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REVIEW

DOGS

Prevalence of *Anaplasma*, *Ehrlichia* and *Rickettsia* infections in dogs in Iran: A meta-analysis study

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Abstract

The current study aims to assess the prevalence of different genera of *Rickettsiales*, intracellular bacteria, in dogs across various regions of Iran. *Rickettsiales*, as zoonotic pathogens, can have various manifestations in different species, including immunosuppression, anaemia and neuropathy. Due to their close interactions with people and livestock, dogs can serve as reservoirs and transmit these pathogens to humans and other animals. The data from this research can be valuable for managing and controlling related diseases and complications in Iran and possibly neighbouring countries. Pertinent data for this study was gathered without time limitations until 1 March 2022, from different databases. Of all the inspected studies, 26 were eligible based on the inclusion criteria. The Egger test result and asymmetry in the funnel plot revealed significant publication bias; therefore, the meta-analysis model was corrected with the trim-and-fill method. After correction, the prevalence of rickettsial infections among dogs varies by genus – 20.1% for *Anaplasma* spp. and 10% for *Ehrlichia* spp. – with an overall prevalence estimated at 18.3% by random-effects analysis. The highest and lowest estimated pooled prevalences were associated with the southwestern (38.5%) and southern (0.3%) provinces of Iran, respectively. The pooled prevalence of rickettsial infection was higher in female dogs (OR = 1.198; $p < 0.978$; 95% CI, 0.842–1.705) and in dogs ≤2 years (OR = 1.014; $p < 0.312$; 95% CI, 0.598–1.72), but the difference was not statistically significant. Ultimately, the pooled prevalence of rickettsial infections among dogs is relatively low compared to other countries like Qatar, Iraq, Saudi Arabia and Turkey, which are nearby Iran. Given the significant clinical outcomes of this disease, necessary measures for prevention should be taken.

KEYWORDS
Anaplasma, *Ehrlichia*, Iran, meta-analysis, *Rickettsia*

1 | INTRODUCTION

Rickettsiales is an order of intracellular alphaproteobacteria with commensal characteristics, including genera, such as *Rickettsia*, *Ehrlichia* and *Anaplasma* (Bourtzis, 2008). Typically, these organisms are transmitted to mammalian hosts by parasitic invertebrate vectors like ticks, fleas and mites (Werren et al., 2008). The replication of rickettsial agents occurs in the cytoplasm or vacuoles of infected cells through binary fission. Daughter cells are released into the cytoplasm, moving to adjacent host cells through the membrane via actin-mediated polymerization (Salje, 2021). Dogs are considered reservoirs for various vector-borne diseases, encompassing *Rickettsia*, *Ehrlichia* and *Anaplasma* genera (Iatta et al., 2021). Zoonotic agents within this order can cause severe human diseases such as rickettsioses, anaplasmosis and ehrlichiosis (Lu et al., 2017). *Rickettsiales* employ different mechanisms to manipulate the host and evade potential immunological responses, including the manipulation of metabolic pathways, apoptosis and reproduction (Renvoisé et al., 2011).

Each species in the *Rickettsiales* order infects specific cells in the host's body. These primary cells are found in the bloodstream, including lymphocytes, monocytes, platelets or other origins like epithelial cells (Greene, 1999). Depending on the genus of the bacterium, different primary or secondary pathological signs are expected. For instance, *Ehrlichia canis* can impair phagolysosome formation to prevent a suitable immune response (McBride & Walker, 2011). Furthermore, it causes the production of antiplatelet antibodies and haemolytic anaemia, resulting in a combination of thrombocytopenia and anaemia (Waner et al., 2000). Due to the immunosuppression caused by ehrlichiosis, meningitis can be a secondary complication with neurological signs such as ataxia, seizures and twitching (Codner et al., 1992; Safari et al., 2022). Lowering the risk of tick bites is a suitable preventative method. Medicines used for treating rickettsial diseases depend on the genus of the infecting agent and usually include tetracycline derivatives, particularly doxycycline and oxytetracycline, along with other antibiotics, such as azithromycin, rifampin and chloramphenicol, along with supportive treatment based on the patient's clinical situation (Greene, 1999). Different diagnostic methods can detect various genera of rickettsial infections, including bacterial culture, direct visualization, serological assays and the polymerase chain reaction (PCR) of whole blood, which is particularly helpful during the early and asymptomatic stages of infection (Dawson et al., 2005). However, due to the difficulties of the first two methods (Bacterial culture and direct visualization), serological assays and PCR of whole blood appear to be more appropriate alternatives, particularly in the symptomatic phase and later stages of the disease.

Genera like *Anaplasma*, *Ehrlichia* and *Rickettsia* are highly prevalent in tropical and subtropical countries, including Iran, owing to favourable climate conditions and limited adoption of preventive measures. The estimation of the prevalence of these *Rickettsiales* genera in dogs has been previously conducted in various regions neighbouring Iran, such as Iraq (Iatta et al., 2021), Turkey (Kirkan et al., 2013), Saudi Arabia (Alanazi et al., 2021) and Qatar (Alho et al., 2017). These studies make it challenging to accurately gauge their impact on animal and human populations because of variations in diagnostic methodologies and the absence of universally applicable risk factors in each study, specific to the respective countries. Consequently, the implementation of preventive methods to minimize infection risks faces obstacles.

This article thoroughly explores *Rickettsiales*, delving into their commensal features, transmission methods and notable zoonotic potential, particularly in the context of dogs. It illuminates the intricate background of *Rickettsiales*, shedding light on their replication mechanisms, target cells and associated pathological signs. Emphasizing the importance of a nuanced understanding of these facets, the introduction smoothly transitions to the study's primary objective: conducting the inaugural systematic review and meta-analysis on the prevalence of *Anaplasma*, *Ehrlichia* and *Rickettsia* genera in dogs across diverse regions of Iran. The narrative seamlessly progresses to the methodology section, elucidating the study's design, search strategy, data extraction and statistical analysis. Subsequently, the study provides a preview of results, encompassing search outcomes, characteristics of eligible studies, pooled prevalence, risk factors and publication bias assessment. This comprehensive approach significantly contributes to a holistic understanding of rickettsial infections in Iranian dogs. The conclusion underscores the study's relevance for veterinary and public health, acknowledges limitations transparently and proposes avenues for future research.

The present survey is the first systematic review and meta-analysis based on various articles published on the prevalence of the *Anaplasma*, *Ehrlichia* and *Rickettsia* genera aimed to provide a prospect of the prevalence of dog rickettsiosis in different regions of Iran. Due to the apparent proximity of dogs to humans and the fact that many of these bacteria are zoonotic and able to infect human hosts, recognizing the prevalence of these important pathogens in the country can be valuable for both veterinary and medical endeavours.

2 | MATERIALS/METHODS

The current study was conducted using the preferred reporting items for systematic reviews and meta-analysis (Jokar et al., 2022; Abdous et al., 2021).

2.1 | Bibliographic search strategy

Articles related to the study were systematically selected without time limitations until 1 December 2022, from a comprehensive range of databases, including seven English databases (PubMed, Springer, Google Scholar, Science Direct, Scopus, Web of Science and ProQuest) and five Persian databases (ElmNet, Civilica, Magiran, Irandoc and Scientific Information Database – SID). Additionally, essays and congress articles were sourced from Irandoc, Civilica and ElmNet. The search strategy incorporated MeSH terms: ('Epidemiological survey') AND ('Prevalence' OR 'prevalence') AND ('*Rickettsia*' OR '*Rickettsia* spp.')

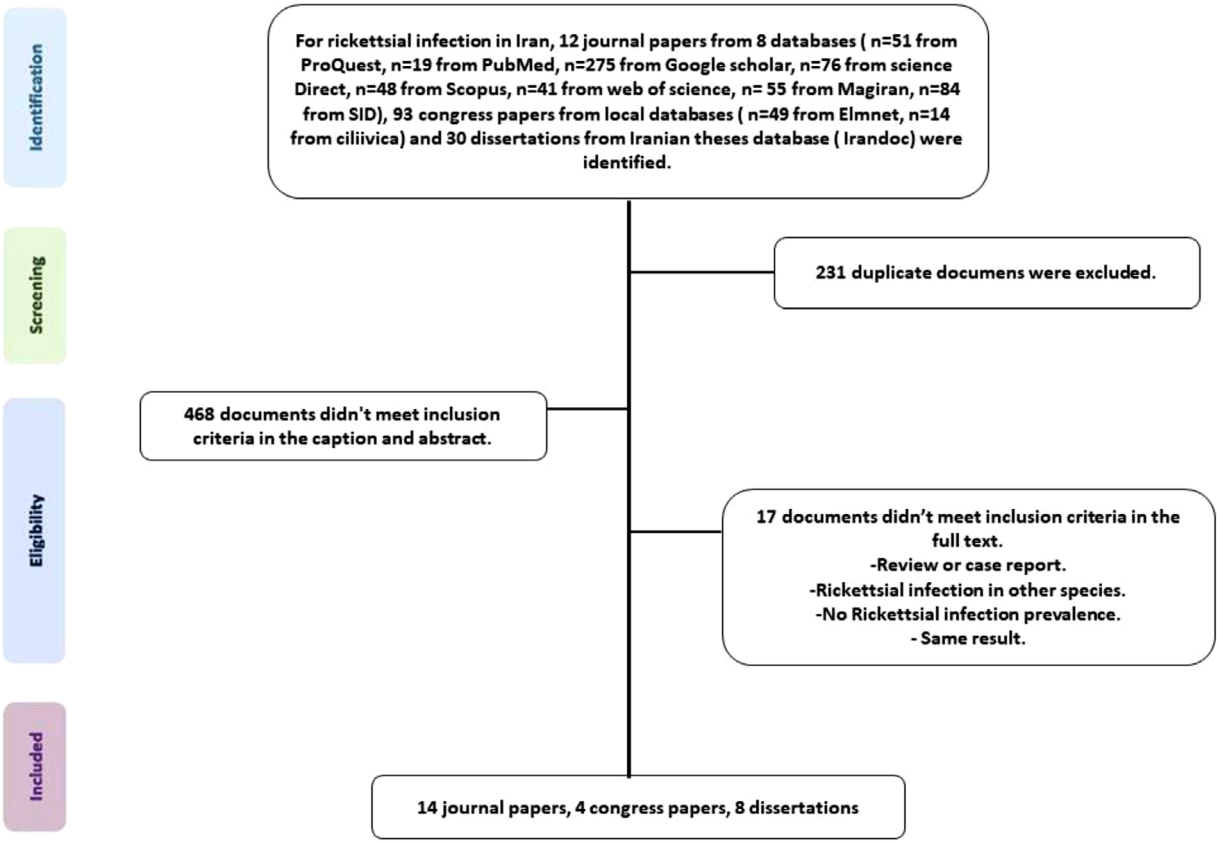

FIGURE 1 PRISMA flowchart presenting the selection of articles analysed in the systematic review and meta-analysis.

AND ('*Ehrlichia*' OR '*Ehrlichia* spp.') AND ('*Anaplasma*' OR '*Anaplasma* spp.') AND ('Dog') AND ('Iran') for English and Persian sources, respectively.

Furthermore, to minimize data loss, the study considered related articles and cross-referenced the references of identified studies. The PRISMA Flowchart visually elucidates the study selection process (Figure 1).

2.2 | Data Extraction and synthesis

To systematically collect data from relevant studies, an Excel-based data extraction form was utilized. This form captured crucial information, such as the first author's name, publication year, study location, sample size, the total number of positive cases for rickettsial diseases and data on sex and age groups. To ensure transparency and reproducibility, additional details were provided on the comprehensive methodology. This included an elaboration on the databases used for the literature search, the specific search terms employed, inclusion/exclusion criteria and the rigorous study selection process (Table 1).

2.3 | Inclusion and exclusion criteria

The literature was systematically reviewed by meticulously evaluating the abstracts and titles of articles associated with different genera of rickettsial infection. Throughout this process, the names of the authors and the journal sources were transparently disclosed to the reviewers. The essential data for analysis, including the author's name, study year, study location, methodology and sample sizes within each group, were diligently recorded in an electronic datasheet.

In instances where articles were not accessible through authorized databases, the research team proactively reached out to the authors for supplementary information. To ensure the integrity of the analysis, all studies underwent rigorous review, and the data listed in the table underwent thorough screening following our inclusion criteria. Duplicates were systematically expunged, and the complete content of each survey was independently examined by two reviewers, with any discrepancies promptly resolved through the mediation of a third specialist (Jokar, Bokaie et al., 2021, 2023).

Our inclusion criteria were thoughtfully defined, comprising the following aspects: (1) studies conducted within the dog population in Iran; (2) studies reporting the prevalence of different genera of rickettsial infections; (3) studies utilizing serological and molecular methods for diagnosing rickettsial diseases. Conversely, the exclusion criteria were explicitly outlined, encompassing: (a) any study not cross-sectional in nature (e.g., experimental and case reports); (b) studies conducted on species other than dogs; (c) studies failing to mention the prevalence of rickettsial infection; (d) studies not employing serological and molecular methods for detecting *Rickettsiales*; (e) any study utilizing self-developed diagnostic methods with low specificity and sensitivity; (f) studies where the methodology for detecting *Rickettsiales* was not listed in the article.

TABLE 1 Article met the eligibility criteria of this systematic review and meta-analysis.

Study ID	District	Province	Diagnostic method	Total number	Total positive number	Quality score
Hamidinejat et al. (2019)	Southwest	Khuzestan	PCR	103	59	8
Yousefi et al. (2019)	North	Tehran	PCR	150	3	4
Rassouli and Aghazamani (2015)	North	Tehran	Blood smear	61	3	5
Rassouli and Aghazamani (2015)	North	Tehran	Blood smear	61	8	4
Iatta et al. (2021)	Southeast	Sistan va Baluchestan	PCR	403	13	5
Iatta et al. (2021)	Southeast	Sistan va Baluchestan	PCR	403	2	4
Iatta et al. (2021)	Southeast	Sistan va Baluchestan	PCR	403	8	4
Jalali (2010)	Northeast	Razavi Khorasan	PCR	250	9	4
Pahlavan (2016)	Southwest	Khuzestan	PCR	103	59	5
Motaghipisheh et al. (2016)	Southeast	Kerman	PCR	100	9	8
Maazi et al. (2014)	North	Tehran	IFA	240	40	8
Derakhshandeh et al. (2016)	South	Fars	PCR	98	3	8
Akhtardanesh et al. (2010)	Southeast	Kerman	IFA	123	18	6
Baharie Yazdi et al. (2018)	Southwest	Khuzestan	PCR	184	57	4
Ansari-Mood et al. (2015)	Northeast	Razavi Khorasan	IFA	250	2	6
Jalali (2010)	Southwest	Khuzestan	ICA	198	19	8
Mazzi (2010)	North	Tehran	PCR	40	8	5
Saffar Benis et al. (2021)	North	Semnan	PCR	134	35	4
Akhtar-danesh et al. (2012)	Southeast	Kerman	PCR	100	6	8
Jalali (2010)	North	Semnan	PCR	100	15	4
Ansari-Mood et al. (2015)	North	Tehran	PCR	110	12	2
Mehranfar (2020)	North	Tehran	PCR	58	10	4
Davoodi (2020)	Southeast	Kerman	PCR	67	8	4
Ezati (2019)	Southeast	Kerman	PCR	98	9	4
Khanjan (2021)	Northeast	Razavi Khorasan	PCR	54	7	2
Shahsavari (2021)	North	Tehran	PCR	33	3	2

Abbreviations: ICA, immunochromatography assay; IFAT, indirect fluorescent antibody assay; PCR, polymerase chain reaction.

These criteria were systematically applied to select studies for the meta-analysis, ensuring a robust and comprehensive approach to evaluating the prevalence of rickettsial infections in the dog population in Iran.

2.4 | Quality assessment

An evaluation tool used in this systematic review and meta-analysis is the Newcastle–Ottawa scale, which assesses each study based on eight items. The quality of the study was assessed as low (<3), moderate (3–5) and high (6<) according to the NOS assessment score (Lo et al., 2014; Shams et al., 2022). Therefore, studies of acceptable quality (high and moderate) were deemed suitable for meta-analysis.

2.5 | Statistical analysis

In this meta-analysis study, random- and fixed-effects models were employed to assess the overall prevalence and corresponding 95% confidence intervals (Abdous et al., 2021; Jokar, Rabiee et al., 2021). The interdependence between the prevalence of different genera of rickettsial infections and associated risk factors, such as gender (male or female) and age (less or more than 2 years), was evaluated by calcu-

lating the odds ratio (OR) with 95% confidence intervals (CIs) for each study, using the random-effects model (Jokar, Rahmanian et al., 2021).

To anticipate and address heterogeneity among the results, statistical methods, including Cochran's *Q* test and the I^2 index, were implemented (Jokar et al., 2022, 2023). For transparency, it is important to note that these methods were employed to assess the potential sources of variation among the surveyed studies. To provide a clear representation of each study's impact, collective prevalence and heterogeneity among the surveys, forest plots were utilized. Additionally, a meta-regression analysis was performed to examine the influence of factors contributing to the observed heterogeneity.

In terms of publication bias assessment, Funnel plots and Egger's regression were used (Jokar, Rahmanian et al., 2021, 2023). For visual representation of *Anaplasma*, *Ehrlichia* and *Rickettsia* infection prevalence across Iran and to map the distribution of prevalence in individual provinces, ArcGIS 10.3 software was employed. It is noteworthy that the trial version of the StatsDirect statistical application was used, available for public use (http://statdirects.com), to conduct the meta-analysis.

3 | RESULT

3.1 | Search results and eligibility studies

In the present survey, 742 studies were identified using the previously defined search terms in the previously listed databases. Two hundred thirty-one articles were omitted due to duplication and inconsistencies in the inclusion and exclusion criteria stated in the caption and abstract, resulting in the exclusion of 468 others. Seventeen articles were excluded for non-compliance with the inclusion and exclusion criteria, specifically due to a lack of clear diagnostic methodology in the full text. Ultimately, 26 articles met the evaluation criteria considered for this study.

3.2 | Characteristics of the eligible studies

The selected surveys include 14 journal articles, 4 congress articles, and 8 dissertations. Two types of serological methods were utilized in the studies: the indirect fluorescent antibody (IFA) test ($n = 2$) and the PCR ($n = 24$). Altogether, the studies reported a prevalence of 6 from all 31 provinces of Iran.

3.3 | Pooled prevalence of rickettsial infections in Iran

Out of 3924 examined cases, 425 had positive results for different genera of rickettsial diseases utilizing the PCR and IFA diagnostic methods. Due to the limited number of studies available for the seroprevalence of *Rickettsia* spp. (only two studies), it was not feasible to conduct a meta-analysis on their effect sizes. The prevalence of different genera yielded the following results: *Anaplasma* spp. showed a prevalence of 20.1% (95% CI, 12.4–27.7) with a *Q* statistic of 285.89 ($p < 0.0001$) and an I^2 of 97.2%, whereas *Ehrlichia* spp. had a prevalence of 10% (95% CI, 7–13.1) with a *Q* statistic of 210.68 ($p < 0.0001$) and an I^2 of 93.4%. *Rickettsia* data indicated a CI range between 0 and 9.9 (Table 3). Altogether, the collective prevalence of rickettsial infections among dogs in Iran was estimated at 18.3% (95% CI, 12–24.5) (Figure 2), employing a random-effects-filled meta-analysis. The prevalence estimates were highly heterogeneous among the inspected surveys *Q* statistic = 9743.679 (df = 32), $p < 0.0001$ and $I^2 = 95.3\%$. Multivariate meta-regression analysis exhibited homogenous districts, provinces, diagnostic methods, type of age and type of pathogen (Table 2). Univariate meta-regression analyses implied heterogeneity due to the districts ($p = 0.006$), though no noticeable diversity was found in the approach of provinces, diagnostic method, age and type of pathogen ($p = 0.032$) (Table 2). Additionally, a schematic map of rickettsial pathogen distribution in dogs was created derived from surveys conducted in different provinces of Iran (Figure 3). The maximum and minimum prevalences of different genera of rickettsial infection regarding a meta-analysis of subgroups were estimated in the southwest and southern regions of Iran as 38.5% and 0.3%, correspondingly. The overall rickettsial infection has been demonstrated in Table 3, showing the collective prevalence in eight geographical regions of Iran. Total prevalence based on the type of diagnostic method was as follows: based on PCR 13.7% (95% CI, 10.4–17, *Q* statistic = 458.29, $p < 0.0001$ and $I^2 = 95.9\%$), blood smear 8.3% (95% CI, 0.4–16.3, *Q* statistic = 2.55, $p < 0.11$ and $I^2 = 60.8\%$), IFA 10.5% (95% CI, −1.6 to 22.6, *Q* statistic = 57.42, $p < 0.0001$ and $I^2 = 96.5\%$) and islet cell cytoplasmic autoantibodies (ICA) 9.6% (95% CI, −2.3 to 9, *Q* statistic = 0, $p < 0.0001$ and $I^2 = 0$) (Table 3).

Total prevalence based on the type of the pathogen followed *Anaplasma phagocytophilum* 27.8% (95% CI, 7–48.6, *Q* statistic = 234.34, $p < 0.0001$ and $I^2 = 98.3\%$), *Ehrlichia ewingii* 4.9% (95% CI, −0.5 to 10.3, *Q* statistic = 0, $p = 0$ and $I^2 = 0$), *Anaplasma platys* 10.9% (95% CI, 4.4–17.4, *Q* statistic = 41.7, $p < 0.0001$ and $I^2 = 92.8\%$), *E. canis* 10.4% (95% CI, 7.2–13.6, *Q* statistic = 209.42, $p < 0.0001$ and $I^2 = 93.8\%$) and *Rickettsia rickettsii* 10% (95% CI, −2.3 to 9.9) (Table 3).

3.4 | Risk factors

Dogs over 2-year old had a higher exposure rate than those two or under (OR = 1.093; $p = 0.684$; 95% CI, 0.696–1.716), which was not statistically significant. Similarly, female dogs had a higher exposure rate (OR = 1.157; $p = 0.7$; 95% CI, 0.572–2.34), but it was not statistically significant. Detailed aspects of the assessed risk factors are presented in Table 4.

3.5 | Publication bias

In order to assess the presence of publication bias, the funnel plot and Egger's test were used. Additionally, significant publication bias was

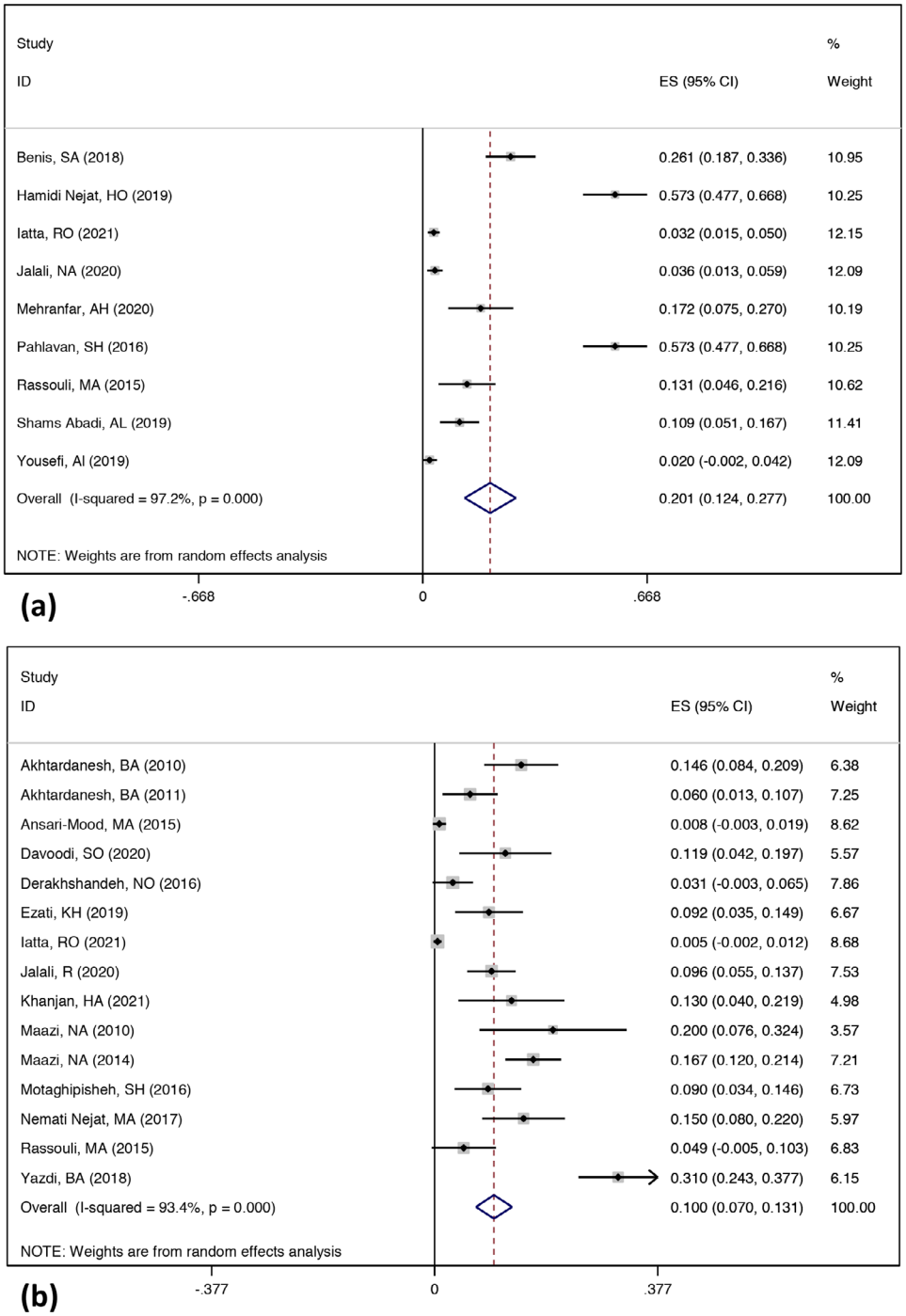

FIGURE 2 Forest plot diagrams showing portion meta-analysis plot of anaplasmosis (a) and ehrlichiosis (b) seroprevalences among dogs in Iran (random-effects).

corrected using the trim-and-fill method. The Egger test (bias = 5.11, 95% CI = 3.58–6.64, $p < 0.01$) and the asymmetry in the funnel plot interpretation indicate significant publication bias in studies, as shown in Figure 4. Based on the trim-and-fill method, the pooled seroprevalence was calculated using the random-effects model as 18.3% (95% CI, 12–24.5).

4 | DISCUSSION

Rickettsiae are mainly transmitted to vertebrate hosts through arthropod vectors, and infected vectors pass on the bacteria through birth or transstadial transmission (Abdad et al., 2018). Curing a rickettsial infection is challenging due to inadequacies in diagnostic approaches,

TABLE 2 Result of multivariate and univariate meta-regression models for detecting probable sources of heterogeneity.

The probable source of heterogeneity	Multivariate		Univariate	
	Coefficient	*p*-Value	Coefficient	*p*-Value
Districts	−0.755754	0.058	−0.0695538	0.006
Province	−0.0063965	0.810	−0.033537	0.109
Diagnostic method	−0.0243209	0.559	−0.0222193	0.520
Type of gender	−0.0871419	0.378	−0.0844902	0.080
Type of pathogen	0.0225434	0.555	−0.0393026	0.032
Total number	−0.0003801	0.505	−0.000414	0.110

Abbreviations: ICA, immunochromatography assay; IFAT, indirect fluorescent antibody assay; PCR, polymerase chain reaction.

treatment with unsuitable antibiotics and costly methods of treatment (Osterloh, 2021). It is also possible for some species of Rickettsiae to endure antibiotic therapy and re-emerge several years later as disorders like Brill–Zinsser disease in humans (Lutwick, 2001).

The surveillance of dogs infected with different genera of rickettsial agents can help prevent and control related diseases by implementing measures such as monitoring affected individuals and restricting their contact with other animals, thereby reducing the risk of transmission.

In a study in Mato Grosso State, Brazil, the prevalence and risk factors of *Ehrlichia* spp. and *Rickettsia* spp. were inspected. Additionally, 70.9% and 47.5% of the examined dogs were positive for each infection, respectively (Melo et al., 2011).

In a similar study, Low et al. (2020) described rickettsial infections as 'endemic' in most Southeastern Asian countries. As a more recent comparison in Turkey, a country neighbouring Iran, this study investigated the serological distribution of some vector-borne diseases in dogs, including rickettsial agents. It was declared in the mentioned study that 30.2% of the examined dogs were infected with at least one of the pathogens, and *E. canis*, with 19.8%, had the highest prevalence among all the surveyed pathogens (Pahlavan, 2016).

Another survey on the dog population of Saudi Arabia, a country near Iran, showed 57.1% of 40 dogs were infected with *A. platys*, which is higher than in Iran (Alanazi et al., 2021). Another study in Qatar showed that 3.2% out of 64 dogs were infected with rickettsial diseases, which is lower than in Iran (Alho et al., 2017).

The current study is a meta-analysis inspecting the prevalence of different genera of *Rickettsiales* in dogs in Iran. For this review, 26 studies were considered, which presented 424 positive cases out of 3924 examined patients in 6 provinces of Iran. The overall Rickettsial infection prevalence was 18.3% (95% CI, 12–24.5), which is lower than the measured prevalence in Turkey and Southeastern Asia.

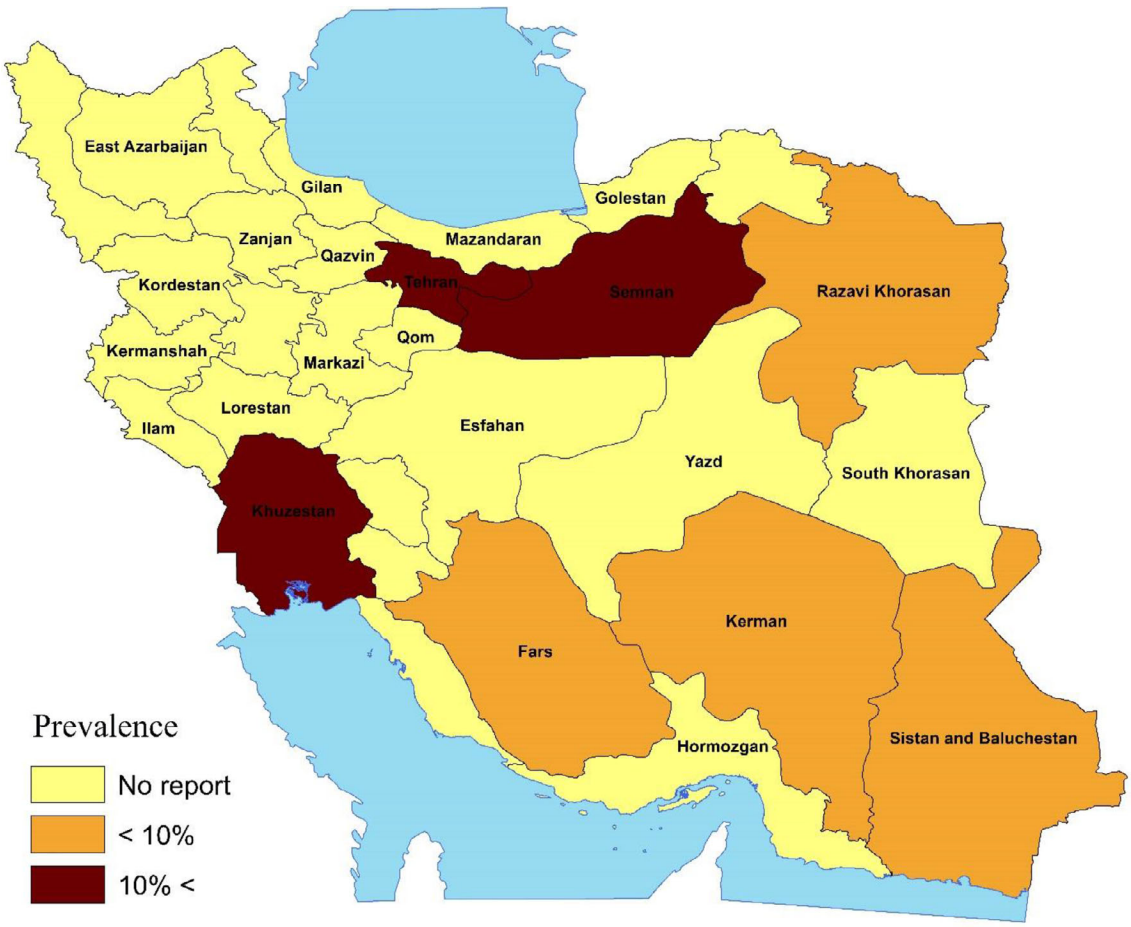

FIGURE 3 Seroprevalence of rickettsial infection in dogs among different provinces of Iran.

TABLE 3 Sub-group analysis of rickettsial infection seroprevalence in Iran.

Variable/ Sub-groups	Number of data sets	Positive samples/Total samples	Pooled prevalence (95% CI)	Heterogeneity		
				I^2 %	Tau^2	*p*-Value
District						
Southwest	4	194/588	38.5% (14.4–62.6)	97.9%	0.0590	$p < 0.01$
North	10	137/987	13.1% (7.5–18.6)	88.4%	0.0066	$p < 0.01$
Northeast	3	18/554	0.3% (0–7.1)	81.8%	0.0007	$p = 0.04$
Southeast	5	73/1697	9.6% (6.6–12.6)	22.8%	0.0003	$p = 0.26$
South	1	3/98	0.3% (0–6.5)	-	-	-
Diagnostic method						
PCR	20	335/2991	13.7% (10.4–17)	95.9%	0.0047	$p < 0.01$
Blood smear	2	11/122	8.3% (0.4–16.3)	60.8%	0.0020	$p = 0.11$
IFA	3	60/613	10.5% (0–22.6)	96.5%	0.0109	$p < 0.01$
ICA	1	19/198	9.6% (5.5–13.7)	-	-	-
Type of genus						
Anaplasma spp.	9	208/1372	20.1% (12.4–27.7)	97.2%	0.0124	$p < 0.01$
Ehrlichia spp.	15	161/1697	10% (7–13.1)	93.4%	0.0028	$p < 0.01$
Rickettsia spp.	2	11/436	9.6% (0–9.9)	-	-	-
Type of species						
Anaplasma phagocytophilum	5	141/527	27.8% (7–48.6)	98.3%	0.0547	$p < 0.01$
Ehrlichia ewingii	1	3/61	4.9% (0–10.3)	-	-	-
Anaplasma platys	4	67/845	10.9% (4.4–17.4)	92.8%	0.0036	$p < 0.01$
Ehrlichia canis	14	191/1957	10.4% (7.2–13.6)	93.8%	0.0029	$p < 0.01$
Rickettsia rickettsi	2	11/436	9.6% (0–9.9)	-	-	-

Abbreviations: ICA, immunochromatography assay; IFAT, indirect fluorescent antibody assay; PCR, polymerase chain reaction.

TABLE 4 Risk factors of dogs rickettsial infection among dogs in Iran.

Risk factors	Number of data sets	P% (infected/total)		OR (95% CI)	OR heterogeneity		
					I^2 %	Tau^2	*p*-Value
Sex	9	Female	18.76% (91/485)	1.198 (0.842–1.705)	0	1.62	$p = 0.978$
		Male	11.49% (66/574)				
Age	7	≤2 years	20.45% (54/264)	1.014 (0.598–1.72)	15.4%	7.09	$p = 0.312$
		>2 years	13.79% (60/435)				

Note: P% (prevalence %).

Out of the 26 evaluated studies in the current survey, 20 used PCR as the diagnostic method, which is complicated to perform but more accurate than other approaches. In three studies, IFA was utilized. Two studies used blood smear, a more accessible method, and only in one study was ICA used. In the assessed surveys, five districts of Iran were evaluated for each of the five different pathogen types: *A. phagocytophilum*, *E. ewingii*, *A. platys*, *E. canis* and *R. rickettsii*. According to the assessed studies, the southwestern part of Iran had the highest pooled prevalence of rickettsial exposure among the inspected regions. The pooled prevalence of rickettsial exposure was higher in female dogs and in dogs ≥2 years, but the difference is not statistically significant. The lack of association between age and sex with the prevalence of rickettsiosis in our study may be attributed to several factors. Rickettsiosis is primarily transmitted by arthropod vectors like ticks and fleas, and exposure to these vectors can occur regardless of age or sex, as individuals of all demographics can come into contact with these disease carriers during outdoor activities or in various environments. Additionally, rickettsiosis may not exhibit a significant age or sex bias in terms of susceptibility, as the risk of exposure can depend more on behavioural factors, such as outdoor exposure and tick avoidance

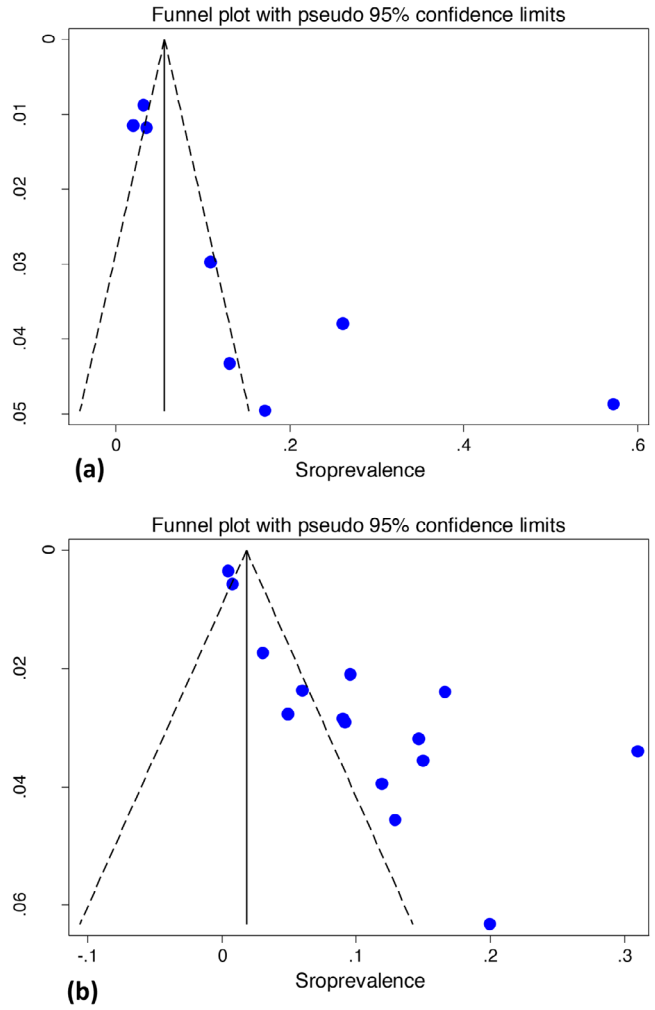

FIGURE 4 Funnel plot with pseudo 95% confidence limits for detection of publication bias among included studies related to seroprevalence of anaplasmosis (a) and ehrlichiosis (b).

measures, rather than age or sex. Moreover, other underlying factors, including genetic predisposition or individual immune responses, could play a more crucial role in determining susceptibility to the infecting agent. Further research may be needed to uncover the specific factors that influence the prevalence of rickettsiosis in different demographic groups.

Dogs residing in the southwestern regions of Iran are at an elevated risk of exposure, potentially due to the region's favourable climatic conditions. Warmer temperatures accelerate the life cycle of arthropod vectors like ticks and fleas, increasing their population and transmission efficiency. High humidity levels create favourable conditions for these vectors, promoting their longevity and feeding activity. Precipitation patterns influence the abundance of wildlife hosts, which can act as reservoirs for rickettsia.

Seasonal variability, often linked to temperature and humidity, impacts vector activity and disease transmission. Geographic location and climate suitability play a role, with certain regions providing ideal conditions for rickettsiosis transmission.

The zoonotic aspect of ehrlichiosis between dogs and humans holds significant implications. Simultaneous identification of granulocytic ehrlichiosis (GE) in both humans and dogs strongly suggests that the agents responsible for GE in canines and humans are similar, as indicated in one study (Greig et al., 1996). Furthermore, research has revealed antibodies in humans that may be indicative of ehrlichiosis caused by *E. canis* or closely related species (Vieira RF da et al., 2013). The information gleaned from this study on rickettsial disease prevalence in dogs, along with its observed variations in distinct regions and demographic groups, holds significant implications for practical applications in the field. The identification of a higher prevalence in specific regions, particularly the southwestern part of Iran, facilitates targeted surveillance efforts, enabling local veterinary authorities to focus on monitoring, early detection and timely intervention in these areas. Moreover, understanding the correlation between elevated exposure risk and favourable climatic conditions, especially in the southwest, allows for the development of climate-informed preventive strategies, such as intensified vector control measures tailored to regional climates. Veterinarians can utilize age- and gender-related insights to inform tailored practices, potentially enhancing preventive care for specific demographic groups. The zoonotic potential of rickettsial infections emphasizes the need for public health awareness campaigns, particularly in high-prevalence regions, contributing to preventive measures for both pet owners and the general public. The limitations of this study, such as the insufficient number of articles for *Rickettsia* spp. and a low number of articles in the south of Iran, pose challenges for conducting a meta-analysis. To address these limitations, we suggest avenues for further research, encouraging exploration of additional serotypes and a broader geographic scope to achieve a more comprehensive understanding of *Rickettsia* distribution in dogs across Iran. Finally, data can play a pivotal role in exploring and implementing prophylactic options, including vaccine development or targeted treatment plans, thereby contributing to the reduction of overall rickettsial infection prevalence in the canine population (Osterloh, 2021). This insight underscores the importance of understanding and monitoring rickettsiosis in dogs concerning public health practices in Iran, especially in regions with a higher prevalence of these pathogens.

5 | CONCLUSION

In conclusion, this study reveals a notable rickettsial infection prevalence of 18.3% among dogs in Iran, with the southwestern part of the country exhibiting the highest pooled prevalence. Although this rate is lower than those reported in Turkey and Southeastern Asia, the findings underscore a region-specific risk, particularly in the southwest. Although the pooled prevalence is higher in female dogs and those aged 2 years or older, these differences lack statistical significance. The heightened exposure risk in the southwestern regions aligns with favourable climatic conditions, emphasizing the influence of climate on disease prevalence. These insights can inform targeted surveillance and control strategies, climate-informed preventive measures and tailored veterinary practices. The study's implications

extend to public health awareness and highlight the need for ongoing research to comprehensively understand *Rickettsia* distribution in dogs across Iran. Ultimately, these findings contribute to evidence-based decision-making for more effective disease management, prevention and control in veterinary practice and public health initiatives (Jokar et al., 2022; Abdous et al., 2021).

AUTHOR CONTRIBUTIONS

Study concept and design: Mohammad Jokar and Arman Abdous. *Acquisition of data*: Arman Abdous and Farzane Shams. *Analysis and interpretation of data*: Mohammad Jokar, Aryan Abbassioun and Vahid Rahmanian. *Drafting of the manuscript*: Mehdi Rahnama, Farzane Shams, Arman Abdous, Mehran Farhoodi and Mohammad Sadegh Kamjoo. *Critical revision of the manuscript for important intellectual content*: Mohammad Jokar. *Statistical analysis*: Mohammad Jokar and Vahid Rahmanian. *Study supervision*: Mohammad Jokar, Mehran Farhoodi and Vahid Rahmanian.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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ETHICS STATEMENT

No ethical approval was required as the current work is a review.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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