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Analysis of cross-sectional studies of leptospirosis in donkeys: A systematic review and meta-analysis

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ABSTRACT.- Morais D.A., Limeira C.H., Nunes B.C., Neto P.S.B., Falcão B.M.R., Brasil A.W.L., Santos C.S.A.B., Azevedo S.S. & Alves C.J. 2024. **Analysis of cross-sectional studies of leptospirosis in donkeys: A systematic review and meta-analysis.** *Pesquisa Veterinária Brasileira 44:e07488, 2024.* Unidade Acadêmica de Medicina Veterinária, Universidade Federal de Campina Grande, Av. Universitária 110, Patos, PB 58708-110, Brazil. E-mail: sergio.santos@professor.ufcg.edu.br

Leptospirosis is a neglected zoonosis that infects donkeys and other animal species, with economic and public health concerns. Donkeys have an important role in the development of societies, the reduction of their effective population in recent years, and the little attention given to the diseases that affect them, reducing their productivity and performance. This study aimed to investigate the pooled prevalence of *Leptospir*a spp. infection in donkeys worldwide through a systematic review and meta-analysis. Overall, 21 surveys met the eligibility criteria, with an overall combined prevalence of 34.90% (95% CI = 23.58% – 48.23%). Cochran's Q test (p<0.01) was used to identify heterogeneity between studies, classified as high heterogeneity by the Higgins and Thompson test (I^2 = 95.4%). Egger's test did not identify the presence of publication bias (p=0.9892). This scenario suggests the need for standardization of epidemiological studies for leptospirosis in this species, such as the use of probabilistic sampling, collection of minimal information on the animals used, and the establishment of a cutoff point for the serological diagnostic test (microscopic agglutination test – MAT) and essential serogroups to be used in serology to determine reliable epidemiological indicators. In addition, there is a need for molecular studies and isolation of *Leptospira* spp. in donkeys for better elucidation of the disease epidemiology.

INDEX TERMS: Public health, zoonoses, prevalence, epidemiology, Equidae, donkeys, leptospirosis.

RESUMO.- [**Análise de estudos transversais da leptospirose em asininos: revisão sistemática e meta-análise.**] A leptospirose é uma zoonose negligenciada que infecta asininos e outras espécies animais, com preocupações econômicas e de saúde pública. Motivado pela importância dos asininos no desenvolvimento das sociedades, pela redução de sua população efetiva nos últimos anos e pela pouca atenção dada às doenças que os acometem, reduzindo sua produtividade e desempenho, este estudo teve como objetivo investigar a prevalência agrupada da infecção por *Leptospira* spp. em asininos em nível mundial através de uma revisão sistemática e meta-análise. No geral, 21 inquéritos preencheram os critérios de elegibilidade, com prevalência global combinada de 34,90% (IC 95% = 23,58% – 48,23%). O teste Q de Cochran (p<0,01) foi utilizado para identificar a heterogeneidade entre os estudos, classificada como alta pelo teste de Higgins e Thompson (I^2 = 95,4%). O teste de Egger não identificou presença de viés de publicação (p=0,9892). Esse cenário sugere a necessidade de padronização dos estudos epidemiológicos para leptospirose nessa espécie, como a utilização de amostragem probabilística, coleta de informações mínimas sobre os animais utilizados, além do estabelecimento de um ponto de corte para o teste diagnóstico sorológico (teste de aglutinação microscópica – SAM) e sorogrupos essenciais a serem utilizados em sorologia para determinar indicadores epidemiológicos confiáveis. Além disso, há necessidade de estudos moleculares e isolamento de *Leptospira* spp. em asininos para melhor elucidação da epidemiologia da doença.

TERMOS DE INDEXAÇÃO: Saúde Pública, zoonoses, prevalência, epidemiologia, Equidae, asininos, leptospirose.

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INTRODUCTION

The donkey species has a long history in the development of human societies, mainly due to its importance in the movement of people and goods and its participation in agricultural and forestry activities (Raw et al. 2021). A decrease in the effective population of donkeys, estimated at 52 million animals worldwide, was observed (FAO 2020). This reduction is attributed to the rural exodus, mechanization of agriculture, increased consumption of donkey meat (Farias et al. 2021), and the recent exploitation of their skin for producing E-jiao by the Chinese industry (Waters 2019), suggesting the possibility of increased risk of extinction (Tatemoto et al. 2021).

Despite their significant social contribution, little attention is paid to diseases that affect donkeys and reduce their productivity and performance (Daddy et al. 2020), including leptospirosis, an infectious anthropozoonosis with worldwide distribution that infects domestic, wild, and synanthropic animals (Lara et al. 2019). It is caused by pathogenic species of the genus *Leptospira*, the most important being *L. interrogans*, with more than 200 serovars identified, each with a preferred host (Vincent et al. 2019).

In horses, *Leptospira* spp. presents tropism through the ocular pathways and genito-urinary tract (Faisal et al. 2012) and may also cause uveitis, the birth of weak offspring, stillbirths, and abortions (Artiushin et al. 2012). Furthermore, the intensity of tissue damage is closely related to the strain involved and host adaptability (De Brito et al. 2018).

Considering the rusticity attributed to donkeys, they may act as silent reservoirs for *Leptospira* spp., which makes them a potential source of infection for humans and other animals (Daddy et al. 2020). Once infected, they can shed the bacteria through urine or placental remains, allowing direct transmission through contact with these materials and/or indirect transmission due to contact with contaminated water or soil (Paixão et al. 2016).

In humans, leptospirosis determines variable clinical manifestations, ranging from a subclinical condition or febrile illness to severe clinical conditions with a high percentage of lethality (Bezerra et al. 2010). It can assume an occupational nature by affecting professionals such as veterinarians, farmers, butchers, rural traders, and sanitation or rodent control workers, who are routinely exposed to open water sources and/or animals (Grevemeyer et al. 2017).

For diagnostic elucidation, laboratory tests are necessary, which can be direct, with the identification of the agent, or indirect, which are based on the detection of specific antibodies (Burriel 2010). The microscopic agglutination test (MAT) is the most frequently used laboratory test to confirm the clinical diagnosis, determine the herd-level prevalence, and perform epidemiological studies (Oliveira Filho et al. 2014) besides having its high specificity as the main advantage (Bourhy et al. 2013).

Despite the significant number of donkeys in various regions of the world, added to their importance and the damage that leptospirosis may cause, very little is known about the infection by *Leptospira* spp. in this species (Alvarado-Esquivel et al. 2018). This fact can be attributed both to their low commercial value and to the fact that breeders consider that they do not require care because they are very resistant animals (Pessoa et al. 2014).

Therefore, given the scarcity of studies addressing leptospirosis in donkeys, its zoonotic importance, and the close relationship between humans and this species (Lara et al. 2019), this study aims to search the literature available electronically for a systematic review, followed by a meta-analysis of the results found on the prevalence of leptospirosis in donkeys.

MATERIALS AND METHODS

Ethical approval. Since all the data were obtained from database literature searches, this study did not perform any animal experiments. The submission to the Ethics Committee on Animal Use (CEUA) was unnecessary.

Study design. This study is characterized as a systematic literature review in which we sought to synthesize the main research information related to infection by *Leptospira* spp. in donkeys and conduct a meta-analysis of prevalence data available in studies published in indexed journals. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology (Moher et al. 2009) was used to standardize the stages of elaboration of the systematic review.

Data sources and research strategies. The process of identifying published articles related to leptospirosis in donkeys was developed in five scientific databases: PubMed, SciELO, Science Direct, Scopus, and Web of Science. The following combination of English search terms was used to search the titles and abstracts of articles: {leptospira OR leptospirosis} AND {donkeys OR *Equus asinus*}.

Citations from the studies thus identified, containing title and abstract, were saved in the BibTex format and exported to the Mendeley® bibliographic manager, from which duplicates were excluded for later selection. Searches were conducted from May 3 to 7, 2022 and an update in June 2024. There were no restrictions regarding language or the country where the study was conducted.

Additionally, a meticulous manual scan was performed using the bibliographic references of each paper to identify and possibly include titles of works that suited the criteria and were not among the findings in the aforementioned databases.

Article eligibility. Case reports, cross-sectional studies describing the clinical, epidemiological (prevalence, species, sex, age, purpose of breeding, characteristics of the breeding area, frequency of positives and risk factors), and diagnostic methods used to identify donkeys naturally infected with *Leptospira* spp. were eligible.

The types of publications included were complete articles, brief communications, and case reports that addressed questions within the following criteria: (I) clinical symptomatology in donkeys; (II) disease frequency in donkey populations; and/or (III) diagnosis of leptospirosis in donkeys.

Literature reviews, research notes, editorials, experimental trials, and other types of publications that did not meet the criteria were excluded. Additionally, all seroepidemiological surveys in equids that presented the number of horses, donkeys, and mules without distinction of species and studies with only the total frequency of positives, without the explicit sample number, were also excluded.

Study selection and data extraction. The studies were selected independently by two researchers, based on the analysis of titles and abstracts, followed by full reading. Because of this thorough review, other studies were excluded for not meeting the eligibility criteria. Occurrences of disagreements between the two researchers were resolved through a consensus.

Data extraction was conducted independently by two researchers, and the information was added to a previously developed spreadsheet. Quantitative data included references (authors and year of publication),

the country where the study was conducted, sample size, number of positive animals, frequency (%), and diagnostic methods. In surveys that used more than one diagnostic test with the same samples, only the serological test (MAT) was included in the meta-analysis to avoid overestimating the combined prevalence estimate.

Data analysis. For the analysis of quantitative data, a 95% confidence interval (95% CI) was considered. Heterogeneity was assessed using Cochran's Q test and quantified using Higgins and Thompson's I^2 test. Pooled estimates and 95% CI were calculated based on the inverse variance random effects model using the restricted maximum-likelihood method. The visual evaluation of the funnel chart and Egger's test were also used as alternatives to identify possible publication biases. All analyzes were conducted using the R environment version 3.5.1 (R Core Team 2020), with the "meta" statistical package (Balduzzi et al. 2019).

RESULTS

A total of 63 articles were initially identified in five databases: PubMed (n=16), SciELO (n=3), Science Direct (n=4), Scopus (n=17), and Web of Science (n=23). After checking for duplicates, 29 articles were excluded. Manual scanning through the references resulted in the finding of seven additional publications that had not been retrieved by the databases. Thus, 41 papers were eligible for title and abstract reading. After this step, there were 27 articles for a full reading. Of these, six were studies that analyzed animals of the genus *Equus* (horses, donkeys, and mules) but did not present the specific number of donkeys tested and positive; therefore, these six articles were excluded.

Of the total number of studies surveyed, 21 met the eligibility criteria (Fig.1). These articles consisted of cross-sectional surveys with sufficient data for a quantitative synthesis and meta-analyses. No case reports of leptospirosis in donkeys were found for the qualitative synthesis.

The included studies were conducted in four continents: Africa (7), America (9), Asia (3), and Europe (2); nine different countries: South Africa (1), Brazil (7), Croatia (1), Egypt (4), Iran (2), Itália (1), Morocco (1), Mexico (1), Saint Kitts and Nevis (1), and Senegal (1), Türkiye (1), and published from 1960 to 2023. A total of 1,925 donkeys of different ages and sexes were analyzed, with the detection of *Leptospira* spp. in 666 animals.

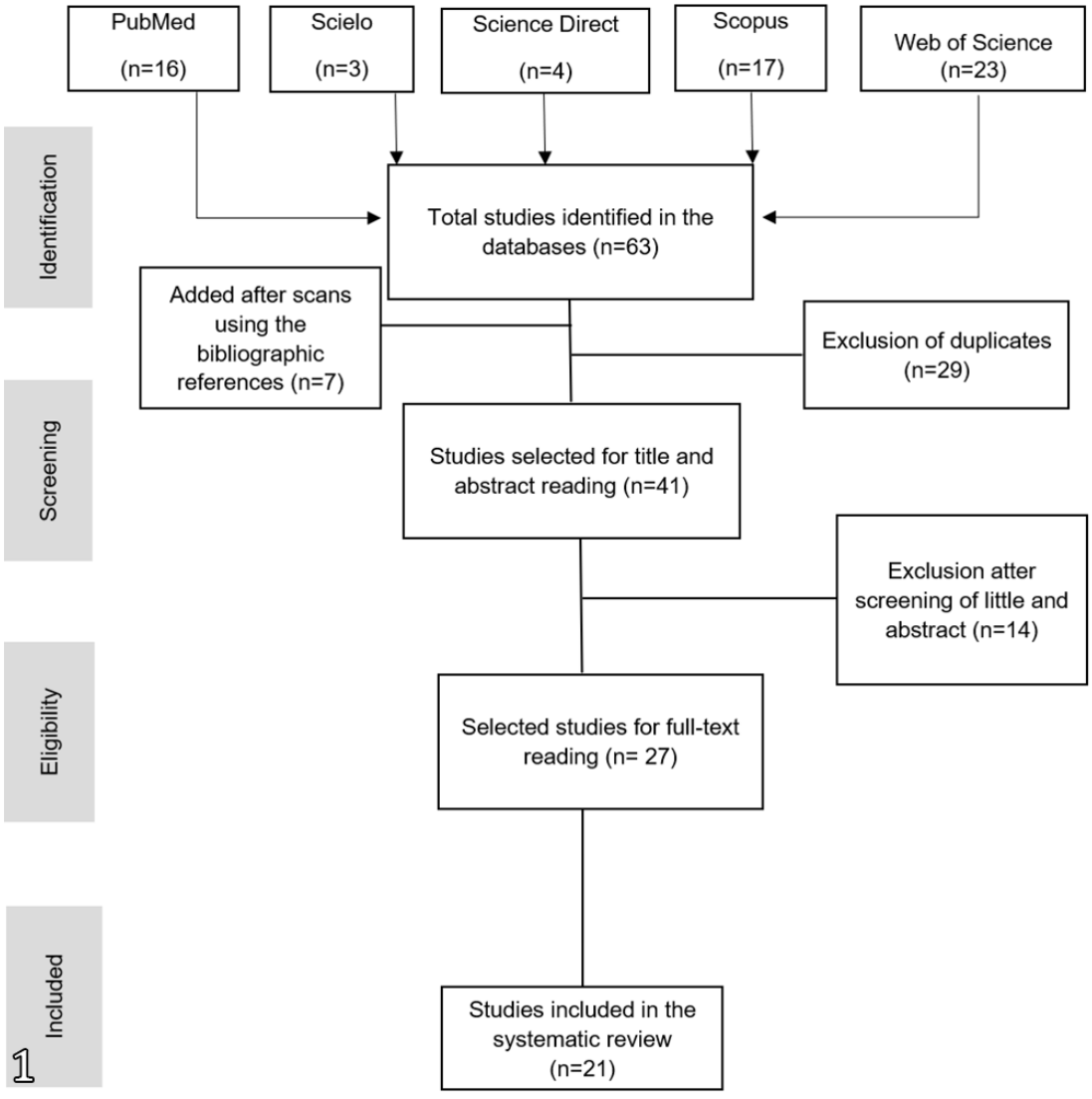

Fig.1. Flowchart of the search in five databases, selection, and inclusion in the systematic review of studies on leptospirosis in donkeys.'

The main information from the studies included in the meta-analysis is described in Table 1. The combined overall prevalence among all 21 included studies was 34.90% (95% CI = 23.58% – 48.23%). Cochran's Q test ($p<0.01$) identified heterogeneity between studies, which was classified as a high heterogeneity by the Higgins and Thompson test ($I^2 = 95.4\%$). A meta-analysis was conducted with separation according to the method used for diagnosis to elucidate this high heterogeneity since one of the 21 studies had used the PCR technique and the others the MAT. Nevertheless, it was identified that the high heterogeneity remained.

Figure 2 shows the forest plot with the 21 studies included in the meta-analysis, where the points within the graph are the prevalence of each study individually, the horizontal line is the confidence interval, and the diamond label shows the combined prevalence. Prevalence results and confidence intervals are shown in the fourth and fifth columns, respectively. The last column represents the weight each participating study added to the result.

The funnel plot (Fig.3) and the application of Egger's test were used to verify the presence of publication bias. Although the visualization of the chart demonstrates an asymmetrical distribution of the 21 included studies, Egger's test did not identify the presence of publication bias ($p=0.9892$), and the asymmetry of the funnel chart could be attributed to other factors, such as the high heterogeneity found (Sterne et al. 2011).

DISCUSSION

In this systematic review and meta-analysis, the few studies reached in the initial search reveal the scarcity of research addressing the theme, reaffirming that leptospirosis is a neglected disease in donkeys. One of the limitations that caused the exclusion of some is that most surveys involving

Table 1. Summary of information found in the analysis of cross-sectional studies of leptospirosis in donkeys

Reference	Year	Country	Tested	Diagnostic	Cutoff	Positive (%)	Most frequent serogroups
Ali & Saeid	2012	Iran	80	MAT	1:100	33 (41.25)	-
Alvarado-Esquivel et al.	2018	Mexico	194	MAT	1:100	151 (77.84)	Icterohaemorrhagiae, Sejroe, Canicola, Grippotyphosa, Ballum, Semaranga, Panama
Barsoum et al.	1978	Egypt	125	MAT	1:128	90 (72)	Autumnalis, Canicola, Icterohaemorrhagiae, Pomona
Benkirane et al.	2016	Morocco	15	MAT	1:20	3 (20)	Australis, Javanica
Bezerra et al.	2010	Brazil	60	MAT	1:100	51 (85)	Copenhageni, Pyrogenes, Autumnalis, Icterohaemorrhagiae
Brewer et al.	1960	Turkiye	50	MAT	1:100	29 (58)	Grippotyphosa, Hebdomadis, Autumnalis
Daddy et al.	2020	South Africa	365	MAT	1:100	42 (11.51)	Australis, Tarassovi
Grevemeyer et al.	2017	Saint Kitts and Nevis	124	PCR	-	22 (17.74)	-
Grubišić et al.	2011	Croatia	94	MAT	-	24 (25.53)	Australis, Pomona, Ictherohaemorrhagiae
Hajikolaei et al.	2005	Iran	90	MAT	1:100	36 (40)	Grippotyphosa, Icterohaemorrhagiae, Ballum, Pomona, Sejroe, Canicola
Lara et al.	2019	Brazil	85	MAT	1:100	53 (62.35)	Icterohaemorrhagiae, Grippotyphosa, Sejroe, Australis, Shermani, Pomona, Copenhageni, Autumnalis, Canicola, Bataviae
Maronpot & Barsoum	1972	Egypt	31	MAT	-	9 (29.03)	-
Morais et al.	2019a	Brazil	349	MAT	1:50	69 (19.77)	Icterohaemorrhagiae, Australis, Autumnalis, Sejroe, Pomona, Celledoni, Tarassovi
Morais et al.	2019b	Brazil	28	MAT	1:100	6 (21.43)	Australis, Tarassovi
Morais et al.	2023	Brazil	30	MAT	1:50	12 (40)	Icterohaemorrhagiae, Ballum, Canicola, Semaranga, Grippotyphosa
Oliveira Filho et al.	2014	Brazil	7	MAT	1:100	2 (28.57)	Batavie, Grippotyphosa
Pires et al.	2023	Brazil	109	MAT	1:100	14 (12.84)	Tarassovi, Sejroe
Ragona et al.	2016	Italy	31	MAT	1:100	0 (0)	-
Roqueplo et al.	2019	Senegal	20	MAT	1:100	16 (80)	Icterohaemorrhagiae, Australis, Pyrogenes, Canicola, Grippotyphosa, Autumnalis, Sejroe, Cynopteri
Samir et al.	2015	Egypt	26	MAT	-	2 (7.69)	Grippotyphosa, Celledoni
Sebek et al.	1989	Egypt	12	MAT	-	2 (16.67)	-

(-) Unavailable information.

the family *Equidae* did not bring specific information about donkeys, compromising their inclusion in the analysis.

Despite meeting the proposed eligibility criteria, the selected studies did not share the same methodology. This explains, therefore, the heterogeneity found, classified as methodological (Santos & Cunha 2013). The use of non-random sampling in part of the studies may have directly influenced the results obtained, considering that this type of selection allows the determination of important epidemiological indicators. However, when selecting the sampling units according to non-probabilistic criteria, it undertakes generalizations of results, influencing the prevalence (Ramalho et al. 2020). In addition to the type of sampling used, the number of animals sampled showed high variability, such as a high number of animals (n=365) in the study by Daddy et al. (2020) and a small number (n=7) in the study by Oliveira Filho et al. (2014).

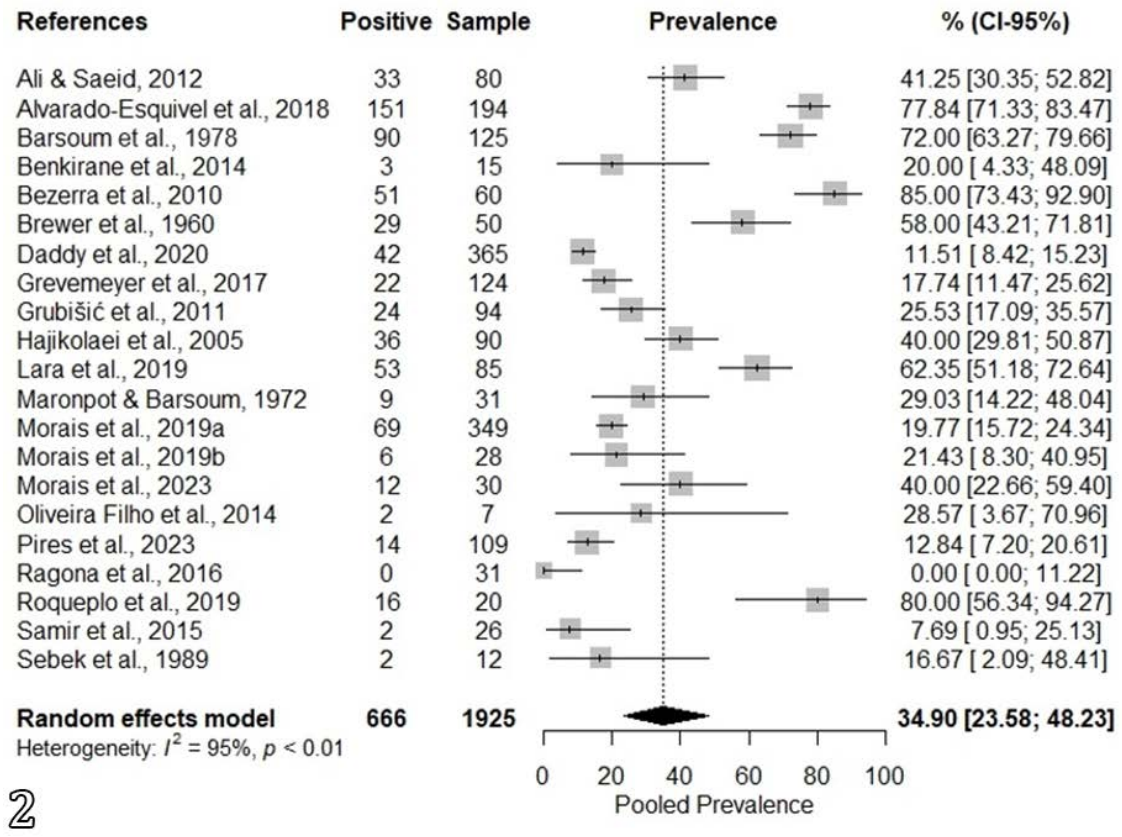

Fig.2. Forest plot with the summary of meta-analysis of cross-sectional studies of leptospirosis in donkeys.

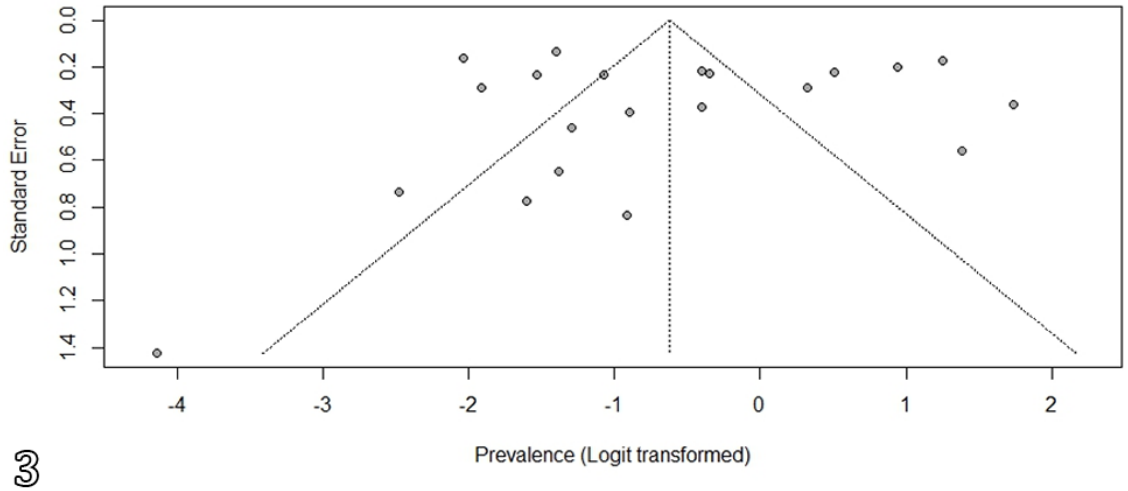

Fig.3. Funnel chart used to verify the presence of publication bias in cross-sectional studies of leptospirosis in donkeys.

As for the years of publication, a difference of 63 years was observed between the oldest study (Brewer et al. 1960) and the most recent one (Morais et al. 2023, Pires et al. 2023). As for age, most surveys did not bring this data from the animals that tested positive but only from the total, making it impossible to analyze the relationship between age and infection. This is a limitation since the selection of sampling units without considering the effect of age can result in an under or overestimation of the prevalence (Ramalho et al. 2020).

When analyzing the information contained in Table 1, the variable prevalence is observed among the studies, with values of 0% (Ragona et al. 2016) in donkeys in Italy, up to 85% (Bezerra et al. 2010) in Brazil, both using the MAT as a diagnostic method. According to Morais et al. (2019a), this range of variation in the prevalence of seropositive donkeys for *Leptospira* spp. can be understood due to the variety of factors that influence the occurrence of the disease, with emphasis on the environmental conditions of the study sites, the cutoff point and the serogroups used in the MAT, the existence of clinical suspicion, hygienic-sanitary management of the herds, degree and type of exposure to other animals, in addition to the sampling performed.

The meta-analyses of prevalence studies mostly show high heterogeneity identified since the majority do not follow the probabilistic sampling achieved. In addition, there is a lot of diversity between locations, generating heterogeneity. In this study, an attempt was made to reduce this by dividing the meta-analyses into subgroups (such as by continent and diagnostic methods), but it remained high. As only one study used the PCR technique, it is not possible to analyze whether there is heterogeneity in this technique alone. This data reveals the lack of literature on DNA research and isolation of *Leptospira* spp. in this species.

The overall combined prevalence of 34.90% (95% CI = 23.58% – 48.23%) among the 21 included studies indicates the detection of antibodies or leptospiral DNA in donkeys from eleven countries distributed over four continents, which may be a risk to human health given the proximity to these animals (Lara et al. 2019). No clinical signs suggestive of the disease were reported. This can be attributed to a disease with a silent course and nonspecific clinical signs since its agent is maintained in the environment, mainly by animals with chronic infection (Oliveira Filho et al. 2014).

The serological tests showed some methodological differences in detecting *Leptospira* spp. in donkeys, such as the cutoff point and the serogroups used. As for the cutoff point varied between 1:20 and 1:100. Regarding the serological diagnosis, 17 of the 21 studies reported which serogroups were identified specifically in donkeys (Table 1). The serogroup Icterohaemorrhagiae was reported in eight studies, being the most frequent serogroup in five, followed by Australis, which was present in seven studies and the most frequent in four. Occurrences of serogroups in donkeys that have greater virulence for humans are of greater concern due to the close contact between humans and donkeys.

CONCLUSIONS

The present study demonstrated a significant pooled prevalence for leptospirosis in donkeys.

The heterogeneity identified among the surveys included in this review is expected because of the existing differences among the cross-sectional surveys. This scenario suggests the need for standardization of epidemiological surveys for leptospirosis in donkeys, such as the use of probabilistic sampling and the collection of minimal information on the animals used, such as gender, age, and zootechnical use. In addition, establishing a cutoff point for the microscopic agglutination test (MAT) and essential serogroups to be used in the species would provide the determination of reliable epidemiological indicators.

Furthermore, there is a need for molecular studies and isolation of *Leptospira* spp. in donkeys for better elucidation of the disease epidemiology.

Authors' contributions.- Davidianne A. Morais: Conceptualization, methodology and writing-original draft; Clécio H. Limeira: Methodology; Bruno C. Nunes: Methodology; Pirajá S.B. Neto: Methodology; Brunna M.R. Falcão: Methodology; Arthur W.L. Brasil: Data analysis; Carolina S.A.B. Santos: Writing-review; Sérgio S. Azevedo: Supervision and writing-review; Clebert J. Alves: Conceptualization, supervision and writing-review.

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Conflict of interest statement.- The authors declare having no conflict of interest.

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