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Research article

Systematization of the prevalence of *Anaplasma* spp. in canines and meta-analysis of *A. platys* and *A. phagocytophilum*

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ABSTRACT

Objective. To estimate the general prevalence of *Anaplasma* spp. and the specific prevalence of *A. platys* and *A. phagocytophilum* in canines through studies published between 2000 and 2018. **Materials and methods.** Systematic review with 14 search strategies to ensure exhaustivity and reproducibility in the stages specified in the PRISMA guide. Quality was evaluated with STROBE. Frequencies were calculated and global and specific prevalences were estimated by country, period of study and diagnostic test employed, with 95% confidence intervals. A forest plot was made showing the individual and global prevalences *A. platys* and A. *phagocytophilum* by PCR, ELISA, and IFI, compared using a Z-Test. **Results.** Thirty studies with 18.472 canines were included, mostly from Brazil, the United States, and Germany. In the studies with IFI, the prevalence was 39.0% (95% CI= 37.0-41.0); with ELISA 9.3% (95% CI=8.8-9.8); and with PCR ,7.1% (95% CI= 6.4-7.8). The prevalence with PCR was statistically higher in America at an 11.9% (95% CI= 10.5-13.3) compared to Africa, at an 5.5% (95% CI= 1.2-9.7), Asia, 4.1% (95% CI= 3.1-5.1), and Europe, 3.5% (95% CI = 2.5-4.5). The prevalence of *A. platys* with PCR was 16.1% (95% CI= 14.2-17.9), and of *A. phagocytophilum*, 3.7% (95% CI= 2.8-4.6). **Conclusions.** The study showed a high prevalence of the infection, with a greater presence of *A. platys*, in a low number of publications worldwide, with highly heterogeneous results when considering countries, diagnostic techniques, and species involved.

Keywords: Anaplasma, canines, meta-analysis, prevalence (*Source: DeCS*).

RESUMEN

Objetivo. Estimar la prevalencia general de *Anaplasma* spp. y la prevalencia específica de *A. platys* y *A. phagocytophilum* en caninos, mediante estudios publicados entre 2000 y 2018. **Materiales y métodos.** Revisión sistemática con 14 estrategias de búsqueda, garantizando exhaustividad y reproducibilidad en fases de la guía PRISMA. Se evaluó la calidad con STROBE. Se calcularon frecuencias y se estimó la prevalencia global y las específicas según país, periodo y prueba diagnóstica, con sus intervalos de confianza del 95%. Se realizó Forest Plot para la prevalencia individual y global de *A. platys* o A. *phagocytophilum* según PCR, ELISA e IFI, las cuales se compararon con base en el Estadístico Z. **Resultados.** Se incluyeron 30 estudios con 18.472 caninos, la mayoría de Brasil, Estados Unidos y Alemania. En IFI se halló una prevalencia de 39.0% (IC95%= 37.0-41.0), en ELISA 9.3% (IC95%= 8.8-9.8) y en PCR 7.1% (IC95%=6.4-7.8). La prevalencia basada en PCR fue estadísticamente mayor en América con 11.9% (IC95%=10.5-13.3) frente a África con 5.5% (IC95%=1.2-9.7), Asia 4.1% (IC95%=3.1-5.1) y Europa 3.5% (IC95%=2.5-4.5). La prevalencia de *A. platys* con PCR fue 16.1% (IC95%=14.2-17.9) y de *A. phagocytophilum* 3.7% (IC95%= 2.8-4.6). **Conclusiones**. Se halló una elevada prevalencia de infección, con mayor importancia de *A. platys*, en un bajo número de publicaciones en el ámbito mundial y con una elevada heterogeneidad según el país, la técnica diagnóstica y la especie implicada.

Palabras clave: *Anaplasma*, caninos, metanálisis, prevalencia (*Fuente: DeCS*).

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INTRODUCTION

Anaplasmosis is an infectious, hemoparasitic disease caused by gram-negative, obligate intracellular, immobile bacteria with coccoid morphology. Their target is hematopoietic cells, especially neutrophils and platelets. These bacteria replicate within a vacuole derived from the membrane of the vertebrate or invertebrate host's eukaryotic cell. Usually transmitted by arthropods, these can affect humans and numerous species of domestic and wild animals which, according to reports, include dogs, horses, goats, sheep, cats, ruminants, and birds, among others which could play an important role in the persistence and dissemination of the disease (1,2).

Anaplasmosis is found in tropical and subtropical areas whose conditions favor vector survival and reproduction. The disease is endemic to the Midwestern, Eastern and Northeastern regions of the United States, as well as to the Western coastal regions where most of the outbreaks are seasonal and simultaneous with the occurrence of ticks. In countries as the United Kingdom, Norway, Sweden, Switzerland, and Germany, infections have been reported in ruminants, canines, and humans, whereas in Asia and South America its study has been less frequent. The need to know about the occurrence and distribution of Anaplasmosis stems from its importance as a zoonotic disease, its wide geographic distribution and the complexity of the clinical profiles related (3,4). Particularly in dogs, approaching the disease is highly important given its increasing occurrence in pets from different regions, the high number of adoptions and the close relationship between canines and humans, which turns the situation into an epidemiologic event that requires a deep exploration of the agent involved (5).

In canines, the main etiological agents are *Anaplasma phagocytophilum* and *Anaplasma platys* (6,7). The infection caused by *A. phagocytophilum* is transmitted by hard ticks of the genus *Ixodes*, resulting in canine granulocytic anaplasmosis. The infection caused by *A. platys* is transmitted mainly by *Rhipicephalus sanguineus*, originating canine infectious cyclic thrombocytopenia (CICT). The main signs of this disease in dogs are fever, depression, lameness, anorexia, joint inflammation, neurological signs, blood counts and urinalyses with traces of thrombocytopenia, non-regenerative anemia, leukopenia, hyperglobulinemia and proteinuria at various stages of the infection (8,9). The clinical signs associated with *Anaplasma* spp are not usually very specific; therefore, its clinical diagnosis entails some difficulties. In addition, some reports indicate that dogs infected by *A. platys* can develop cyclic thrombocytopenia that may become serious enough to produce hemorrhage, including petechiae and ecchymoses, but it is believed that most dogs control the infection immunologically (10).

The diagnosis includes a Giemsa-stained blood smear, which has low sensitivity to low bacteremia or transient infections. Enzyme-linked immunosorbent and immunofluorescence assays with good sensitivity and specificity are also available but restricted by the fact that antibodies are generally absent during two weeks after the signs of the disease first appear, persist for up to eight months after the elimination of the agent, and cross-reactions with other agents of the families Anaplasmataceae and Rickettsiales may occur (2).

Lastly, PCR is used worldwide as a tool in the diagnosis of infectious diseases and the characterization of pathogens. Its usefulness resides in the rapid and accurate identification of diseases that would otherwise be difficult to detect, using universal primers targeted at bacterial 16S ribosomal DNA and sequence analyses (11). Previous publications have shown the specificity, low cross-reactivity with other species and good reproducibility with low coefficients of intra- and inter-assay variation of PCR, which make it possible to overcome the limitations of other diagnostic methods, since it allows the detection and quantification of the DNA of *Anaplasma* spp. in canine blood, crucial for the detection, diagnosis, and monitoring of the infection (12). The infection may even become highly heterogeneous due to its asymptomatic nature, nonspecific hematological and biochemical laboratory findings, variations in the diagnostic usefulness of the techniques employed, and environmental factors, particularly those related to the presence of specific vectors with epidemiological influence in different regions worldwide (13).

All things considered, it is relevant to develop a systematic review that allows the elaboration of a global profile of the prevalence of *Anaplasma* spp. in canines, and the characterization of its occurrence by species of *A platys* or A. *phagocytophilum*. The latter is one of the main causative pathogens in dogs under domestic conditions and may be related to the occurrence of infections in humans (14). It is also relevant to compare prevalence by place, period of study and diagnostic test employed, so that subsequent sanitary and research actions may be taken. In addition, systematic reviews are structured, explicit, systematic, exhaustive and reproducible explorations of studies linked to a research question that enable an increasing extrapolation of results, improve the precision in the estimation and comparison of prevalences, and become a key tool in decision-making in the context of health and in the evaluation of research needs. Besides, these are often used as a starting point for consensus groups, and panels of experts or commissions with regulatory responsibilities and high impact on health to perform their job (15).

The objective of this study was to estimate the general prevalence of *Anaplasma* spp. and the specific prevalence of *A platys* and *A. phagocytophilum* in canines through studies published between 2000 and 2018.

MATERIALS AND METHODS

Type of study. A systematic review of literature and meta-analysis. The guidelines for both the design of the search strategy and the selection of articles are contained correspond to the *PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses)* stages.

Identification. An exhaustive search of scientific literature was conducted in PubMed, Scielo, and Lilacs, using combinations of the terms *Anaplasma* or *Anaplasmosis* with the synonyms for prevalence included in the Health Sciences Descriptors (DeCS), namely, frequency, occurrence, epidemiology, surveillance, outbreaks, and incidence, for a resulting total of 14 different search strategies in Spanish and English.

Screening. According to the criteria applied, the title/abstract of the articles included the search terms and were observational studies of prevalence, with canines as the main population and with an explicit report of the prevalence, that is, population and number of positives. Neither time nor selection restrictions were established for the search. As the first study found was published in 2001 and the search protocol was last updated in April 2018, the period between 2000 and 2018 was accordingly established as the time window for this study. Some syntax used in the search and selection were: (Anaplasma[Title]) AND Prevalence[Title/Abstract], (Anaplasmosis[Title]) AND Ocurrence[Title/Abstract], (ti: (anaplasma)) AND (ab: (prevalencia)).

Selection. Studies that were not original research works of the editorial type nor topic reviews were excluded, and also were those with incomplete information - regarding the name of the diagnostic test employed, for example - case studies or case series with small samples (10 or less).

Inclusion. The studies that met the protocol above-mentioned were synthesized qualitatively and quantitatively in an Excel database that included the variables TITLE, AUTHORS, YEAR OF PUBLICATION, NUMBER OF CANINES EVALUATED, NUMBER OF POSITIVE CANINES and DIAGNOSTIC TEST EMPLOYED. In some studies, it was possible to analyze additional variables such as INFECTING SPECIES and PRESENCE OF COINFECTIONS.

Reproducibility analysis and methodological quality evaluation. The reproducibility of both the search and selection of the studies was guaranteed by consensus and referral to a third party. Regarding reproducibility of the data extraction, the Excel database was revised independently by two researchers, finding a *Kappa* index of 1.00 in the qualitative variables and an intraclass correlation coefficient of 1.00 for the quantitative ones. For the evaluation of the methodological quality of the studies, the criteria applied is contained in the STROBE (*Strengthening the Reporting of Observational studies in Epidemiology*) guide for cross-sectional studies.

Data analysis. The study variables were described using absolute and relative frequencies. A forest plot was made showing the prevalence of the infection reported in each study, grouped in accordance with the diagnostic test, with 95% confidence intervals. In addition, specific prevalences were also estimated by period of study, continent where it was carried out, and diagnostic test employed. Also, for any possible case, the specific prevalence of *A. platys* and *A. phagocytophilum* infections was estimated with 95% confidence intervals, using a random effects model (which includes intra- and inter-study variability in the estimation) given the heterogeneity of the individual reports according to this RI coefficient (I^2 - Proportion of the total variance in response to the variance between studies). The specific prevalence of each species was compared using a Z-Test or a confidence interval to analyze the difference of proportions.

Ethical aspects. Based on Resolution 8430 of 1993 issued by the Ministry of Health of Colombia, the study is classified as risk-free research since it involved the use of documentary or secondary sources.

RESULTS

After applying all search strategies, the initial search resulted in 1,314 studies found in all databases. Out of these, 408 publications that included the search terms in the title, abstract, or both, were screened; only 30 studies met the search and inclusion protocols (Figure 1).

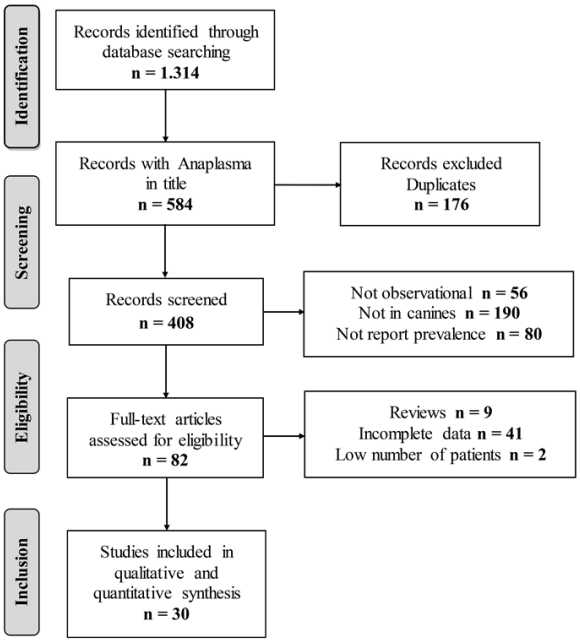

Figure 1. Flow gram of selection of the studies.

The studies were published between 2005 and 2017 with a higher proportion of publications as of 2011 with 66.7% (n = 20). Out of the total of studies, 43.3% (n = 13) was carried out in European countries; 36.7% (n = 11) in the American continent; 13.3% (n = 4) in Asia; and 6.7% in Africa (n = 2). The countries with the highest number of studies were Brazil, the United States, and Germany. PCR was reported to have been used in 56.6% (n = 17) of the studies; ELISA in 36.6% (n = 11); and IFI in 20.0% (n = 6). PCR and ELISA were used simultaneously in one study; PCR and IFI in three of them (Table 1).

The systematic review was conducted in a population of 18,472 canines with 70.7% (n=13.067) between 2011 and 2017.Regarding the location, 55.4% were (n=10.237) in Europe; 29.5% (n=5.442) in the American continent; 13.0% (n=2.397) in Asia; and 2.1% (n=396) in Africa. From such population, 65.2% (n=12.044) of the canines were screened or diagnosed with ELISA; 30.1% (n=5.554) with PCR; and 13.3% (n=2.453) with IFI (Table 1).

The studies showed a good methodological quality after meeting 70% or more of the criteria specified in the STROBE guide. However, the compliance of some criteria, such as those related to the control of selection and data biases, the performance of additional analyses exploring associated factors, and the discussion of possible generalizations of results, was not very explicit (Figure 2).

Table 1. Description of the studies according to year, country, test and number of individuals included.

Author	Year	País	Test	N
Haibin H (16)	2005	Venezuela	PCR	43
Barutzki D (17)	2006	Germany	IFI	1.124
Levi O (18)	2006	Israel	IFI	195
Solano L (19)	2006	Italy	PCR	460
Jensen J (20)	2007	Germany	PCR e IFI	111
Beall M (21)	2008	U.S	PCR y ELISA	731
M'Ghirbi Y (22)	2009	Tunisia	IFI	286
Pantchev N (23)	2009	France	ELISA	919
Barber R (24)	2010	U.S	PCR	109
Sakamoto L (25)	2010	Japan	PCR	1.427
Carrade D (26)	2011	U.S	ELISA	2.431
Kohn B (27)	2011	Germany	IFI y PCR	522
Barth C (28)	2012	Germany	ELISA	448
Cardoso L (29)	2012	Portugal	ELISA	1.185
Ferreira G (30)	2012	Brazil	PCR	256
Mircean V (31)	2012	Romania	ELISA	1.146
Xia Z (32)	2012	China	ELISA	600
Berzina I (33)	2013	Latvia	ELISA	470
Costa L (34)	2013	Brazil	PCRq	511
Ebani V (35)	2013	Italy	PCR e IFI	215
Lasta C (36)	2013	Brazil	PCR	199
Santos H (37)	2013	Brazil	PCR	398
Volgina N (38)	2013	Russia	ELISA	522
Kramer F (39)	2014	Poland	ELISA	3.094
Lanza M (40)	2014	Spain	PCR	21
McCown M (41)	2014	Colombia	ELISA	498
Santamaria A (42)	2014	Panama	PCR	201
Dahmani M (43)	2015	Guiana	PCR	65
Dahmani M (44)	2015	Algeria	PCR	110
Yuasa Y (45)	2017	Taiwan	PCR	175

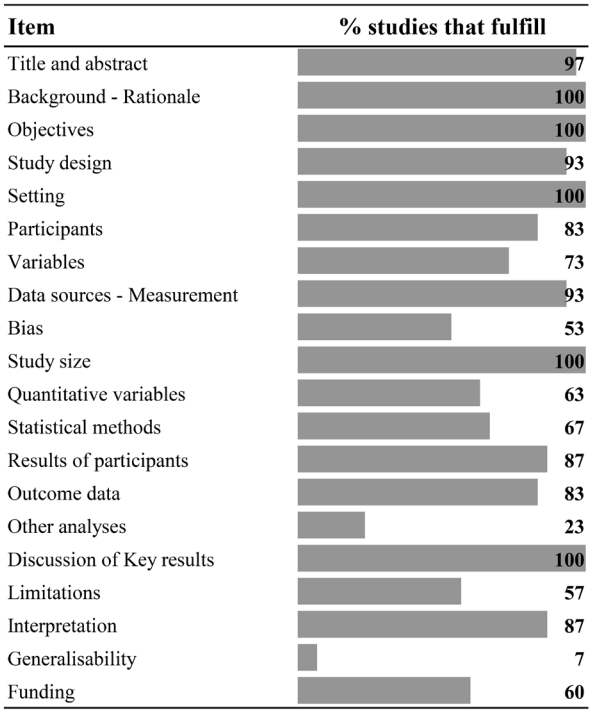

Figure 2. Evaluation of the methodological quality of the included studies.

In the 2,453 individuals screened with IFI, the seroprevalence of infection found, at a 39.0% (95% CI=37.0-41.0), was statistically higher than the one obtained with the other tests: in the 12.044 canines tested with ELISA, seroprevalence was estimated at a 9.3% (95% CI=8.8-9.8), and in the 5,096 canines analyzed with PCR, at 7.1% (95% CI=6.4-7.8). All tests showed a high heterogeneity, with a prevalence between 0.0% and 50.1% (Figure 3).

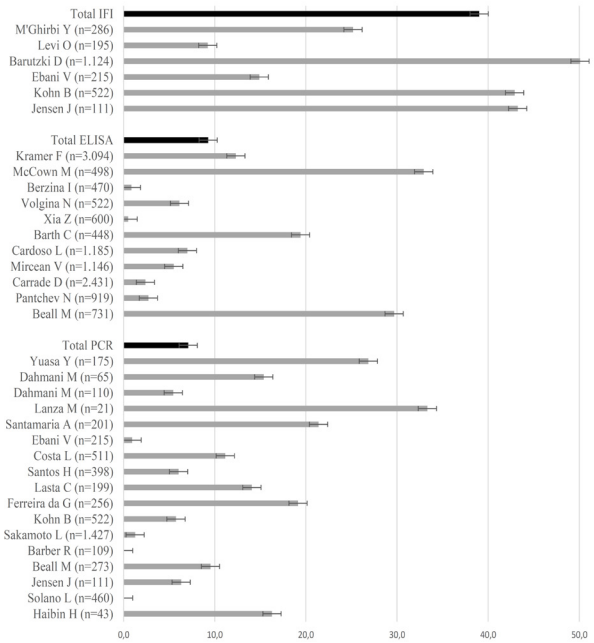

Figure 3. Overall infection prevalence by study and diagnostic technique (95% confidence intervals).
Note: the total number is greater than the population of canines evaluated by the studies that simultaneously applied two tests.

After analyzing the prevalence by period of study, the most recent studies - between 2011 and 2017, showed lower values, except for the PCR-based studies published between 2001 and 2010, whose prevalence was 2.4% (95% CI=1.8-3.0) in contrast to an 11.3% (95% CI=10.1-12.5) between 2011 and 2017 (Figure 4).

When analyzed by place of study, a statistically higher prevalence was found in Africa. However, the prevalence based only on PCR resulted in statistically higher results in the American continent with 11.9% (95% CI=10.5-13.3) in comparison to Africa with 5.5% (IC95% =1.2-9.7), Asia 4.1% (95% CI = 3.1-5.1) and Europe 3.5% (95% CI=2.5-4.5) (Figure 4).

The report on the specific prevalence of the studies included was deficient regarding the area of origin, the species involved, the frequency of coinfections, and the presence or absence of signs in the canines. In this sense, some studies reported a higher prevalence in the rural areas during the rainy season, followed by rural areas in the dry season, and lastly, urban areas (34).

In the studies that reported the prevalence of infection by presence of signs, no significant differences were observed since this was estimated at a 44.9% in dogs with signs and at a 41.9% in asymptomatic dogs (20); at a 46.9% in sick dogs and at a 39.8% in healthy animals (27); at a 4.5% in healthy canines and 9.2%

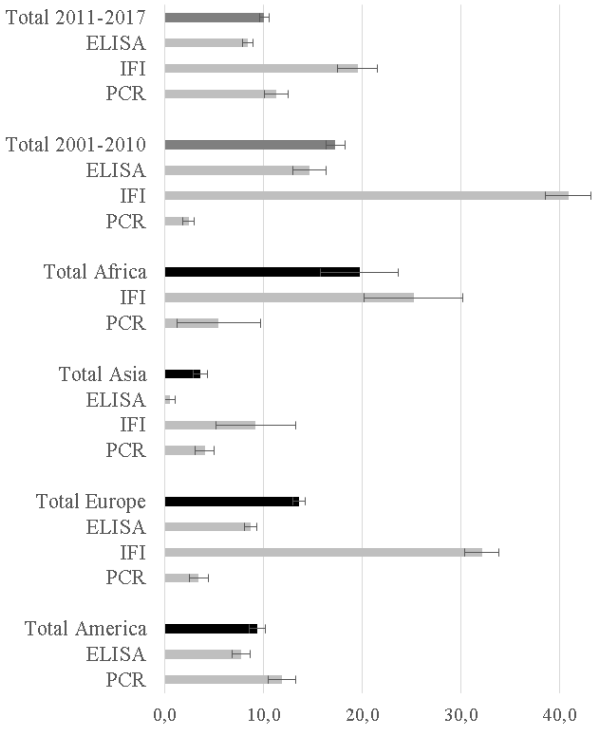

Figure 4. Prevalence of infection according to period, place and diagnostic test.

in animals with canine vector-borne diseases (30). Only one study reported the prevalence of *A. bovis* at 1.3% (95% CI=0.5-1.6) (25).

The prevalence of *A. platys* in 1,581 individuals evaluated with PCR was heterogeneous (RI coefficient = 0.90) with studies reporting prevalences between 5.5% and 33.3% and a global prevalence at a 16.1% (95% CI=14.2-17.9). This was statistically higher than the prevalence of *A. phagocytophilum*, which in 1,706 canines evaluated with PCR was 3.7% (95% CI= 2.8-4.6), equivalent to a difference between 10.3% and 14.5% (Z-Test for the difference of proportions = 11.95. Vp = 0.000) (Figure 5).

In turn, the prevalence of *A. phagocytophilum* was statistically lower in studies that used PCR in comparison to those that used ELISA and IFI. In 10,859 canines analyzed with ELISA, the seroprevalence was 9.4% (95% CI=8.9-10.0), with a difference of proportions between 4.7% and 6.8% (Z-Test = 7.8. Vp=0.000) compared to PCR. In 2,453 canines diagnosed with IFI, the seroprevalence was 39.0% (95% CI= 37.0-40.9), which is between 33.1% and 37.5% higher than the results with PCR (Z-Test = 26.0. Vp = 0.000). Lastly, the seroprevalence of *A. phagocytophilum* with IFI was between 27.5% and 31.6% higher than with the use of ELISA (Z-Test = 37.1. Vp = 0.000) (Figure 5).

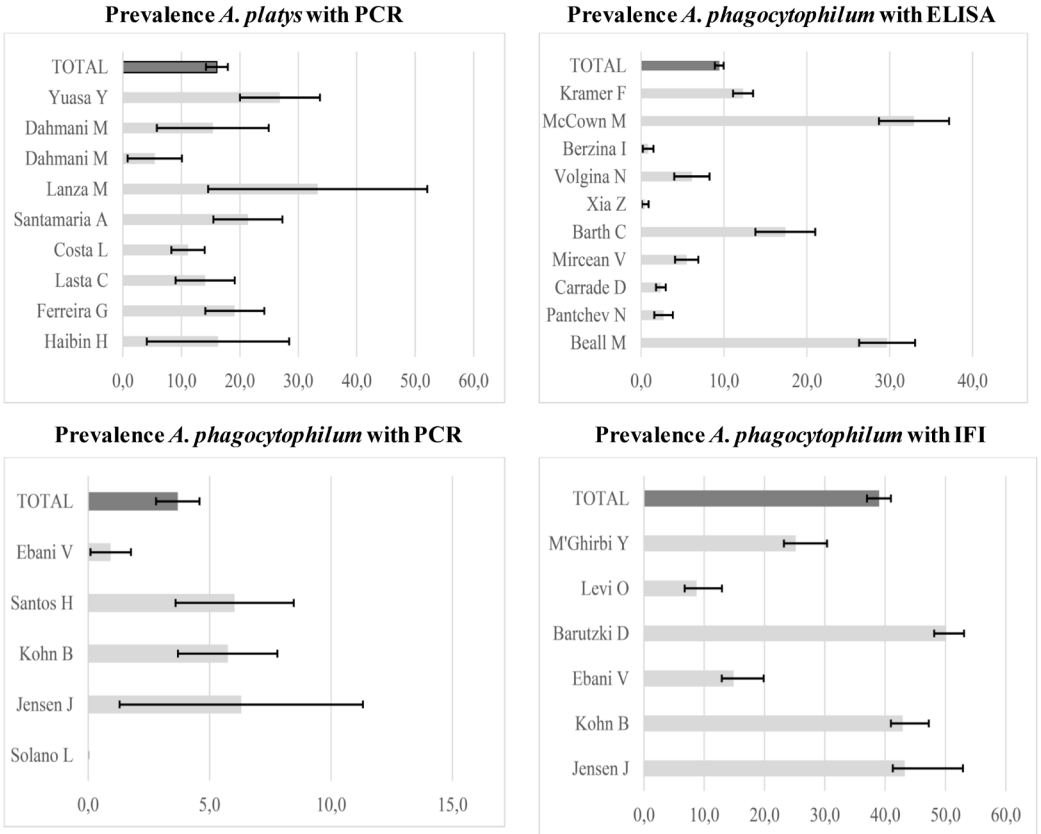

Figure 5. Meta-analysis (Forest Plot) of the prevalence of *A. platys* and *A. phagocytophilum* according to the diagnostic test.

DISCUSSION

Thirty articles published within a period of 13 years were included, which described the evaluation of the infection in 18.472 canines, mostly from Brazil, the United States, and Germany. These studies displayed good methodological quality, which translates into a high validity of this meta-analysis. In countries like Brazil, the occurrence of the disease may be due to the geographical location and climate, given that infections by *Anaplasma* spp. have been reported with greater incidence in tropical and subtropical areas (43). In fact, tropical moist climates provide a suitable environment for vectors such as ticks and mosquitoes. Regarding natural reservoirs, there is a strong geographical variation between the diverse diseases transmitted by ticks. Canine populations are susceptible to most tick-transmitted pathogens that infect mammals, including humans, becoming thus large reservoirs and sentinels for infectious and zoonotic diseases (41).

In European countries such as Germany, anaplasmosis has been reported as an emerging zoonosis. *Anaplasma* spp. is widely distributed geographically, extending throughout the Northern hemisphere from Canada to China. Seropositive results in people from various European countries, as well as the presence of the pathogen in ticks and in macro and micromammals from the region, is a proof of the distribution of *A. phagocytophilum* throughout the continent.

Regarding time, a clear seasonality of *Anaplasma spp.* is observed both in Europe and in the United States. Most cases recorded occur in the summer and late fall, which coincides with the appearance of tick nymphs and adults. The difference between seropositive and clinical cases is attributed to incorrect diagnoses and the existence of pathogen variants of *Anaplasma* spp. (13)

A prevalence of 39.0% was found in the studies that employed IFI, 9.3% in those with ELISA, and 7.1% with PCR, with a high heterogeneity attributable to the places of study and the test *per se*, which evidences the risk of finding false positives in screening programs based on the estimation of seroprevalence. The most appropriate method must be chosen in order to establish the number of dogs with past, active or persistent infections of anaplasmosis. Positive results of *Anaplasma* spp. have been reported with the IFI, whereas PCR resulted in negative findings. It is then when it should be determined if there is a past infection with the presence of antibodies and the absence of antigens (20,27,35).

The microscopic visualization of Giemsa-stained blood smears is the baseline diagnostic technique and the most common method applied in the identification of *Anaplasma* spp. in animals with clinical symptoms. However, in chronic phases, in asymptomatic individuals, or in carrier stages, the disease does not express a high parasitemia, so its detection with staining is not possible. It is an economical and simple method, useful to detect levels of parasitemia from 0.1 to 0.2%, that is, only levels greater than 106 infected erythrocytes per milliliter of blood are detectable. Besides, the process is tedious, not appropriate when there is a large number of samples and not useful when trying to differentiate species (48).

IFI is one of the most used techniques and has often been considered a sensitive test. However, it is sometimes considered unhelpful because of false positive reactions. On the other hand, the detection of antibodies by ELISA is sensitive as well as specific and provides the possibility of a better interpretation of the results when compared with the techniques mentioned above. It allows the identification of the immune status of animal populations and determination of the seroprevalence of the infection. Nevertheless, there are reports on cases of cross-reactivity between *A. platys* and *A. phagocytophilum*, as they are related species that share antigenic epitopes (48).

Lastly, PCR is the test of greater sensitivity and specificity, which makes it possible to overcome the limitations of other tests, such as the high proportion of false results and cross-species reactions. This is essential to support clinical diagnoses, identify a certain animal as a carrier and estimate the prevalence of the general infection and by species (48).

Based on the studies that used PCR, a statistically higher prevalence was found in America with 11.9%, compared to Africa with 5.5%, Asia with 4.1% and Europe with 3.5%. These heterogeneous results may be attributable to the country of study, while evidencing the need to conduct studies in each context, in order to gain insight on the relationship between environmental characteristics, hosts, and vectors specific to each place (aspects not described in the systematized studies). Such need becomes even more important when considering other possible reasons for the varied infection distribution, such as the type of population selected, the endemicity of the place of study, the presence or absence of clinical signs, and whether the health authorities of each country demand notification of its occurrence (20,27,35).

With PCR, the prevalence of *A. platys* was 16.1%, while the prevalence of A. *phagocytophilum* was 3.7%. The first species was thus proven to be the main causative agent in canines, unlike others such as *A. phagocytophilum* , which predominates in humans, horses, donkeys, wild swine and small ruminants such as goats and sheep (14,46); *A. marginale* and *A. centrale,* which are common in bovines; and *A. ovis,* which causes a disease only in sheep and goats (47).

Ehrlichia canis and *Borrelia burgdorferi* were observable in most of the coinfection cases. Regarding the former, a study in Brazil reported 16.4% of infection by *E. canis*, 19.4% by *A. Platys* and 5.5% of coinfection by both microorganisms (30); in Panama, there was a prevalence of 64.2% by *E. canis*, 21.4% by *A. platys,* and 7.5% of coinfection (42); and in three cities of Colombia, a prevalence of 25% was reported in relation to *E. canis*, 11% to *A. phagocytophilum* and 6% to the coinfection (41). In the case of *B. burgdorferi*, a study in Germany found seroprevalences of 4.9% with this agent, 19.4% with *A. phagocytophilum* and a 2.0% of coinfection (28); the seroprevalence of coinfection in Latvia was estimated at a 36% in the canines infected with *B. burgdorferi* (33); and in Poland the coinfection by *A. phagocytophilum* and *B. burgdorferi* was 1.7% (39). These data demonstrate the importance of using techniques that allow the identification and differential diagnosis of the species, mainly in cases of anemia and thrombocytopenia.

Among the limitations, it is worth highlighting that the report on prevalences by area of origin, species involved, frequency of coinfections, and presence or absence of signs in canines was deficient or highly heterogeneous. Certain independent variables, as well as the ones mentioned above, are useful in prevalence studies to identify potential associated factors and consolidate hypotheses for analytical studies.

In conclusion, a high prevalence of the global infection was found, with a predominance of *A. platys*, in a low number of publications worldwide and with high heterogeneity in the occurrence of the infection according to countries, diagnostic techniques and species involved. These findings provide relevant information that may promote the development of epidemiological research and sanitary actions involving canine population.

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Conflict of interests

None of the authors declare conflicts of interest for the publication of this study.

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