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Systematic Review

Worldwide Overview of *Neospora* spp. Infection in Equids Diagnosed by Serological Tests: Systematic Review and Meta-Analysis

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Abstract: Equids can be infected by *Neospora* spp., and its infection is associated with neurological and reproductive disorders. This systematic review and meta-analysis aimed to assess the prevalence of anti-*Neospora caninum* antibodies in horses, donkeys and mules worldwide between 2012 and 2022. Five scientific databases were consulted: MEDLINE, ScienceDirect, PubMed, LILACS and SciELO, using the descriptors "protozoan" or "neospora" AND "horses" or "donkeys" or "mules". A total of 1909 studies were screened, and 25 studies met the inclusion criteria. The pooled prevalence of *Neospora* spp. infection in equids, based on a total of 11,076, was 8.34% (95% CI: 4.78–14.17%). Further analysis by species revealed no statistically significant difference in the prevalence of *Neospora* spp. infection across equids: 8.5% (95% CI 5.03–13.99%) in horses, 7.36% (CI 2.08–22.94%) in donkeys and 6.07% (95% CI 0.71–36.83%) in mules. Regarding diagnostic tests, the prevalence of *Neospora* spp. infection determined by the indirect fluorescent antibody test (IFAT) was 8.08% (95% CI: 3.74–16.61%), by the enzyme-linked immunosorbent assay (ELISA) was 7.91% (95% CI: 3.71–16.08%), and by the modified agglutination test (MAT) was 20% (95% CI: 15.37–25.60%). This meta-analysis provided valuable and comprehensive insights into the prevalence of *Neospora* spp. infection in horses, donkeys, and mules using serological detection methods. The results highlight the wide distribution of *Neospora* spp. infection in equids across all continents, indicating the importance of this infection. Transmission of the parasite can occur both horizontally (directly between animals) and vertically (from mother to offspring), further emphasizing the significance of understanding and managing this infection in equid populations.

Keywords: donkeys; horses; mules; neosporosis; prevalence; serodiagnosis

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1. Introduction

Neosporosis is considered a significant disease affecting cattle and dogs worldwide. It is caused by protozoa belonging to the genus *Neospora* and is recognized as one of the primary causes of abortion in cattle and sheep, resulting in significant economic losses in livestock [1–6]. The life cycle of the parasite primarily involves a definitive carnivorous host (*Canidae*) that consumes meat from an intermediate herbivorous host. After infection, the definitive hosts shed oocysts in the feces, which can then infect intermediate hosts through oral ingestion. As a result, the tissues of these intermediate hosts are compromised by the cysts, which subsequently become infective to carnivorous hosts. Notably, *Neospora caninum* is known for its efficient transplacental transmission, making it a significant concern [3,4,7].

Equids can become infected by two species of the genus *Neospora* spp. [8], those being *Neospora hughesi* and *Neospora caninum* [9]. The infection by these parasites has

been associated with neurological and reproductive disorders [10]. Considering the global significance of equine breeding, as these animals are utilized for sports, reproduction, leisure, and work purposes, it is crucial to ensure their health status is suitable for carrying out these activities [11–13].

The serological diagnosis methods are of utmost importance for epidemiological studies to bring accurate data. The main methods used for *Neospora* spp. serological diagnosis are microscopic agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA) and immunofluorescence antibody test (IFAT). The IFAT has the most reliability amongst the three, with the most sensitivity and specificity [14], while ELISA may vary between commercial kits in its diagnostic ability [14]. Lastly, the MAT technique has the lowest sensitivity of the three [14].

Determining the exposure of equids to *Neospora* spp. is essential for enhancing our understanding of its epidemiology, which, in turn, is necessary for implementing effective prevention strategies [15]. In this regard, a systematic review and meta-analysis were conducted to evaluate the prevalence by qualitative serological methods of anti-*Neospora* spp. antibodies in horses (*Equus caballus*), donkeys (*Equus asininus*), and mules (*Equus mulus*) worldwide.

2. Results

The database search yielded a total of 1209 studies, out of which 232 were identified as duplicates. Among the remaining 904 studies, a screening of titles and abstracts narrowed down the selection to 69 studies. After conducting a thorough quality assessment and carefully reviewing each article for adherence to the inclusion criteria (including year of publication, serological test utilized, and accessibility), a final set of 25 studies were found to provide relevant insights into the seroepidemiology of *Neospora* spp. in equids.

Initially, a meta-analysis was conducted using data from 25 selected studies. The analysis revealed a combined prevalence of *Neospora* spp. infection in equids of 8.34% (95% CI: 4.78–14.17%). Heterogeneity was observed in the studies, as indicated by the Cochran Q test ($p < 0.01$), and further confirmed by the Higgins and Thompson test, classifying the heterogeneity as high (I2 = 97.3%) (Figure 1).

Utilizing the random-effects model, which accounts for the heterogeneity among the included studies, three additional meta-analyses were conducted to explore the sources of heterogeneity (Table 1). In the first analysis, the primary studies were stratified by continent, revealing the highest seroprevalence in Asia (20.48%; 95% CI: 7.66–44.43%). The second analysis categorized the studies based on species (horses, donkeys, or mules), indicating a slightly higher seroprevalence in horses (8.50%; 95% CI: 5.03–13.99%), although statistical significance was not observed. The third analysis examined the diagnostic methods used, with the modified agglutination test (MAT) demonstrating the highest seroprevalence (20.00%; 95% CI: 15.37–25.60%).

The funnel plot graph depicts the distribution of the 25 studies included in this meta-analysis, revealing an asymmetric pattern of dots. This asymmetry indicates a possible presence of publication bias (Figure 2). To further examine this, the Egger test was employed, but it did not yield statistically significant results ($p = 0.08$), suggesting no conclusive evidence of publication bias.

Table 1. Subgroup analyses of the global seroprevalence of *Neospora* spp. in equids, according to continent, species and diagnostic methods.

	Number of Studies	Sample	Positive	Combined Prevalency (95% CI)	Heterogeneity	
					p	I^2
Overall combined prevalence	25	11,076	1178	8.34% (4.78–14.17%)	<0.01	97.30%
Continent						
Africa	1	144	10	6.94% (3.78–12.43%)	Not applicable	
Asia	6	3816	603	20.48% (7.66–44.43%)	<0.01	98.30%
Central/North America	2	865	24	3.07% (1.65–5.64%)	0.14	55.00%
Europe	4	1451	115	5.48% (1.49–18.19%)	<0.01	95.80%
South America	12	4800	426	6.87% (2.81–15.82%)	<0.01	97.30%
Specie						
Donkeys	10	3954	397	7.36% (2.08–22.94%)	<0.01	97.10%
Horses	19	6950	756	8.50% (5.03–13.99%)	<0.01	96.90%
Mules	3	172	25	6.07% (0.71–36.83%)	<0.01	83.70%
Diagnosis						
ELISA	7	3746	414	7.91% (3.71–16.08%)	<0.01	95.10%
IFAT	17	7095	717	8.08% (3.74–16.61%)	<0.01	97.90%
MAT	1	235	47	20.00% (15.37–25.60%)	Not applicable	

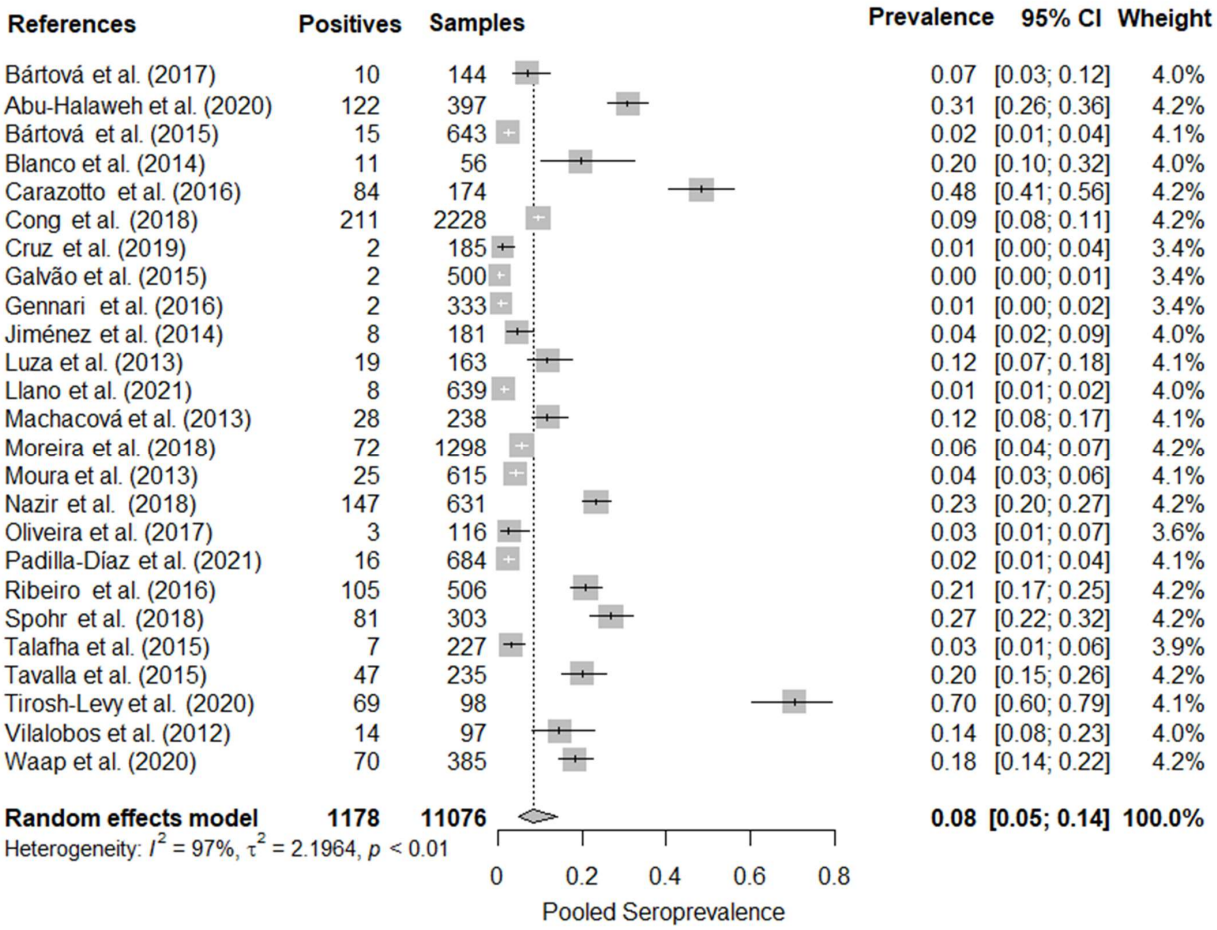

Figure 1. Forest plot of the combined evaluation of the global seroprevalence of *Neospora* spp. in equids [8,16–39].

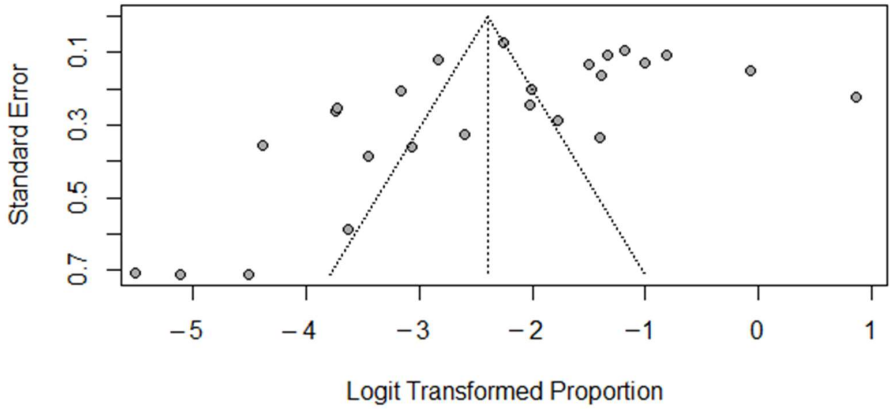

Figure 2. Funnel plot showing the distribution studies (spots) according to the seroprevalence and standard errors of *Neospora* spp. in equids worldwide.

3. Discussion

Neospora spp. has the potential to infect horses, leading to neosporosis, a disease associated with reproductive, neurological, and neuromuscular disorders [8,40]. Infections caused by *Neospora* spp. have been documented globally in horses, manifesting as clinical and subclinical signs. Serological surveys, aimed at detecting anti-*Neospora* IgG antibodies, have been conducted to investigate the prevalence of infection and identify potential risk factors [8,16–39].

The global investigation of anti-*Neospora* spp. antibodies has been conducted extensively. However, when undertaking a seroprevalence study, the selection of an appropriate diagnostic test is a crucial step to ensure the reliability of the research. It is essential to consider a technique with high sensitivity and reasonable specificity to minimize the occurrence of false-positive results [38]. Among the studies reviewed, a predominance of the IFAT was observed, with 17 studies utilizing this method. This was followed by the ELISA employed in seven studies, and the MAT utilized in a single study.

In this study, the indirect fluorescent antibody test (IFAT) was not only employed in a larger number of studies but also exhibited a higher seropositivity rate of 8.08% (CI: 3.74–16.61%) for *Neospora* spp. in horses. On the other hand, the enzyme-linked immunosorbent assay (ELISA) yielded a seropositivity rate of 7.91% (CI: 3.71–16.08%). Despite the higher seropositivity observed in IFAT studies, they also displayed greater heterogeneity. This variation in results may be attributed to the diverse geographical locations of the studies, each with its distinct climatic and environmental factors.

Although the Asian continent exhibited the highest seroprevalence (20.48%), it also displayed the highest level of heterogeneity (98.3%), with only six studies conducted in Asia. The elevated seroprevalence in Asia can be attributed to the diagnostic techniques employed in the studies. Two studies utilized IFAT [16,36], three studies used ELISA [20,30,35], and one study employed MAT [36], which presented 48.5% seroprevalence. The choice of diagnostic technique may have contributed to the high seroprevalence due to the lower sensibility of MAT and lower specificity of ELISA assays, thus leading to increased heterogeneity in the results.

Central/North America exhibited the lowest overall seroprevalence of 3.07%. However, this finding can be attributed to the limited number of studies conducted in the region (only two studies), which also resulted in the lowest heterogeneity among continents (55%). One of these studies was conducted in Mexico, utilizing 684 serum samples and diagnosing *Neospora* spp. through IFAT, which yielded a seroprevalence of 2.34% [32]. The other study was conducted in Costa Rica, employing ELISA assays on 181 equine serum samples, and reported a seroprevalence of 4.4%, nearly double that of the previous study [24]. The disparity in these values could be attributed to the lower sensitivity of the ELISA assays [17].

The majority of the South American studies were conducted in Brazil, with only two out of twelve studies conducted in Colombia. The studies conducted in Colombia reported seroprevalence rates of 1.23% using IFAT assays [25], and one study utilized ELISA assays, reporting a seroprevalence of 19.7% [18].

In Brazil, the seroprevalence of *Neospora* spp. infection ranged from the highest recorded value of 48.27% [20] to the lowest value of 0.4% [22]. The variation in seroprevalence rates can be attributed to various factors, including differences in the specific regions where the studies were conducted. However, in this review, no consistent pattern of seroprevalence disparity was observed among neighboring regions with similar environmental characteristics. Despite their geographical proximity and similar environmental conditions, studies conducted in these regions demonstrated a significant disparity in seropositivity rates.

In the southern region of Brazil, specifically in the state of Santa Catarina, a study reported a seropositivity rate of 4.1% [29]. Conversely, in the neighboring state of Paraná, another study observed a higher seropositivity rate of 14.4% [39]. Moving to the northern region of Brazil, a study conducted in the state of Pará documented a seropositivity rate of 4.97% [28], while in the neighboring state of Roraima, the seropositivity rate was substantially higher at 26.7% [34]. In the state of São Paulo, one study revealed a seropositivity rate of 2.6% [31], whereas in the neighboring state of Minas Gerais, another study reported a much higher seropositivity rate of 23.9% [34]. These findings demonstrate that the significant heterogeneity observed in seroprevalence data within Brazil may not be solely attributable to regional differences. Despite neighboring regions sharing similar environmental characteristics, there are considerable variations in seropositivity rates, indicating the influence of additional factors contributing to the observed heterogeneity.

The European studies included in this review were conducted in Italy [17,27] and Portugal [21,28]. These studies revealed considerable disparities in their findings. In Portugal, the seroprevalence rates were 1.1% using ELISA assays [21] and 9.1% using IFAT [28]. Similarly, in Italy, the seroprevalence rates were 2.3% using IFAT [17] and 11.8% using ELISA assays [27]. Notably, Bartová et al. [17] reported a seroprevalence of 2.3% using IFAT, while also demonstrating a higher seroprevalence of 10.9% using ELISA. This underscores the importance of employing highly sensitive and specific techniques in studies to ensure the generation of accurate and reliable data.

Among the 25 included studies, a total of 11,076 equines were examined, consisting of 6950 horses, 3954 donkeys, and 172 mules. The overall pooled seroprevalence of *Neospora* spp. in equines was estimated at 8.34% (95% CI: 4.78–14.17%). The seroprevalence varied among the different species, with rates of 6.07% (95% CI: 0.71–36.83%) in mules, 7.36% (95% CI: 2.08–22.94%) in donkeys, and 8.50% (95% CI: 5.3–13.99%) in horses. However, no statistically significant differences were observed between the species regarding *Neospora* spp. infection.

The global distribution of neosporosis in horses has been evident across all continents, emphasizing the significance of this neurological and reproductive infection. It underscores the need for further investigations on this parasite, particularly in the African continent, where limited studies have been conducted. The Asian continent also requires additional studies due to its high seroprevalence rates and the regular consumption of equid-derived meat and dairy products. The impact of neosporosis on equid farming, with its adverse effects on reproductive rates, necessitates a deeper understanding of the disease and the implementation of appropriate management strategies [20].

4. Materials and Methods

4.1. Data Base

The current systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, implementing the following methodological steps: identification, selection, eligibility, and inclusion [41].

We conducted a comprehensive search for published studies on *Neospora* spp. in equids using five reputable scientific databases: MEDLINE, ScienceDirect, PubMed, LILACS, and SciELO. The search process employed the following descriptors: (protozoan OR neospora) AND (horses OR donkeys OR mules). The identified publications were exported in "BibTex" and "RIS" file formats to the Mendeley® bibliographic manager, where duplicates were eliminated. Three independent researchers reviewed the titles and abstracts of the primary studies. The inclusion criteria encompassed articles published between 2012 and 2022, without any language restrictions. The search was conducted on 23 October 2022.

4.2. Selection Criteria

The articles were assessed for eligibility, and any discrepancies were carefully evaluated. The following inclusion criteria were established for the systematic review and meta-analysis: (1) original peer-reviewed studies without any geographic limitations, (2) studies focusing on the seroprevalence of *Neospora* spp. infection in horses, donkeys, and mules, (3) comprehensive and easily accessible papers, (4) studies providing information on the total sample size and the number of seropositive animals, and (5) studies employing serological methods such as Immunofluorescence Antibody Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA), and Modified Agglutination Test (MAT). Following a meticulous selection process, studies that did not meet these requirements, had ambiguous results, lacked serological evaluation, or presented indeterminate features were excluded from the analysis (Figure 3).

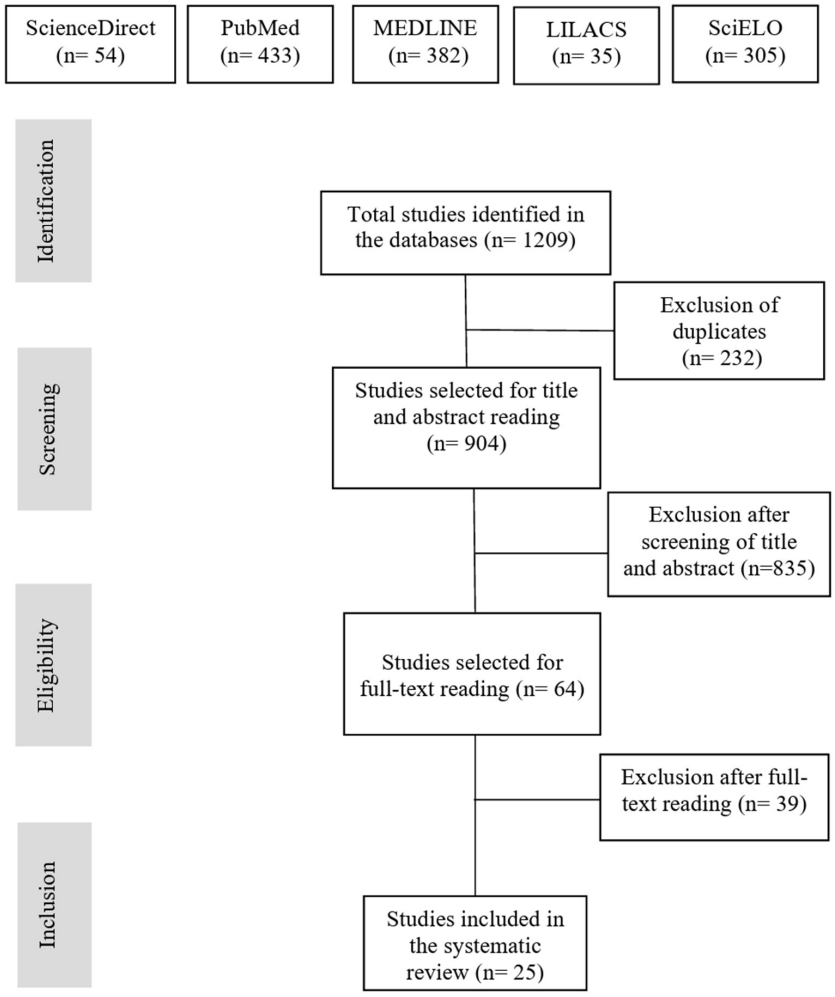

Figure 3. PRISMA flowchart, inclusion and exclusion criteria of studies on global seroprevalence of *Neospora* spp. in horses, donkeys and mules.

4.3. Meta-Analysis Approach

For the meta-analysis, a random-effects model was employed to assess the prevalence of infection by species. The heterogeneity among the included studies was evaluated using Cochran's Q test and the I2 index, which quantifies heterogeneity on a scale of 0 to 100%. Subgroup analyses were conducted to explore heterogeneity based on factors such as year, continent, country, species, and the diagnostic test utilized [40]. To examine publication bias, Egger's regression test was applied, and a funnel plot was generated. The statistical analysis was conducted using R software version 3.5.1 [42] with the assistance of the "meta" statistical package [43]. A p-value of less than 0.05 was considered statistically significant.

5. Conclusions

This systematic review and meta-analysis have compiled crucial and comprehensive data pertaining to *Neospora* spp. infection in horses, donkeys, and mules through serological detection from 2012 to 2022. The reproductive and neurological disorders caused by this infection not only pose health risks but also lead to significant economic losses in equid breeding. Therefore, it is imperative to assess the risk factors associated with *Neospora* spp. infection and implement effective surveillance policies and health-education programs for equid producers. These measures aim to mitigate the risk of protozoan infection and safeguard the health and productivity of equids.

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