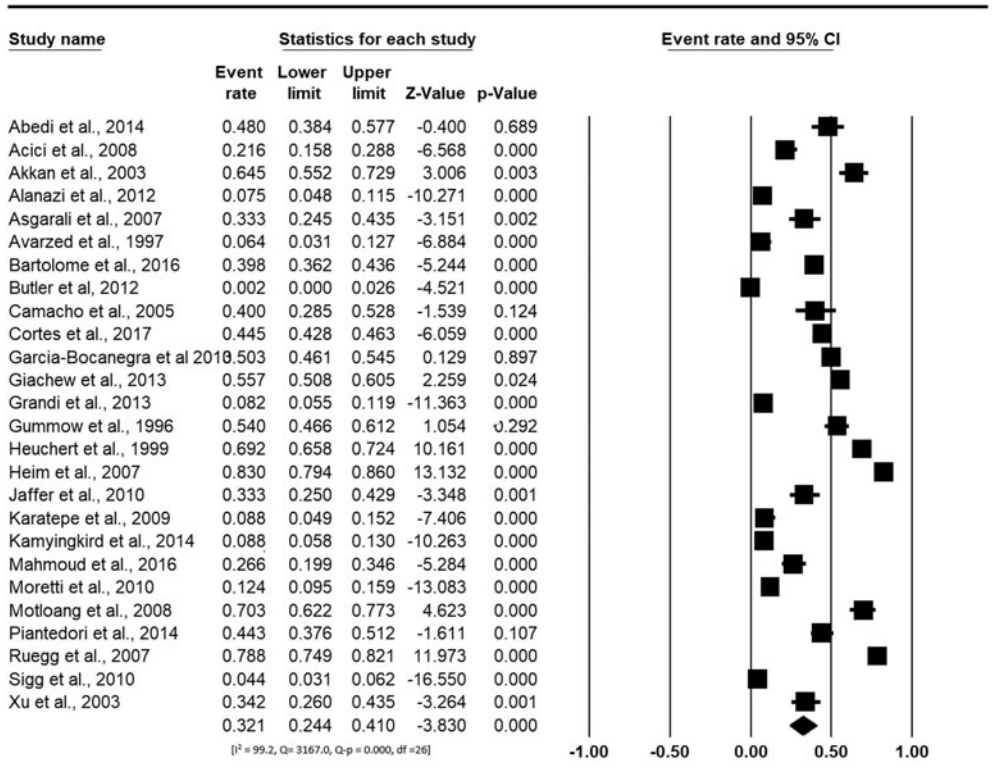

Fig. 8. Forest plot of the prevalence estimates of *Theileria equi* using IFAT as a diagnostic technique in equids from 1990 and 2019. Note: The squares show the individual point estimate. The diamond at the base indicates the pooled estimates from the total studies.

understanding of the epidemiology of the parasite and more efficient control of the vectors. Furthermore, the testing of equids before their export as recommended by the OIE may have further helped to decrease the burden of the disease and help in the curtailment of the spread of the disease between different regions of the world. Additionally, we speculate that the decrease in EP over the time period could also be attributed to differences in the diagnostic techniques over the years.

We have attempted to present a systematic review and meta-analysis of exposure of equids to EP to gain more insight on the global epidemiology of the disease. Due to the pooling of data, we acknowledge that this will lead to significant heterogeneity as a result of the differences in the characteristics among the identified studies despite the use of random-effect model. Some of the limitations include paucity of data which varies across region, publication bias, uneven distribution of prevalence across countries and low sample size in some studies. Therefore, results must be interpreted with caution as apparent prevalence may vary from the actual estimates. Nevertheless, we believe that our report is very close to true estimates of the global exposure of equids to agents of EP.

Conclusion

To the best of our knowledge, this study represents the first systematic review and meta-analysis on the global exposure of equids to agents of EP to better understand the distribution of the disease across the world in the last three decades. All eligible studies incorporated in this systematic review were cross-sectional, further studies incorporating case–control and cohort studies will be required to expand our knowledge horizon on the risk factors and exposures to this disease. Lastly, they are urgent needs for discovering candidate antigens for improved diagnostic tools for the control of equine babesiosis most especially in Africa and the Middle East. Therefore, further studies to fill in this knowledge gap are expedient.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182020001407

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Conflict of interest. No conflict of interest exists among the authors.

Ethical standards. Not applicable.

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