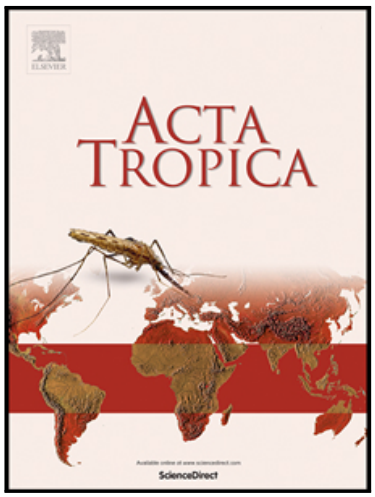

Neosporosis in sheep: a systematic review and meta-analysis of global seroprevalence and related risk factors

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Neosporosis in sheep: a systematic review and meta-analysis of global seroprevalence and related risk factors

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In addition, I want to state that I (Valeria A. Sander) am the last author and the corresponding author of the manuscript, I am not the first author. I have made this comment previously on the online submission of the manuscript mentioned above, but I want to be sure no mistake will be made about this issue. Thus, the correct order and affiliations of authors are as follows:

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HIGHLIGHTS

- The global seroprevalence of ovine neosporosis is 13% (95% CI, 10-15)
- Seroprevalence is higher in Africa and America than in other continents
- Seroprevalence is negatively associated with absolute latitude
- Seroprevalence of ovine neosporosis varies depending on the used serological method
- Age and extensive production system were identified as risk factors (positive)

ABSTRACT

Neosporosis is recognized as the main cause of abortions in cattle worldwide and there is an increasing concern about its role in ovine reproductive losses; however, epidemiological studies regarding neosporosis in sheep are still limited. This meta-analysis aimed to estimate the global pooled seroprevalence and associated risk factors of ovine neosporosis. In the current report, a comprehensive strategy of search and data collection from 7 worldwide databases was performed. A final set of 73 studies (80 datasets) published from 2000 to 2021 were selected based on inclusion criteria, comprising data on 35,740 sheep (corresponding to 37,565 evaluated samples) from 30

countries worldwide. The global pooled seroprevalence of *Neospora caninum* infection in sheep estimated by the random-effects model was 13% (95% CI, 10-15) and showed high heterogeneity (Q = 5147.15, I^2 = 98%, p< 0.001). Furthermore, by meta-analyses of subgroups it was demonstrated for the first time that seroprevalence significantly varied between continents (highest in Africa; 20%, 95% CI, 4-44), WHO regions (highest in African Region; 42%, 95% CI, 36-48), countries (highest in Colombia; 79%, 95% CI, 61-92%) and diagnostic methods (highest by IFAT; 17%, 95% CI, 12-23). Meta-regression indicated significant increasing trends in the prevalence of ovine neosporosis with decrease in geographical latitude (coefficient = -0.013; p<0.001), whereas longitude did not influence it (coefficient = -0.001; p=0.365). Regarding associated risk factors, older sheep were more likely to be infected with *N. caninum* than younger ones (OR 1.42; 95% CI 1.08–1.87), and sheep bred under intensive or semi-intensive systems resulted less susceptible to be seropositive than those bred under extensive system (OR 0.65; 95% CI 0.42-0.99 and OR 0.74; 95% CI 0.62–0.89, respectively). Conversely, no apparent association was found between seroprevalence and other variables, such as sex (OR 1.06; 95% CI 0.9–1.24), the presence of dogs on the farm (OR 1.15; 95% CI 0.63–2.12) or the presence of abortion (OR 1.80; 95% CI 0.87–3.74). In conclusion, the seroprevalence of ovine neosporosis is widely and heterogeneously distributed throughout the world, and it is negatively associated with increasing geographical latitude. In addition, age and extensive production system represent risk factors, which suggest that the horizontal transmission route is relevant for this host species. It is recommended to pay more attention to this disease and emphasize the global need for more indexed studies concerning the seroprevalence and risk factors of ovine neosporosis to better understand the epidemiology of this coccidian infection.

Keywords: Neosporosis, Meta-analysis, Prevalence, Epidemiology, *Ovis aries*, ovine, Odds Ratio

Running title: Meta-analysis of *Neospora caninum* seroprevalence in sheep

1. Introduction

Neospora caninum is an apicomplexan parasite with a complex heteroxenous life cycle, in which wild canids and dogs act as definitive hosts, while a wide range of mammals, including ruminants, act as intermediate hosts (Donahoe et al., 2015). Ruminants acquire neosporosis through the ingestion of water or food contaminated with sporulated oocysts shed by dogs (horizontal transmission) or by the transplacental passage of tachyzoites from the infected mother to the foetus (vertical transmission). Two types of transplacental transmission have been described: (i) exogenous transplacental transmission, which occurs when the dam becomes infected during pregnancy; and (ii) endogenous transplacental transmission, which occurs after the recrudescence of chronic infection in the dam (Dubey et al., 2007).

Neosporosis is recognized as one of the major infectious causes of abortion and reproductive failure in cattle, leading to economic losses estimated at U$S 1.300 billion annually in the livestock industry worldwide (Reichel et al., 2013). It has been generally considered a disease of cattle and dogs; however, recent findings have suggested that *N. caninum* infection is vertically transmitted and causes fetal loss, abortion and neonatal death also in sheep (Della Rosa et al., 2021; González-Warleta et al., 2014; Hecker et al., 2019; Howe et al., 2008; Moreno et al., 2012; Romo-Gallegos et al., 2019; Sánchez-Sánchez et al., 2021). However, the relevance of neosporosis in sheep reproductive efficiency is still under discussion and needs further investigation (Bartley et al., 2019; Dubey et al., 2017; Rodrigues et al., 2020). In fact, despite the number of articles accounting for the occurrence of neosporosis in sheep has been rising continuously since the first report of a *N. caninum*-induced abortion has been published (Dubey & Lindsay 1990), prevalence studies of ovine neosporosis are still limited (Dubey &

Schares 2011), which may lead to an underestimation of its significance in the sheep industry.

To date, only one systematic review and meta-analysis has evaluated the global seroprevalence of ovine neosporosis (Romanelli et al., 2021). Despite the article by Romanelli et al. (2021) depicts valuable information, most of the analyzed data corresponded to Brazil, including only nine datasets from other countries worldwide. This lack of data from several countries and continents among the included studies led to some limitations on its results and conclusions: the pooled seroprevalence of ovine neosporosis was not assessed by continents or countries (except for Brazil) and the sources of heterogeneity among data were not clearly identified. Moreover, no risk factor associated with ovine neosporosis was statistically confirmed by Romanelli et al. (2021). Thus, the global epidemiological situation of *N. caninum* infection in sheep remains unclear.

In the lack of a commercial vaccine or effective chemotherapeutic treatment against *N. caninum* infection, measures against this disease rely on farms management of flocks (Sánchez-Sánchez et al., 2021). Therefore, the updated systematic collection and statistical analysis of epidemiological data and the evaluation of factors influencing the seroprevalence of ovine neosporosis are crucial to develop appropriate strategies to control *N. caninum* infection in sheep. In the present study, a systematic review and meta-analysis was conducted, including information published from 2000 to 2021 in 7 worldwide databases, to estimate the pooled seroprevalence of ovine neosporosis not only globally, but also for the first time by continents, World Health Organization (WHO) regions and countries, in order to evaluate differences between these geo-political regions. A comprehensive analysis was also conducted to assess differences in the estimate of pooled seroprevalence of *N. caninum* infection in sheep between the

most commonly employed serological methods and their potential causes. In addition, a meta-regression was performed to evaluate the currently unknown relationship between geographical latitude and longitude and seroprevalence of ovine neosporosis. Finally, in the present study, several risk factors associated with ovine neosporosis were also analyzed by meta-analytical methods for the first time.

2. Material and Methods

2.1. Design and protocol registration

The current study was structured according to the recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al., 2009). PRISMA checklist is included as Supplementary (Supp) Table 1. The protocol of this systematic review was registered and is currently available at PROSPERO under the code CRD42019121309 (https://www.crd.york.ac.uk/prospero).

2.2. Search strategy

To assess the global seroprevalence and associated risk factors of *Neospora caninum* infection in sheep, a thorough literature search between articles published up to February 2021 was carried out in the following databases: PubMed, MEDLINE, Scopus, LILACS, SciELO, Google Scholar and ScienceDirect. This study was conducted using controlled vocabulary (i.e. MeSH terms) and keywords, such as "*Neospora caninum*", "neosporosis", “seroprevalence", "prevalence", "epidemiology", "occurrence", "sheep", "*Ovis aries*", "ewe", "flock", alone or combined with Boolean operators (AND, OR), with no additional language restrictions. As an example, the Pubmed electronic search strategy is shown: ("Neospora caninum"[All Fields] OR "neosporosis"[All Fields]) AND ("seroprevalence"[All Fields] OR "prevalence"[All

Fields] OR "epidemiology"[All Fields] OR "occurrence"[All Fields]) AND ("sheep"[All Fields] OR "Ovis aries"[All Fields] OR "ewe"[All Fields] OR "flock"[All Fields]). Reference lists of all the relevant studies were examined by the corresponding author (VAS) using backwards and forwards citation strategies to prevent missing data. Unpublished studies and grey literature (e.g. conference abstracts, thesis/dissertations, poster abstracts, etc.) were excluded.

2.3. Eleqibility criteria

The inclusion criteria were: (1) peer-reviewed original articles without geographical limitation; (2) cross-sectional studies that estimated the seroprevalence of *N. caninum* infection in sheep; (3) studies with available full texts; (4) studies that provided the total number of sheep tested and the number of sheep that tested positive. Each article that did not fulfill the above-mentioned inclusion criteria was excluded from further consideration.

2.4. Study selection and data extraction

After the removal of duplicated articles, the reference lists were transferred into an Excel file to be assessed for eligibility by two independent researchers (VL and LFMM). Discrepancies between the authors at any stage of the selection process were resolved by consulting a third reviewer (VAS). Data extraction was done using a form based on the template provided by Cochrane Collaboration. The following information was recorded: first author, year of publication, country of setting, World Health Organization (WHO) region, continent, study design, diagnostic method, number of tested sheep (sample size), number of *N. caninum*-positive sheep (positive samples), the informed seroprevalence of *N. caninum* infection. In addition, detailed data about the

serological methods used to determine seroprevalence were extracted: type of methodology, antigen, conjugate, cut-off value and manufacturer (if any). Also, data on geographical latitude and longitude were extracted for each city/area included in individual studies. To obtain this information, the GPS Coordinates database was used (https://gps-coordinates.org/).To further analyze the associated risk factors of ovine neosporosis, data concerning sex and age of sheep, the type of production system, the presence of dogs on the farm and the history of abortion were also extracted from the eligible studies. Articles that had incomplete information concerning the number of *N. caninum*-positive sheep and the total number of the sheep in the exposed or unexposed groups, were excluded from the meta-analyses of risk factors and were only included in the meta-analysis of pooled seroprevalence and subgroup analyses.

Articles that evaluated: a) age as a risk factor but included categories that could not be standardized as young (≤1-year-old) and adult (>1-year-old); b) type of production system as a risk factor but included production systems that could not be categorized as "intensive", "semi-intensive" or "extensive"; or c) type of production system as a risk factor that did not include "extensive system" as a category, were also excluded from the meta-analyses of risk factors. Finally, the studies included in the meta-analyses of risk factors were standardized to allow a comparative assessment between the articles considering: sex (male vs. female); age (adult vs. young); type of production system (intensive or semi-intensive vs. extensive); the presence of dogs on the farm (yes vs. no); and the presence of abortion (yes vs. no).

2.5. Quality assessment

In the present study, the methodological and other qualities of the included articles were assessed by using a modified version of the Newcastle-Ottawa Scale (NOS)

adapted for cross-sectional studies by Modesti et al. (2016). The scores of 6–7, 3–5, and 1–2 indicated high, moderate, and low quality, respectively. In this regard, the higher scores were awarded to studies of higher quality.

2.6. Data synthesis and statistical analysis

The meta-analysis was performed with the help of MetaXL2.0 software (Epigear International Pty Ltd., Wilston, Queensland, Australia). Before performing the meta-analysis, the Freeman–Tukey double arcsine transformation was used to stabilize the variance (Rodrigues et al., 2020). The presence of heterogeneity was assessed by computing Cochran's Q and was quantified by using Higgin's statistic (I^2) (Anvari et al., 2020). Estimates of the degree of heterogeneity using the I^2 index were considered low (25%), moderate (50 %), and high (75 %) (Higgins et al., 2003). The random-effects model described by DerSimonian and Laird (2015) was selected based on the extent of heterogeneity in the retrieved studies. Forest plots were used to show the results of each study and the heterogeneity among studies. The pooled seroprevalence of *N. caninum* infection in sheep was estimated and informed as percentages and the 95% confidence interval (CI) was calculated for each study. Potential sources of heterogeneity were investigated by subgroup analyses. Factors investigated were: WHO region, continent, country of setting, diagnostic method, and NOS quality index. Fisher's exact test was also used to investigate the interaction (X^2) among the subgroups. The raw prevalence data were used in the meta-regression, which was performed to determine whether the latitude and longitude influenced the *N. caninum* seroprevalence in sheep, the metareg command of the STATA 12 package (STATA Corp., College Station, Texas) was used for the analysis. Potential risk factors associated with *N. caninum* seroprevalence in sheep were evaluated by odds ratio (OR) and the corresponding 95% CI. Potential

publication bias regarding pooled seroprevalence was visually assessed using funnel plot and doi plots, and Luis Furuya-Kanamori (LFK) index was determined. Luis Furuya-Kanamori index exceeding [2] indicates major asymmetry (publication bias) (Furuya-Kanamori et al., 2018). Regarding subgroup analyses, publication bias was evaluated when the corresponding study included at least ten datasets (Sterne et al., 2011).The equations used to calculate the statistical parameters above mentioned are detailed in Supp. Data 1. A p-value less than 0.05 was considered statistically significant. In addition, we performed a sensitivity analysis to assess whether the pooled seroprevalence estimate was influenced by individual studies. The choropleth map of global seroprevalence of *N. caninum* infection in sheep was created by using the online software Datawrapper (Lorenz et al., 2012).

3. Results

3.1. Search yield

The identified, included and excluded studies at each stage of the selection process were reported in the form of a PRISMA flow diagram (Fig. 1), along with the reasons for exclusion. A total of 1,298 studies were retrieved from 7 databases, and after the exclusion, 73 articles corresponding to 80 datasets were included in this systematic review and meta-analysis.

3.2. Description of included studies and qualitative assessment

The main characteristics of the included articles are summarized in Table 1. Data concerning 35,740 sheep (corresponding to 37,565 evaluated samples) were obtained from 73 selected articles (80 datasets) published from 2000 to February 2021 to determine the seroprevalence of *N. caninum* infection in sheep. Data were extracted

from 29 locations, corresponding to 30 countries (the countries of Wales and England were grouped as the United Kingdom in the research conducted by Helmick et al. (2002) (Table 1). Countries were categorized into continents, namely, Africa (countries n=4; datasets n=4), North America (countries n=1; datasets n=2), Central America (countries n=2; datasets n=2), South America (countries n=4; datasets n=39), Asia (countries n=7; datasets n=14), Europe (countries n=9/10; datasets n=16) and Oceania (countries n=2; datasets n=3). In addition, the member states of the World Health Organization (WHO) were also analyzed according to WHO regions: African Region (AFR) (countries n=2; datasets n=2), Region of the Americas (AMR) (countries n=7; datasets n=45), European Region (EUR) (countries n=10/11; datasets n=20), Eastern Mediterranean Region (EMR) (countries n=7; datasets n=8), and Western Pacific Region (WPR) (countries n=3; datasets n=7). The Southeast Asia Region had no available datasets regarding seroprevalence of ovine neosporosis.

Most of the studies investigated the seroprevalence by the Enzyme-Linked Immunosorbent Assay (ELISA) (n = 41) or by Indirect Immunofluorescent Assay (IFAT) (n = 29). In addition, 4 articles analyzed seroprevalence by both, ELISA and IFAT methods, and a single article analyzed data by two different ELISA methods (Table 1). The most salient characteristics of the serological methods employed in the included datasets are summarized in Supp. Table 2. It is important to note that among the datasets that analyzed seroprevalence by ELISA, 38/47 employed commercial ELISA kits, whereas only 5/33 datasets that analyzed seroprevalence by IFAT used commercial kits. The cut-off values employed in ELISA determinations varied not only depending on the type of ELISA strategy (competitive/indirect/direct) and manufacturer (if any), but also between the same ELISA commercial kit depending on the author's criterion. In IFAT determinations, the cut-off titers varied from 1:25 to 1:64; however,

the cut-off value of 1:50 was the most generally employed by researchers (25/33).Regarding the quality assessment of studies, most of the articles included in this meta-analysis were qualified as moderate/high-quality studies (n=68, n=74 datasets) whereas 5 (n= 6 datasets) of them were qualified as low-quality studies (Supp. Table 3).

Regarding the sex of animals, 28 datasets informed positive and negative female and male samples, which were included in the corresponding OR analysis. In addition, only 19 articles included data that let us group the animals in the categories of adult (>1-year-old) and young (≤1-year-old). Regarding the production system, one study included transhumance as a breeding practice (Gazzonis et al., 2016), whereas in the rest of the articles that evaluated production systems, extensive, intensive and/or semi-intensive categories were informed, resulting in a final set of 5 articles for each OR analysis. Almost half of the included research articles informed the presence of dogs on the farms as a potential risk factor associated with *N. caninum* seroprevalence in sheep, but only 14 of them reported the number of cases in each category and were included in the OR analysis.

Concerning abortion, 3 studies were specifically conducted in samples from aborted sheep (Helmick et al., 2002; Gharekhani et al., 2013; Špilovská and Reiterová 2008), 1 article in samples from aborted sheep or in samples from sheep with associated risk factors (Rizzo et al., 2018) and in another article, samples were obtained from farms which reported high abortion rates or a high number of ovine deaths (Patarroyo et al., 2013). Finally, 8 studies specified the number of positive and negative sheep based on abortion status, which were included in the OR analysis performed here.

3.2. Quantitative synthesis: Results of the meta-analyses

3.2.1. Seroprevalence

The global pooled seroprevalence of *N. caninum* infection in sheep was 13 % (95% CI, 10-15) based on the random-effects model of meta-analysis (Fig. 2). The included studies demonstrated a strong heterogeneity (Q = 5147.15, I^2 = 98%, p < 0.001). Publication bias was assessed by funnel plot and doi plot, and it was also checked by LFK index, which showed no substantial asymmetry in the analyzed data (LFK index = 1.26) indicating that it may not have a substantial impact on this estimate (Supp. Fig. 1A and 1B, respectively). Sensitivity analysis showed that exclusion of each data at a time neither modified the seroprevalence of *N. caninum* infection in sheep nor the heterogeneity of the results. Moreover, neither the exclusion of the studies that included only samples from abortive sheep (n=5) nor the exclusion of all datasets from Brazil (n=34) significantly modified these parameters (13%, CI 10-16, Q=4771, I^2 = 98%, p < 0.001, Supp. Fig. 2; 9%, CI 7-11, Q=1492, I^2 = 97%, p < 0.001, Supp. Fig. 3, respectively).

Results from the analyses of subgroups are shown in Table 2, and assessments of publication bias are available in Supp. Fig. 4A-R. Data showed that pooled seroprevalence of *N. caninum* infection was significantly different among continents (X^2=898, 6; p<0.0001). The highest seroprevalence was found in Africa (20%; 95% CI, 4-44), followed by South America (18%; 95% CI, 13-23), Central America (12%; 95% CI, 9-14) and North America (9%; 95% CI, 2-18). The pooled seroprevalence of *N. caninum* was 8% (95% CI, 4-14) in Europe, 8% (95% CI, 5-11) in Asia and 1% (95% CI, 0-2) in Oceania. Subgroup analysis based on continents also showed high/moderate heterogeneity for each continent (Table 2). Regarding the subgroup analysis based on WHO regions, a similar profile to that of continents was obtained, and the AFR also showed the highest pooled seroprevalence (42%; 95% CI, 36-48), followed by the AMR (17%; 95% CI, 13-22). The seroprevalences in the remaining WHO regions were: 10%

(95% CI, 4-16) in EMR, 8% (95% CI, 4-12) in EUR and 4% (95% CI, 1-7) in WPR. The pooled seroprevalence of *N. caninum* infection significantly varied among WHO regions (X^2=975.6, 4; p<0.0001), which showed high heterogeneity (I^2>95%), except for the AFR, which demonstrated homogeneity (I^2 = 0%). In addition, the seroprevalence of *N. caninum* infection in sheep was also significantly different among countries (X^2 =1893, 28; p<0.0001), with the highest seroprevalence found in Colombia (79%; 95% CI, 61-92%) followed by Gabon (42%; 95% CI, 32-52) and Senegal (42%; 95% CI, 35-49) (Table 2, Fig.3). In those countries in which pooled seroprevalence could be determined, results showed high heterogeneity (I^2 >82%), except for China, which showed moderate heterogeneity (I^2 >57%) (Table 2).

Regarding the subgroup analysis of diagnostic methods, the pooled seroprevalence of *N. caninum* infection in sheep evaluated by ELISA was 10% (95% CI, 7-12), whereas it was 17% (95% CI, 12-23) when evaluated by IFAT, being both results significantly different (X^2=686.2, 1; p<0.0001). Finally, subgroup meta-analysis was based on NOS quality index of included articles and showed no significant differences (X^2=1.930, 1; p=0.1648) between low-quality NOS-indexed articles (12%; 95% CI, 7-17; I^2>90%) and moderate/high-quality NOS-indexed articles (13%; 95% CI, 10-16; I^2>99%).

No publication bias was found in any of the datasets analyzed regarding meta-analyses of subgroups (Supp. Fig. 4A-R) indicating that it may not have a substantial impact on the corresponding estimates.

3.2.2. Meta-regression and assessment of risk factors

According to the meta-regression, there was a significant negative association between latitude and seroprevalence (coefficient = -0.013; p<0.001, Fig. 4A), whereas

no effect of longitude on seroprevalence of *N. caninum* infection in sheep was found (coefficient = -0.001; p=0.365, Fig. 4B).

The results of this meta-analysis did not show a significant association between the sex of evaluated animals and seropositivity (OR 1.06; 95% CI 0.9–1.24, Fig.5). On the contrary, the meta-analysis demonstrated that older sheep show increased risk of *N. caninum* seropositivity than younger sheep (OR 1.42; 95% CI 1.08–1.87, Fig.6).

According to this meta-analysis, sheep bred under intensive or semi-intensive production systems were less likely to be susceptible to *N. caninum* infection than sheep bred under extensive production system (OR 0.65; 95% CI 0.42-0.99 and OR 0.74; 95% CI 0.62–0.89, Fig.7A and Fig.7B, respectively). No particular association was found between *N. caninum* seropositivity and the presence of dogs on the farms (OR 1.15; 95% CI 0.63–2.12, Fig.8)

Finally, no significant association was found between *N. caninum* seropositive sheep and the presence of abortion (OR 1.80; 95% CI 0.87–3.74, Fig.9).

4. Discussion

Currently, neosporosis is recognized as the main cause of abortions in cattle and there is also an increasing concern about its role in ovine reproductive losses. Contrary to bovine neosporosis, several aspects of neosporosis in sheep remain partially unknown. The results of this meta-analysis revealed that the global estimated pooled seroprevalence of *N. caninum* infection in sheep is 13% (95% CI, 10-15) based on the random-effects model, and that the included studies demonstrated a strong heterogeneity. These data are in accordance with a previous study on ovine neosporosis by Romanelli et al. (2021), but differs from the lower global pooled seroprevalence of

N. caninum infection reported in goats (5.99%) by Rodrigues et al. (2020). Despite both species are closely related, the feeding behavior of sheep could make them more susceptible to became horizontally infected than goats, since sheep usually graze on grass which may be linked to the ingestion of pathogens found close to the ground, such as *N. caninum*, whereas goats browse on weeds and herbs (Ekesbo 2018; Mazinani and Ruth 2020; Moskwa et al., 2018), which may explain the differences in data obtained for both ruminants.

To further analyze the potential causes of the high heterogeneity found in the estimate of global pooled seroprevalence of ovine neosporosis, meta-analyses of subgroups were also performed. To our knowledge, this is the first meta-analysis that estimated the pooled seroprevalence of *N. caninum* infection in sheep by continents, WHO regions and countries. According to the analyses, the seroprevalence varied widely among these geo-political regions. These novel data of neosporosis in sheep are in agreement with the results from meta-analyses conducted on the seroprevalence of *N. caninum* infection in other animal species, such as bovines (Ribeiro et al., 2019), dogs (Anvari et al., 2020) and goats (Rodrigues et al., 2020). The highest seroprevalence of ovine neosporosis was found in Africa, regardless if data were analyzed based on WHO region or on continent, followed by the American Region. Consistently, results indicated that Colombia (South America) was the country with the highest seroprevalence, followed by Gabón and Senegal (Africa). Recently, Anvari et al. (2020) reported similar results in a meta-analysis of global seroprevalence of *N. caninum* infection in dogs**.** On the other hand, America was the continent with the highest seroprevalence of neosporosis in cattle and goats (Ribeiro et. al., 2019; Rodrigues et al., 2020). Despite the potential differences in the evaluated host species and in the assessment of seroprevalence of individual studies included on the previously mentioned meta-

analyses, Africa and America (particularly South America) have been found as the leading regions concerning seroprevalence of neosporosis. Although it is very difficult to define the reasons associated with the differences found in seroprevalence of *N. caninum* infection between geographical regions, adequate environmental and/or meteorological conditions for the survival of oocysts shed in faeces by canids (Dubey & Schares 2011) should definitely be included among them. In the current report, the relationship between geographical latitude and seroprevalence of ovine neosporosis was evaluated for the first time, and a negative association was found. This result is in agreement with data published by Rodrigues et al. (2020) for goats. In this regard, it has been demonstrated that high temperatures and humidity (such as those from the equator) may favor the survival and sporulation of oocysts in the environment, increasing the risk of eventual infection (Dubey et al., 2007). In the present meta-analysis most of the data from regions near the equator corresponded to countries from South America and Africa, which may explain the increased seroprevalence found in those continents. On the contrary, no association was found between geographical longitude and ovine neosporosis, in accordance with data by Rodrigues et al. (2020) for goats. Another fact that must not be ruled out is that more than 70% of livestock keepers from Africa and Latin America are considered small-scale breeders (Salcedo and Guzmán 2014), who breed sheep under extensive pastoral systems (Baltenweck et al., 2020), which may be related to an increased exposure of the sheep to the pathogen (Dubey & Schares 2011), as found in the present report. This issue will be discussed in detail some paragraphs ahead.

Serological data regarding *N. caninum* infection is the first step to evaluate potential control options; however, there is currently no appropriate reference test to define a true-positive or true-negative animal (Ortega-Mora et al., 2007). Among the

different available serological tests, IFAT and ELISA were the most commonly used techniques for the detection of anti-*N. caninum* antibodies not only in sheep but also in cattle (Ribeiro et al., 2019), dogs (Anvari et al., 2020) and goats (Rodrigues et al., 2020). In the current analyses of subgroups, the global seroprevalence of *N. caninum* infection in sheep evaluated by IFAT was significantly higher than that obtained by ELISA, which suggests that the type of diagnostic method used to determine seropositivity may be a source of heterogeneity in the present meta-analysis. This result contradicts data previously published by Romanelli et al. (2021), who reported a slight, but not statistically significant difference between both methodologies. This discrepancy may be related to the fact that a higher number of datasets were included here (80 datasets vs. 24 datasets), which may allow a more robust statistical subgroup analysis. It is important to note that despite the establishment of a standardized methodology to define a true *N. caninum* infected sheep is highly desirable, the selection of the most adequate serological test to determine the prevalence of ovine neosporosis, as well as its methodological conditions, are beyond the scope of this paper and have been previously discussed for other animal species by Campero et al. (2018), Gondim et al. (2017), Guido et al. (2016), Ortega-Mora et al. (2007) and Silva et al. (2007), representing an important challenge ahead. However, some suggestions could be made based on the results of this systematic review and meta-analysis. In the current study, most of the research groups that evaluated seroprevalence by IFATs used tachyzoites from the NC-1 strain as coated antigen and selected 1:50 as the established cut-off value to define a positive sample. It has been previously demonstrated that the same or higher dilution cut-offs by IFAT are appropriate to avoid cross-reactivity between *N. caninum* and *T. gondii* in serum samples from different hosts (Benetti et al., 2009; Hebbar et al., 2022; Lobato et al., 2006; Silva et al., 2007); whereas a cut-off value of 1:100 (or higher) is

recommended for the same purpose when evaluating seropositivity by ELISA (Hebbar et al., 2022). Also, since reactions limited to the apex of the parasite were regarded as non-specific in *N. caninum* IFAT, it has been suggested that for a sample to be considered positive, a complete peripheral fluorescence of the parasite have to be observed (Gondim et al., 2017; Pare et al., 1995). Taken together, these data suggest that the mentioned conditions should be evaluated as *N. caninum* IFAT potential standard conditions. Regarding ELISAs, an interesting approach is represented by tests based on tachyzoite plus bradyzoite specific recombinant antigens (multi-antigenic or chimeric proteins), since this alternative design could avoid cross-reactivity generally seen in the conventional ELISAs based on crude tachyzoite antigens (Gondim et al., 2017) and also could help to detect seropositive animals chronically infected (Björkman et al., 1994; Gondim et al., 2017; Guido et al., 2016; Nishikawa et al., 2002; Silva et al., 2007). It is worth noting that IFAT tests and all currently commercially available ELISAs are based on tachyzoite antigens. These tests are not able to discriminate among animals in the acute and chronic phases of infection, and even more, it is not clear if these tools are accurate enough to completely identify persistently infected animals (reviewed in Alves-Sinnott et al., 2017 and Guido et al., 2016). In this regard, despite specific IgM antibodies are generally associated with recent infections, IgM is not an accurate marker of acute neosporosis, since its levels persist for several months or even years after prime-infection. In this scenario, antibody avidity assessment arises as an interesting serological marker in immunodiagnosis of neosporosis, as low-and high-avidity antibodies mainly increase in recent and chronic conditions, respectively (Hebbar et al., 2022). Finally, an important issue detected in this systematic review and meta-analysis is that most of the commercial ELISAs employed in the datasets included here established a rank of “negative, suspicious and positive” cut-off values, and it

depended on each researcher's criterion to decide if a suspicious sample was considered positive or negative, leading to differences in seroprevalence data. Thus, it is strongly recommended to clearly define and adopt a unified criterion for this topic by the consortium of researchers working on *N. caninum* epidemiology.

The identification of risk factors associated with seroprevalence of *N. caninum* infection in sheep represents an important tool to understand the epidemiology of the disease. However, the only meta-analysis that has previously evaluated this topic did not find any risk factor statistically associated with the prevalence of ovine neosporosis, probably due to the small number of studies included in the analysis and the high heterogeneity among data (Romanelli et al., 2021). In the current report, age and type of production system were identified as risk factors associated with ovine neosporosis for the first time by meta-analytical methods. However, no association was found between the sex of sheep and *N. caninum* seroprevalence. This result was consistent with those reported in most of the studies that have evaluated this potential risk factor in sheep; in fact, only a few studies have found positive (Ibrahim et al., 2016; Paiz et al., 2015) or negative (Faria et al., 2010; Nie et al., 2018) statistical associations between female sheep and seropositivity.

The contribution of the different transmission routes to the perpetuation of ovine neosporosis is still unclear and must be better understood to design strategies to control this disease (González-Warleta et al., 2018). As previously mentioned, in the present meta-analysis a positive association between age and *N. caninum* seroprevalence was found, which suggests that the horizontal transmission route may be of relevance for this host species, as suggested by previous findings by Al Nasr (2013), Figliuolo et al. (2004), Rossi et al. (2011) and Wang et al. (2018). In addition, a negative association was found between intensive and semi-intensive production systems and the

seroprevalence of *N. caninum* infection in sheep. It is possible that sheep bred in non-traditional systems (i.e. intensive and semi-intensive systems) may have a decreased exposure to potential sources of infection and risk factors (Al Nasr 2013; Dubey et al., 2007; Ibrahim 2016) since they usually undergo higher hygienic standards and more adequate managements than in extensive ones (Barling et al., 2001; De Moura et al., 2014; Wang et al., 2018). Additionally, in intensive or semi-intensive production systems dogs or other canids are less likely to access the ovine placenta or carcass, which would facilitate the spread of *N. caninum* infection (Gazzonis et al., 2016). In this regard, it is widely accepted that postnatal *N. caninum* infection in ruminants is caused by the ingestion of oocysts spread to the environment by infected canids (Dubey et al., 2007), which suggests that the presence of dogs on the farms would be associated with increased seroprevalence (Dubey et al., 2007; Dubey & Schares 2011) as it has been reported for bovines by Ribeiro et al. (2019) and for goats by Rodrigues et al. (2020). However, in the present meta-analysis, no association was found between this factor and seropositive sheep. This data might be considered controversial, but it is important to highlight some facts for better interpretation of the result: i) the absence of dogs on the farms refers only to family/herding dogs and it is not related to the absence of feral/ stray dogs which generally have free access to the farm rangelands (Anvari et al., 2020); ii) feral/stray dogs mostly fed raw meat and carcasses; meanwhile family/herding dogs are generally fed cooked and canned food (Anvari et al., 2020); iii) the presence of family/herding dogs may inhibit the visit from other canids on the farm, reducing the risk of *N. caninum* infection in livestock (Barling et al., 2001). Thus, the missing data about the presence of feral/stray dogs living or wandering in the analyzed farm rangelands may lead to misinterpretation of the results when evaluating "the presence of dogs on the farm" as a potential risk factor to *N. caninum* infection, both in individual

studies and meta-analyses. In addition, canids other than dogs might also be involved in the horizontal transmission of *N. caninum*, such as coyotes (*Canis latrans*), dingoes (*Canis lupus dingo*), wolves (*Canis lupus lupus*), maned wolves (*Chrysocyon brachyurus)*, foxes (*Vulpes vulpes*), golden jackals (*Canis aureus*) and wild African dogs (*Lycaon pictus*) (reviewed in Almeria 2013). An interesting tool to determine the environmental contamination by *N. caninum* oocysts in farms (and thus, the potential horizontal transmission of the parasite) is to evaluate the prevalence of neosporosis in indicator species, such as small mammals, insectivores and birds, which once dead, could also lead to the transmission of the parasite if ingested by farm animals, either by accident (ruminants) or on purpose (pigs) (Almeria 2013; De Barros et al., 2017). Noteworthy, "age" and "extensive production system" have been identified as risk factors to *N. caninum* infection in sheep in this meta-analysis, which suggest that horizontal transmission represents an important route of infection in sheep that must be manage through measures to keep not only dogs out of the rangelands but also other wildlife definitive host species and reservoirs.

Endogenous transplacental transmission is the main mechanism responsible for the perpetuation of neosporosis in cattle herds, probably due to two facts: (i) *N. caninum* is the most efficiently transmitted parasite (most of the infected dams deliver clinically healthy but chronically infected calves) and (ii) neosporosis rarely causes abortion in bovines. In sheep, the endogenous transplacental transmission route has been generally considered irrelevant in contrast to the horizontal transmission route, but recent studies on natural infections have reported that rates of vertical transmission are within the range of those reported in cattle with naturally occurring neosporosis, demonstrating that it may be also highly efficient in ovines (Feitosa et al., 2021; González-Warleta et al., 2018). In fact, despite it has been reported that *N. caninum* infection causes

abortions and reproductive failures in naturally infected sheep (Della Rosa et al., 2021; González-Warleta et al., 2014; Hecker et al., 2019; Moreno et al., 2012; Sánchez-Sánchez et al., 2021), the frequency of abortion related to ovine neosporosis remains uncertain. In the current report, the currently unknown relationship between *N. caninum* seroprevalence and the presence of abortion in sheep was evaluated by meta-analytical methods; however, no statistical association was found between these parameters. Accordingly, Howe et al. (2002) have suggested that despite neosporosis should not be excluded as a potential cause of ovine abortion; it appears to be an infrequent one, after examining the seroprevalence of *N. caninum* in aborted sheep from the United Kingdom. Similar results have been reported by Gharekhani et al. (2013), who found that only 2.2 % of aborted ewes were *N. caninum* seropositive in Iran. Moreover, Rizzo et al. (2018) indicated that *N. caninum* infected sheep showed a reduction in the risk of abortion and reproductive problems. On the contrary, Patarroyo et al.(2013) reported a seroprevalence of *N. caninum* infection of 78,6% in aborted ewes from Colombia, representing the highest informed seroprevalence from the datasets included in this meta-analysis; however, the lack of other epidemiological data from that country prevent us to interpret this result. Similar results have recently been published by Sánchez-Sánchez et al. (2021) who found that seropositive sheep were more likely to abort than seronegative ones. Moreover, Della Rosa et al. (2021) confirmed that *N. caninum* infection is an important cause of abortion in naturally infected sheep in flocks from Argentina. Noteworthy, most of the reports about seroprevalence of ovine neosporosis included in the present meta-analysis did not evaluate its potential association with the presence of abortion, the main clinical sign of neosporosis in cattle (Reichel et al., 2013). Since low reproductive performance in ovine population is normally recognized as the single most significant problem in the sheep industry (Ali et

al., 2019), the role of neosporosis in ovine abortion should clearly be established, not only by assessing this factor in every cross-sectional study of seroprevalence of *N. caninum* infection but also by evaluating it by other types of study designs (e.i., longitudinal studies, case-control studies, prospective cohort studies, etc), which should help to find definitive evidence in this regard.

The limitations of the present study included: 1) lack of indexed studies on the seroprevalence of *N. caninum* infection from many countries with a large population of sheep (such as India, Chad, Mongolia, etc.); 2) inadequate research on the association of potential risk factors and seroprevalence of *N. caninum* infection in sheep; 3) insufficient information regarding the presence of clinical signs potentially associated with *N. caninum* infection and the serostatus of sheep in most of the studies. Data presented here should encourage the researchers not only to evaluate the seroprevalence and risk factors of ovine neosporosis but also to publish their results in indexed journals included in worldwide databases.

4. Conclusion

The results of this meta-analysis showed that ovine neosporosis is widely and heterogeneously distributed worldwide. Also, it was demonstrated for the first time that the seroprevalence of this infection is negatively associated with increased geographical latitude. In addition, increased age and extensive production system represent risk factors, which suggest that the horizontal transmission route is relevant for this host species. Taking into account that the presence of domestic dogs on the farms is not statistically associated with seroprevalence of ovine neosporosis, management to reduce horizontal transmission may include control measures to avoid/

reduce the access of feral dogs, wildlife canids and reservoir species to the farm rangelands. In addition, it is strongly recommended to clearly discuss and adopt a unified criterion to define a true positive or true negative infected animal (gold standard methodology and its experimental conditions) by the consortium of researchers working on *N. caninum* epidemiology. To conclude, it is important to note that there is a global need for more indexed studies concerning *N. caninum* seroprevalence and risk factors in sheep, which will be essential in establishing the epidemiological and clinical relevance of this disease.

Conflict of interest

There is no conflict of interest.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure legends

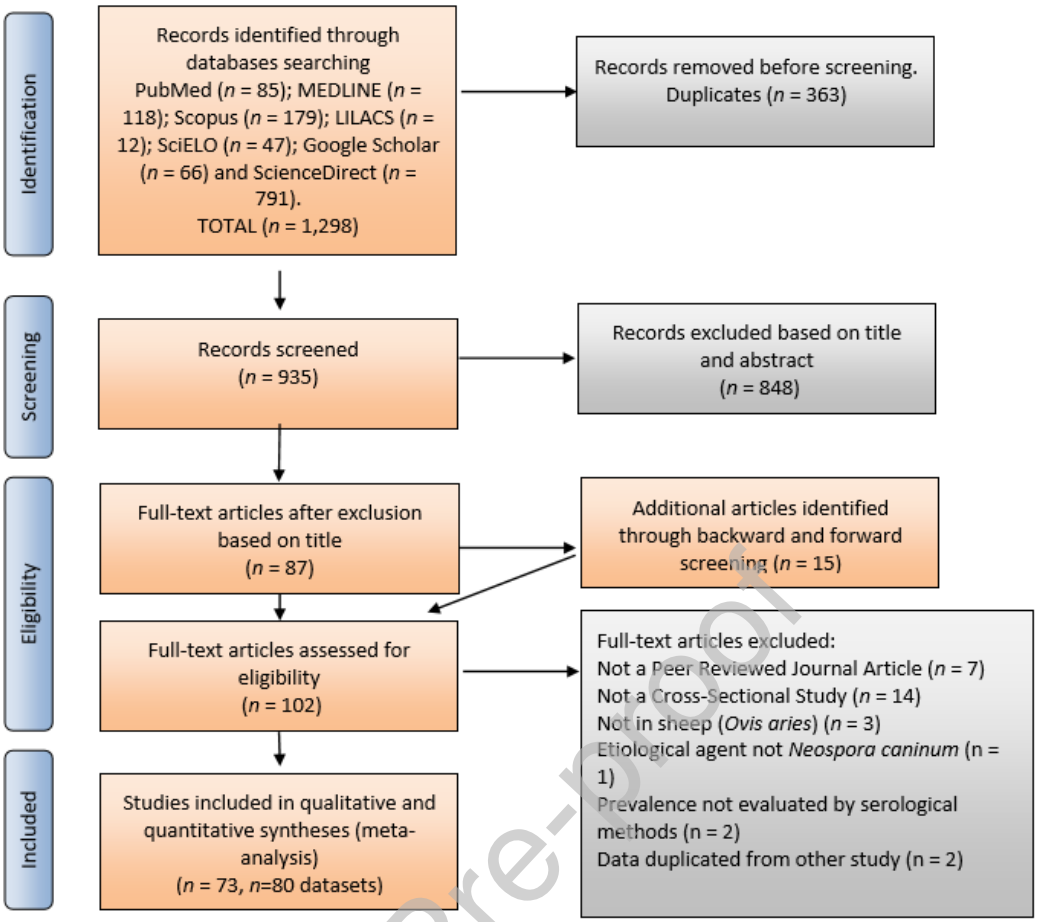


Figure 1. PRISMA flow chart describing search strategy to select studies on the global seroprevalence of ovine neosporosis

Table 1. Summary of abstracted datasets from included studies+

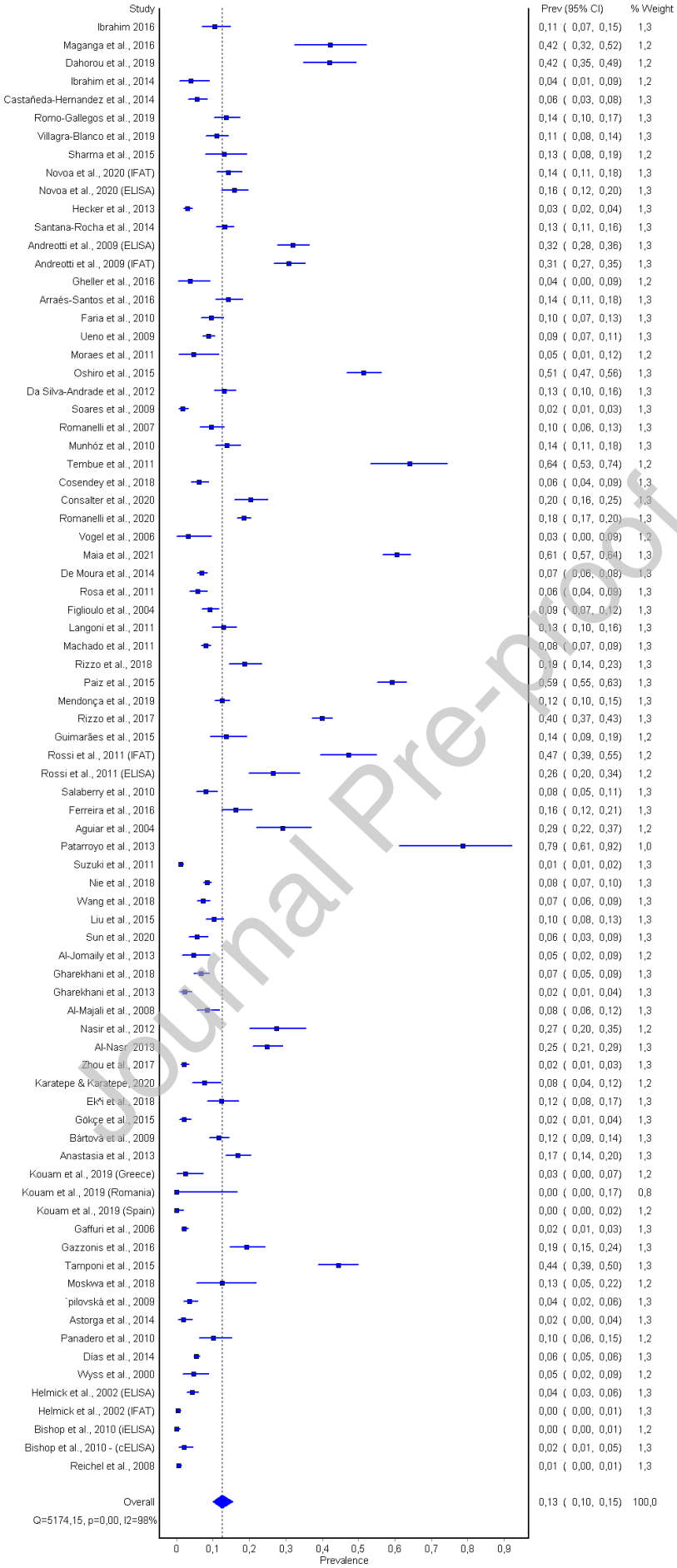


Figure 2. Forest plot of the worldwide seroprevalence of ovine neosporosis. The black dot is the point estimate and the horizontal line is the 95% confidence interval (CI) for seroprevalence plotted for each dataset. The left column shows the bibliographic reference for each dataset, the right columns represent the seroprevalence and 95% CI from each dataset and the weight of the study related to the global estimate. The blue diamond at the bottom of the forest plot is a worldwide pooled seroprevalence of *N. caninum* infection in sheep.

Table 2. Subgroup analyses of pooled seroprevalence of ovine neosporosis based on continent, WHO region, country, diagnostic method and NOS quality index

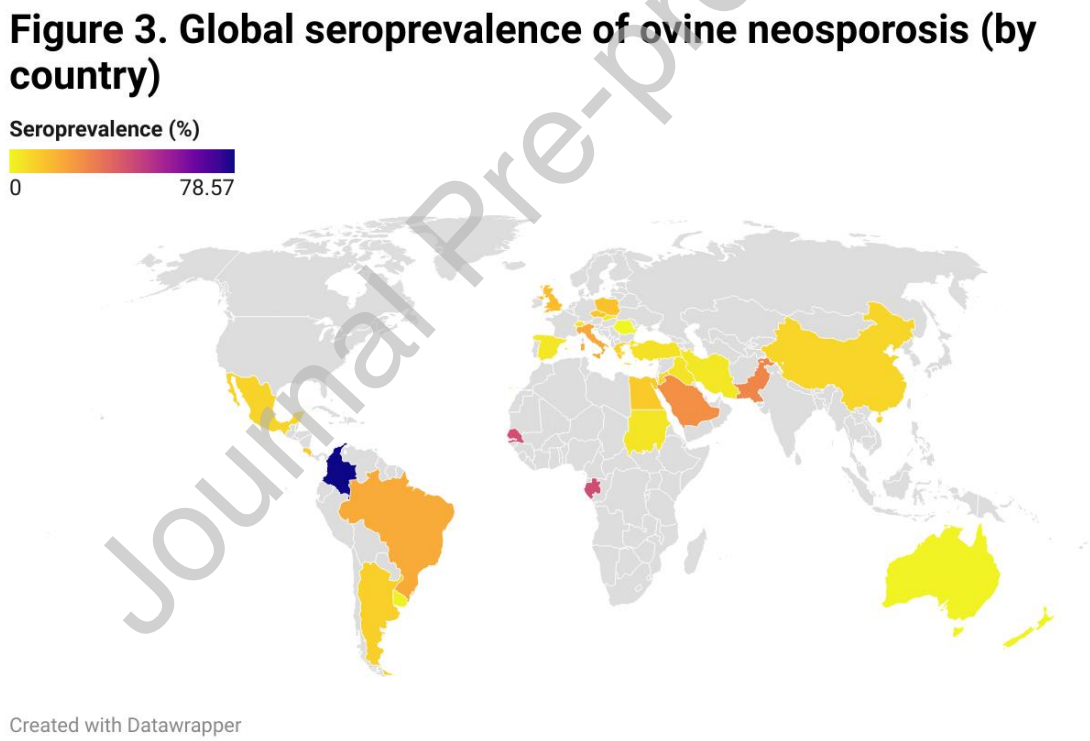
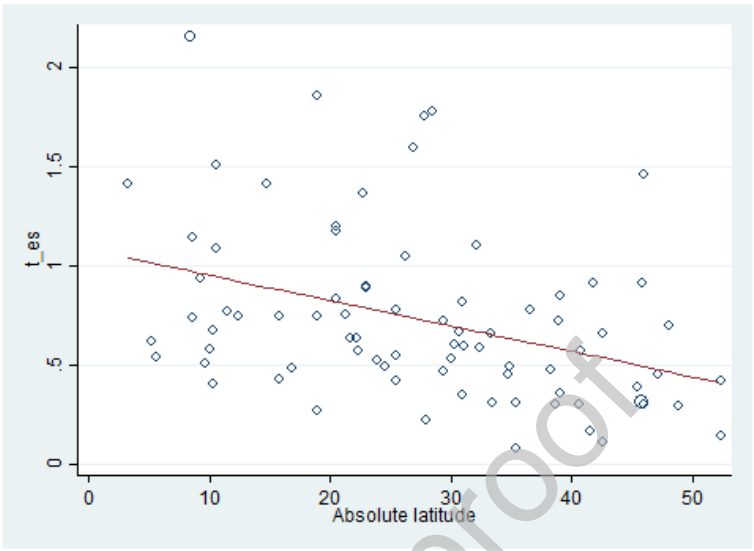


Figure 3. Global seroprevalence of ovine neosporosis (by countries). An interactive choropleth map is also available at https://datawrapper.dwcdn.net/z6EH0/4/

A)



B)

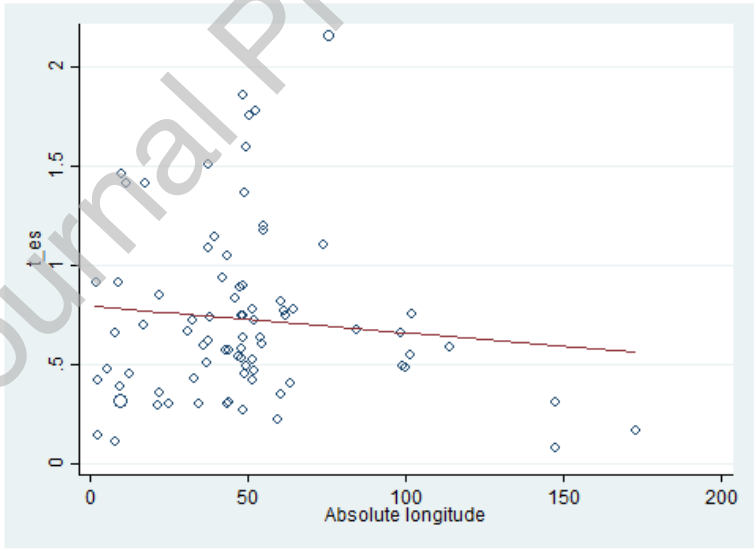


Figure 4. Meta-regression of geographical parameters of the studied region against the *N. caninum* seroprevalence in sheep. A) Latitude; B) Longitude. The circles

represent the individual studies. The continuous lines represent the regression lines. The geographical parameters are plotted on the horizontal axis. The prevalence of *N. caninum* in plotted numbers is plotted on the vertical axis.

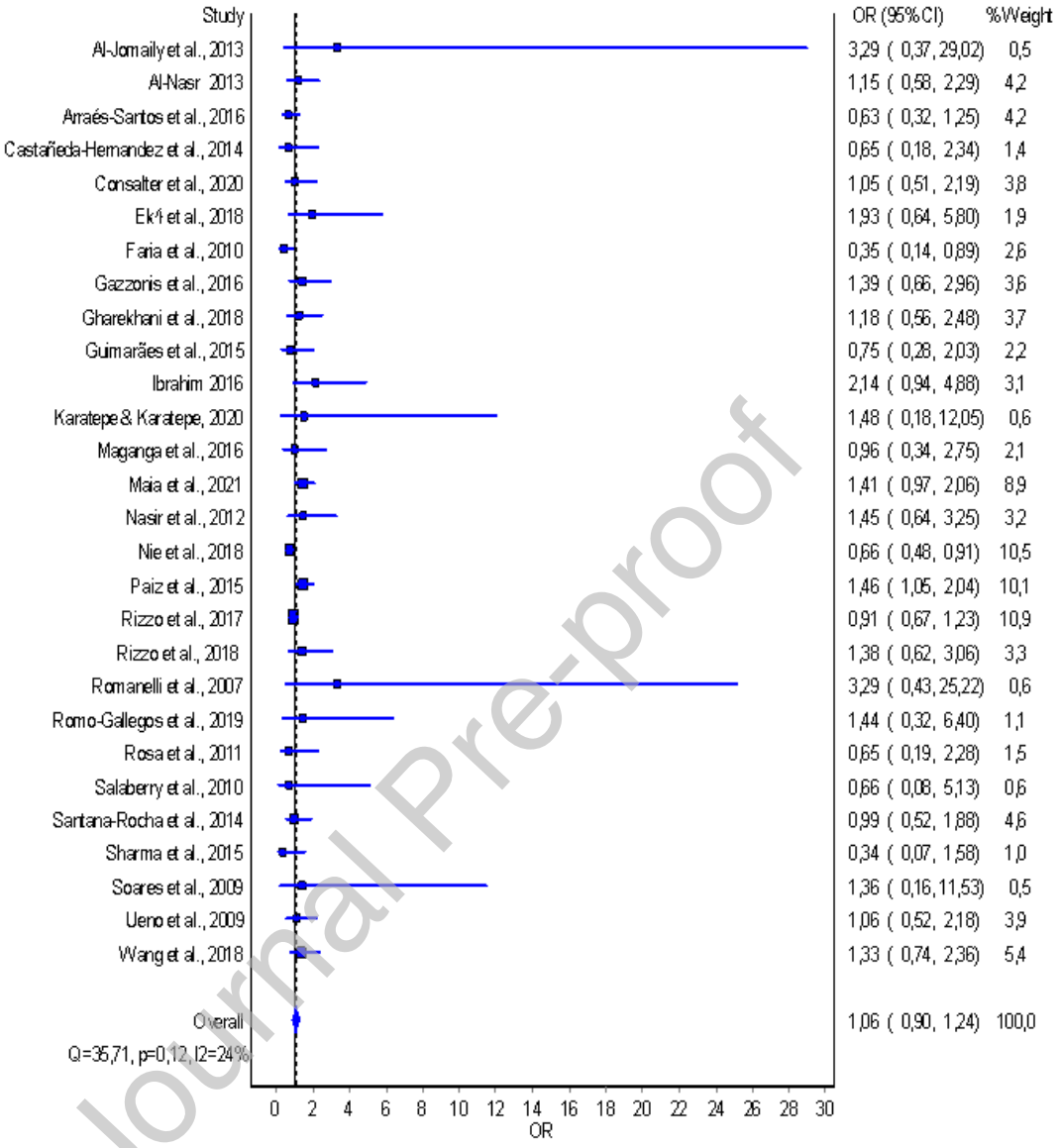


Figure 5. Forest plot of the pooled seroprevalence of ovine neosporosis and its potential association with the sex of sheep [female vs male (reference)] estimated as Odds Ratio (OR).

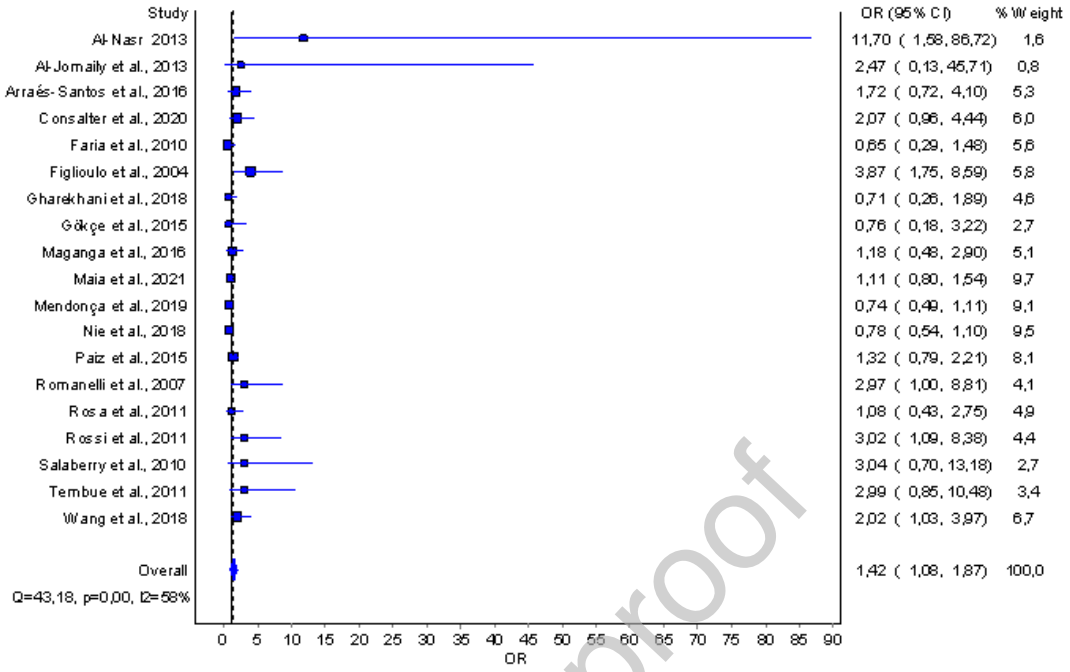


Fig. 6. Forest plot of the pooled seroprevalence of ovine neosporosis and its potential association with the age of sheep [adult vs young (reference)] estimated as Odds Ratio (OR).

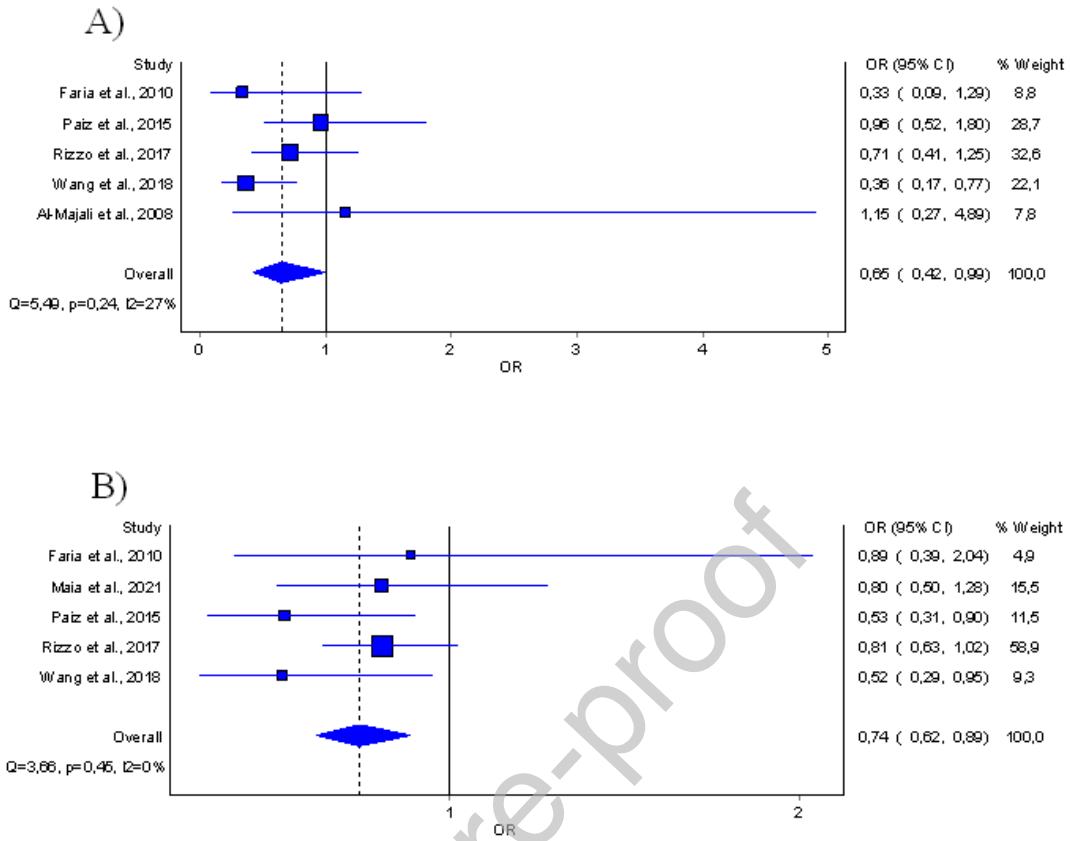


Fig. 7. Forest plot of the pooled seroprevalence of ovine neosporosis and its potential association with the type of production system estimated as Odds Ratio

(OR). A) Intensive system [intensive system vs extensive system (reference)], B) Semi-intensive system [semi-intensive system vs extensive system (reference)].

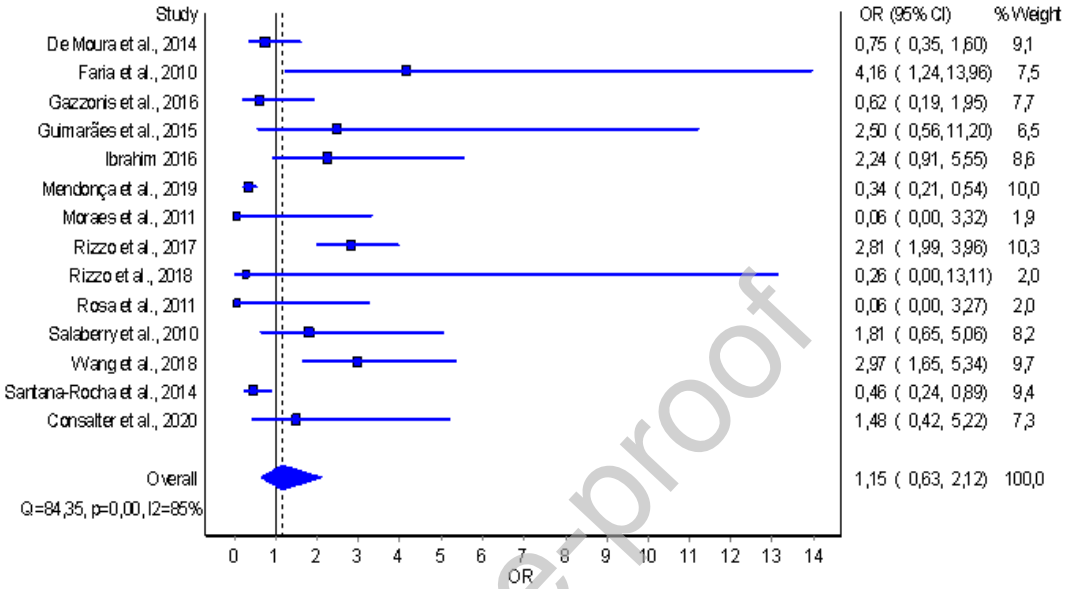


Fig. 8. Forest plot of the pooled seroprevalence of ovine neosporosis and its potential association with the presence of dogs on the farm [yes vs no (reference)] estimated as Odds Ratio (OR).

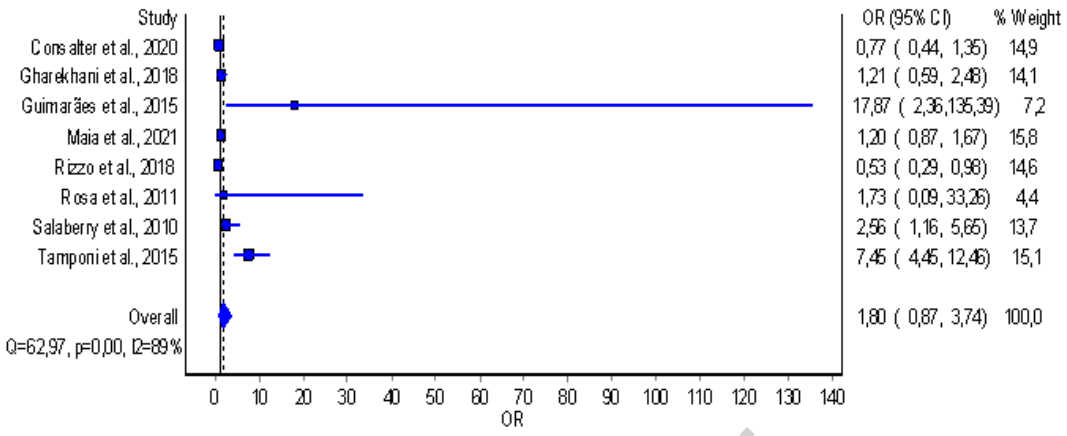


Fig. 9. Forest plot of the pooled seroprevalence of ovine neosporosis and its potential association with the presence of abortion [yes vs no (reference)]

Table 1. Summary of abstracted datasets from included studies

Continent	WHO REGION	Country	Positive Samples	Total samples	Seroprev(%)	Confidence Interval (95%)	Serological Method	NOS Index	Reference
Africa	EMR	Egypt	26	247	10.53	6.98-14.69	ELISA	M/H	Ibrahim 2016
Africa	AFR	Gabon	40	95	42.10	32.32-52.21	ELISA	M/H	Maganga et al., 2016
Africa	AFR	Senegal	73	174	41.95	34.70-49.38	ELISA	M/H	Dahourou et al., 2019
Africa	EMR	Sudan	4	100	4	0.87-8.91	ELISA	M/H	Ibrahim et al., 2014
North Am	AMR	Mexico	18	324	5.56	3.3-8.34	ELISA	M/H	Castañeda Hernández et al., 2014
North Am	AMR	Mexico	50	368	13.59	10.26-17.29	ELISA	M/H	Romo-Gallegos et al., 2019
Central Am	AMR	Costa Rica	43	392	10.97	8.05-14.27	ELISA	M/H	Villagra-Blanco et al., 2019
Central Am	AMR	Grenada	18	138	13.04	7.89-19.23	ELISA	M/H	Sharma et al., 2015
South Am	AMR	Argentina	21	704	2.98	1.84-4.38	IFAT	M/H	Hecker et al., 2013
South Am	AMR	Argentina	61	385	15.84	12.36-19.67	ELISA	L	Novoa et al., 2020
South Am	AMR	Argentina	55	385	14.29	10.96-17.97	IFAT	L	Novoa et al., 2020
South Am	AMR	Brazil	41	141	29.07	21.85-36.87	IFAT	M/H	Aguiar et al., 2004
South Am	AMR	Brazil	136	441	30.84	26.61-35.24	IFAT	M/H	Andreotti et al., 2009
South Am	AMR	Brazil	141	441	31.97	27.69-36.4	ELISA	M/H	Andreotti et al., 2009
South Am	AMR	Brazil	47	332	14.16	10.60-18.13	IFAT	M/H	Arraes-Santos et al., 2016
South Am	AMR	Brazil	61	300	20.33	15.96-25.09	IFAT	M/H	Consalter et al., 2020
South Am	AMR	Brazil	24	388	6.19	3.98-8.82	ELISA	M/H	Cosendey et al., 2018
South Am	AMR	Brazil	64	488	13.10	10-16	IFAT	M/H	da Silva et al., 2012
South Am	AMR	Brazil	92	1308	7.03	5.71-8.49	IFAT	M/H	de Moura et al., 2014
South Am	AMR	Brazil	33	343	9.62	6.71-12.98	IFAT	M/H	Faria et al., 2010
South Am	AMR	Brazil	49	300	16.33	12.35-20.74	IFAT	L	Ferreira et al., 2016
South Am	AMR	Brazil	55	597	9.21	7.02-11.67	IFAT	M/H	Figliuolo et al., 2004
South Am	AMR	Brazil	3	81	3.70	0.05-9.18	IFAT	M/H	Gheller et al., 2016
South Am	AMR	Brazil	8	376	13.74	9.08-19.15	IFAT	M/H	Guimaraes et al., 2015
South Am	AMR	Brazil	49	382	12.83	9.65-16..38	IFAT	M/H	Langoni et al., 2011

South Am	AMR	Brazil	120	1497	8.02	6.69-9.45	IFAT	M/H	Machado et al., 2011
South Am	AMR	Brazil	373	616	60.55	56.66-64.38	IFAT	M/H	Maia et al., 2021
South Am	AMR	Brazil	116	932	12.45	10.40-14.65	IFAT	M/H	Mendonca et al., 2019
South Am	AMR	Brazil	3	64	4.69	0.60-11.55	IFAT	M/H	Moraes et al., 2011
South Am	AMR	Brazil	53	381	13.91	10.61-17.58	IFAT	M/H	Munhoz et al., 2010
South Am	AMR	Brazil	214	416	51.44	46.63-56.24	IFAT	M/H	Oshiro et al., 2015
South Am	AMR	Brazil	353	596	59.30	55.23-63.10	IFAT	M/H	Paiz et al., 2015
South Am	AMR	Brazil	478	1200	39.83	37.08-42.62	IFAT	M/H	Rizzo et al 2017
South Am	AMR	Brazil	53	284	18.66	14.33-23.41	IFAT	M/H	Rizzo et al 2018
South Am	AMR	Brazil	29	305	9.51	6.45-13.08	IFAT	M/H	Romanelli et al., 2007
South Am	AMR	Brazil	332	1800	18.44	16.69-20.27	IFAT	M/H	Romanelli et al., 2020
South Am	AMR	Brazil	21	360	5.83	3.62-8.51	IFAT	M/H	Rosa et al., 2011
South Am	AMR	Brazil	73	155	47.10	39.27-55	IFAT	M/H	Rossi et al., 2011
South Am	AMR	Brazil	41	155	26.45	19.78-33.70	ELISA	M/H	Rossi et al., 2011
South Am	AMR	Brazil	27	334	8.08	5.38-11.27	IFAT	M/H	Salaberry et al., 2010
South Am	AMR	Brazil	105	795	13.21	10.93-15.65	IFAT	M/H	Santana-Rocha et al., 2014
South Am	AMR	Brazil	7	409	1.71	0.64-3.24	IFAT	M/H	Soares et al., 2009
South Am	AMR	Brazil	52	81	64.20	53.40-74.33	IFAT	M/H	Tembue et al., 2011
South Am	AMR	Brazil	90	1028	8.75	7.10-10.56	IFAT	M/H	Ueno et al., 2009
South Am	AMR	Brazil	2	62	3.22	0.05-9.48	ELISA	M/H	Vogel et al., 2006
South Am	AMR	Colombia	22	28	78.57	61.18-92.09	ELISA	M/H	Patarroyo et al., 2013
South Am	AMR	Uruguay	16	1357	1.17	0.67-1.83	ELISA	M/H	Suzuki et al., 2011
Asia	WPR	China	62	600	10.33	8.02-12.90	ELISA	M/H	Liu et al., 2015
Asia	WPR	China	184	2187	8.41	7.28-9.61	ELISA	M/H	Nie et al., 2018
Asia	WPR	China	17	299	5.69	3.31-8.63	ELISA	M/H	Sun et al., 2020
Asia	WPR	China	57	779	7.32	5.59-9.26	ELISA	M/H	Wang et al., 2018
Asia	EMR	Iran	8	358	2.23	0.92-4.06	ELISA	M/H	Gharekhani et al., 2013
Asia	EMR	Iran	37	550	6.72	4.77-8.98	ELISA	M/H	Gharekhani et al., 2018
Asia	EMR	Iraq	6	127	4.72	1.60-9.22	ELISA	M/H	Al-Jomaily et al., 2013
Asia	EMR	Jordan	19	320	8.44	5.62-11.75	ELISA	M/H	Al-Majali et al., 2008
Asia	EMR	Pakistan	35	128	27.34	19.94-35.43	ELISA	M/H	Nasir et al., 2012
Asia	EMR	Saudi Arabia	103	414	24.88	20.83-29.16	ELISA	M/H	Al Nasr 2013

Asia	EUR	Turkey	29	234	12.39	8.45-16.95	ELISA	M/H	Eski et al., 2018
Asia	EUR	Turkey	8	376	2.13	0.87-3.87	ELISA	M/H	Gökçe et al., 2015
Asia	EUR	Turkey	14	180	7.78	4.26-12.19	ELISA	M/H	Karatepe and Karatepe 2020
Asia	EUR	Turkey	13	610	2.13	1.11-3.45	ELISA	M/H	Zhou et al., 2017
Europe	EUR	Czech Rep	63	547	11.52	8.97-14.33	ELISA	M/H	Bártová et al., 2009
Europe	EUR	Greece	77	458	16.81	13.52-20.38	ELISA	M/H	Anastasia et al., 2013
Europe	EUR	Greece	2	80	2.5	0.04-6.45	ELISA	M/H	Kouam et al., 2019
Europe	EUR	Italy	22	1010	2.18	1.36-3.18	ELISA	M/H	Gaffuri et al., 2006
Europe	EUR	Italy	49	255	19.22	14.60-24.30	ELISA	M/H	Gazzonis et al., 2016
Europe	EUR	Italy	135	304	44.41	38.86-50.03	ELISA	M/H	Tamponi et al., 2015
Europe	EUR	Poland	8	64	12.5	5.36-21.88	ELISA	L	Moskwa et al., 2018
Europe	EUR	Romania	0	10	0	0-16.64	ELISA	M/H	Kouam et al., 2019
Europe	EUR	Slovakia	14	382	3.66	1.84-5.81	ELISA	L	Špilovská and Reiterová 2008
Europe	EUR	Spain	4	209	1.91	0.41-4.31	ELISA	M/H	Astorga et al., 2014
Europe	EUR	Spain	132	2400	5.50	4.62-6.45	ELISA	M/H	Díaz et al., 2014
Europe	EUR	Spain	0	90	0	0-1.90	ELISA	M/H	Kouam et al., 2019
Europe	EUR	Spain	18	177	10.17	6.10-15.10	ELISA	L	Panadero et al., 2010
Europe	EUR	Switzerland	7	150	4.67	1.78-8.72	ELISA	M/H	Wyss et al., 2000
Europe	EUR	UK	28	660	4.24	2.83-5.93	ELISA	M/H	Helmick et al., 2002
Europe	EUR	UK	3	660	0.45	0.05-1.14	IFAT	M/H	Helmick et al., 2002
Oceania	WPR	Australia	0	184	0	0-0.93	iELISA	M/H	Bishop et al., 2010
Oceania	WPR	Australia	5	232	2.15	0.06-4.50	cELISA	M/H	Bishop et al., 2010
Oceania	WPR	New Zealand	4	640	0.62	0.13-1.41	ELISA	M/H	Reichel et al., 2008

Abbreviations: Am, America; AFR, African Region; AMR, Region of the Americas; EMR, Eastern Mediterranean Region; EUR, European Region; WPR, Western Pacific Region; UK, United Kingdom; Seroprev, serological prevalence; L, low; M/H moderate/high; ELISA, enzyme-linked immunosorbent assay; IFAT, immunofluorescence antibody test; iELISA, indirect ELISA; cELISA, comparative ELISA; NOS, Newcastle-Ottawa Scale.

Table 2. Subgroup analyses of pooled seroprevalence of ovine neosporosis based on continent, WHO region, country, diagnostic method and NOS quality index

Subgroup variable	Seroprevalence (%)	Confidence Interval (CI. 95%)	Heterogeneity (Q)	I^2 (%)	P value	Interaction test (X^2)	*df*	P value
Continent								
Africa	20.47	3.76-44.10	105.18	97	<0.0001	898	6	< 0.0001
America: North America	8.57	2.21-17.98	13.15	92	<0.0001			
America: Central America	11.56	8.97-14.43	0.48	0	>0.05			
America: South America	17.84	13.09-23.14	3280.60	99	<0.0001			
Europe	8.41	3.83-14.45	849.86	98	<0.0001			
Asia	8.15	5.47-11.28	243.18	95	<0.0001			
Oceania	0.69	0.01-2.07	6.01	67	<0.05			
WHO region								
AFR	42.05	36.20-47.99	0.00	0	>0.05	975.6	4	< 0.0001
EMR	9.85	4.38-16.26	152.10	95	<0.0001			
EUR	7.78	4.15-12.01	917.57	98	<0.0001			
AMR	17.17	12.76-21.93	3320.74	99	<0.0001			
WPR	4.06	1.40-7.41	147.04	96	<0.0001			
Country								
Egypt	10.53	6.98-14.69		n.a.		1893	28	<0.0001
Gabon	42.10	32.32-52.21		n.a.				
Senegal	41.95	34.70-49.38		n.a.				
Sudan	4	0.87-8.91		n.a.				
Mexico	8.57	2.20-17.98	13.15	92	<0.001			
Costa Rica	10.97	8.05-14.27		n.a.				
Grenada	13.04	7.89-19.23		n.a.				
Argentina	9.13	1.59-20.89	74.40	97	<0.0001			
Brazil	18.14	13.25-23.59	2550.97	99	<0.0001			
Colombia	78.57	61.18-92.09		n.a.				
Uruguay	1.17	0.67-1.83		n.a.				
China	8.11	6.70-9.67	6.91	57	>0.05			
Iraq	4.72	1.60-9.22		n.a.				
Iran	3.83	0.55-9.28	10.41	90	<0.01			
Jordan	8.44	5.62-11.75		n.a.				
Pakistan	27.34	19.94-35.43		n.a.				
Saudi Arabia	24.88	20.83-29.16		n.a.				
Turkey	4.95	1.34-10.38	40.05	93	<0.0001			
Czech Rep	11.52	8.97-14.33		n.a.				

Greece	9	0-25.73	16.81	94	<0.0001			
Italy	18.47	0-52.63	24.56	99	<0.0001			
Poland	12.5	5.36-21.88		n.a.				
Romania	0	0-16.64		n.a.				
Slovakia	3.66	1.84-5.81		n.a.				
Spain	3.53	0.92-7.52	339.76	88	<0.0001			
Switzerland	4.67	1.78-8.72		n.a.				
UK	13.48	0-73.74	380.32	99.7	<0.0001			
Australia	0.93	0-3.81	5.66	82	<0.05			
New Zealand	0.62	0.13-1.41		n.a.				
Diagnostic method								
ELISA	9.65	7.29-12.31	1579.99	97	<0.0001	686.2	1	<0.0001
IFAT	16.92	11.90-22.60	2922.10	99	<0.0001			
NOS quality index								
Moderate/High	11.59	7-17.12	50.28	90	<0.0001	1.93	1	0.1648
Low	12.62	9.83-15.70	5123.35	99	<0.0001			

Abbreviations: AFR. African Region; AMR. Region of the Americas; EMR. Eastern Mediterranean Region; EUR. European Region; WPR. Western Pacific Region; NOS. Newcastle-Ottawa Scale, n.a.: not assessed (only one dataset)