Universidade Federal do Maranhão
Programa de Pós-Graduação em Ciência Animal

NEOSPOROSE E TOXOPLASMOSE EM SISTEMAS DE PRODUÇÃO DE CAPRINOS: SOROPREVALÊNCIA, FATORES DE RISCO E MANEJO SANITÁRIO

ARLAN ARAUJO RODRIGUES

Chapadinha – MA
2021

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Dissertação apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Federal do Maranhão, para obtenção do título de Mestre em Ciência Animal.

Orientador: Prof. Dr. Ivo Alexandre Leme da Cunha

Coorientador: Prof. Dr. Thiago Vinícius Costa Nascimento

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A ciência é sábia, siga seus conselhos.
Rick and Morty

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RESUMO

Neosporose e toxoplasmose em rebanhos, especialmente em ovinos e caprinos, são responsáveis por perdas econômicas significativas associadas ao aborto, natimortos e perdas neonatais. No entanto, estudos que abordam a prevalência e fatores de risco para neosporose e toxoplasmose em caprinos são escassos no Brasil e no mundo e ausentes no estado do Maranhão, Brasil. Nesse sentido, os objetivos neste trabalho foram estimar a soroprevalência de neosporose e toxoplasmose associada a fatores de risco nos sistemas produtivos de caprinos no mundo e no estado do Maranhão, Brasil. Para isso, foram realizados três estudos, dois estudos de revisão sistemática da literatura e metanálise de fatores de risco para soroprevalência de *Neospora caninum* e *Toxoplasma gondiii* em caprinos no mundo, e um estudo de soroprevalência de neosporose e toxoplasmose associadas a fatores de risco em sistemas de produção de caprinos no leste do estado do Maranhão, Brasil. No primeiro estudo, realizou-se uma revisão da literatura e meta-análise para soroprevalência de *N. caninum* em caprinos. Esse trabalho foi publicado na Medidcine Veterinary Preventive (volume 185, ano 2020). O estudo foi baseada nos dados de 36 artigos selecionados nas principais bases de publicações científicas do mundo. Concluiu-se que a soroprevalência de *N. caninum* em caprinos está amplamente distribuída em todo o mundo, com 6% (IC 95 % 4,4 - 7,8) dos animais soropositivos, sendo que o continente americano apresenta maior proporção de caprinos soropositivos. No segundo estudo, realizou-se uma revisão da literatura e meta análise para soroprevalência de *T. gondii* em caprinos. O estudo foi baseada nos dados de 75 artigos selecionados nas principais bases de publicações científicas do mundo. Concluiui-se 27,5% (IC 95% 24,15 - 30,95) dos caprinos foram soropositivos para *T. gondii*, sendo que as maiores taxa de animais soropositivos foram relatadas em países onde há presença de gatos entre o rebanho e presença de sistema de manejo semi-intensivo ou extensivo. No terceiro estudo, verificou-se a soroprevalência de neosporose e toxoplasmose associada a fatores de risco nos sistemas produtivos de caprinos no estado do Maranhão, Brasil. Foram coletadas amostras de sangue de 383 caprinos e informações epidemiológicas para estimar os possíveis fatores de risco associados à soroprevalência de *N. caninum* e *T. gondii*, e caracterizar as propriedades estudadas. A soroprevalência de *N. caninum* e *T. gondii* em caprinos no Maranhão foi de 26,4% e 29,8%, respectivamente. A caprinocultura da região Leste Maranhense é uma atividade de baixa eficiência. O rebanho é composto principalmente por animais sem raça definida, com criação extensiva, pouca ou nenhuma adoção de medidas sanitárias ou nutricionais, sendo a vegetação nativa a principal fonte de alimento dos animais. Os principais fatores de risco associados à neosporose foram idade, histórico de aborto, sistema de exploração e presença de cães e gatos, enquanto para toxoplasmose foi idade, categoria, presença de outras espécies e objetivo da criação. Este é o primeiro estudo que estima a soroprevalência de *N. caninum* e *T. gondii* em caprinos a nível mundial e no estado no Maranhão, Brasil. Considerando a importância do *N. caninum* e *T. gondii* na caprinocultura mundial, a compreensão desses dados pode ajudar autoridades epidemiológica adotarem medidas zootecnicas de controle englobando os fatores de risco abordados no presente estudo.

Palavras-chave: caprinocultura, meta-análise, *Neospora caninum*, *Toxoplasma gondii*

ABSTRACT

Neosporosis and toxoplasmosis in herds, especially in sheep and goats, are responsible for significant economic losses associated with abortion, stillbirths and neonatal losses. However, studies that address the prevalence and risk factors for neosporosis and toxoplasmosis in goats are scarce in Brazil and in the world and absent in the state of Maranhão, Brazil. In this sense, the objectives of this work were to estimate the seroprevalence of neosporosis and toxoplasmosis associated with risk factors in the production systems of goats around the world and in the state of Maranhão, Brazil. For this, three studies were carried out, two systematic literature review studies and meta-analysis of risk factors for seroprevalence of *Neospora caninum* and *Toxoplasma gondiii* in goats worldwide, and a study of seroprevalence of neosporosis and toxoplasmosis associated with risk factors in systems of goat production in the state of Maranhão, Brazil. In the first study, a literature review and meta-analysis for the seroprevalence of *N. caninum* in goats was performed. This manuscript was published in Medidcine Veterinary Preventive (volume 185, year 2020). The study was based on data from 36 articles selected from the main scientific publications databases in the world. It was concluded that the seroprevalence of *N. caninum* in goats is widely distributed throughout the world, with 6% (CI 95% 4.4 - 7.8) of seropositive animals, and the American continent has a higher proportion of seropositive goats. In the second study, a literature review and meta-analysis for *T. gondii* seroprevalence in goats was performed. The study was based on data from 75 articles selected from the main scientific publications databases in the world. It was concluded that 27.5% (95% CI 24.15 - 30.95) of goats were seropositive for *T. gondii*, and the highest rate of seropositive animals was reported in countries where there is presence of cats among the herd and presence of semi-intensive or extensive management system. In the third study, the seroprevalence of neosporosis and toxoplasmosis associated with risk factors in the production systems of goats in the state of Maranhão, Brazil was verified. Blood samples from 383 goats and epidemiological information were collected to estimate the possible risk factors associated with the seroprevalence of *N. caninum* and *T. gondii*, and to characterize the properties studied. The seroprevalence of *N. caninum* and *T. gondii* in goats in Maranhão was 26.4% and 29.8%, respectively. Goat farming in the East Maranhense region is a low-efficiency activity. The herd is mainly composed of animals without a defined breed, with extensive breeding, little or no adoption of sanitary or nutritional measures, with native vegetation being the main source of food for the animals. The main risk factors associated with neosporosis were age, abortion history, exploitation system and presence of dogs and cats, while for toxoplasmosis it was age, category, presence of other species and purpose of breeding. This is the first study to estimate the seroprevalence of *N. caninum* and *T. gondii* in goats worldwide and in the state of Maranhão, Brazil. Considering the importance of *N. caninum* and *T. gondii* in world goat farming, understanding these data can help epidemiological authorities adopt zootechnical control measures encompassing the risk factors addressed in this study.

Keywords: goats, meta-analysis, *Neospora caninum*, *Toxoplasma gondii*

1. CAPÍTULO I – CONSIDERAÇÕES GERAIS

1.1. Introdução

Na produção animal, a sanidade é responsável pelo controle de diversas enfermidades nos rebanhos, como neosporose e toxoplasmose, doenças parasitárias causadas pelo *Neospora caninum* e *Toxoplasma gondii*, respectivamente, e associadas a distúrbios reprodutivos nos animais de produção, incluindo os pequenos ruminantes (Fthenakis and Papadopoulos, 2018). Esses parasitas são protozoários coccídeos, seus hospedeiros definitivos são os carnívoros e hospedeiros intermediários as aves e várias espécies de mamíferos. Os principais problemas causados pelo *N. caninum* e *T. gondii* são distúrbios reprodutivos como abortos, má formação fetal e mortalidades neonatal (Dubey, 2016; Dubey et al., 2007; Dubey and Schares, 2011; Gondim et al., 2004). Além disso, o *T. gondii* é um protozoário zoonótico de importância mundial, apontado pela FAO como o quarto maior parasita responsável por infecções alimentares em humanos (FAO, 2014).

N. caninum e *T. gondii* causam grandes prejuízos na produção animal (Freyre et al., 1997; Reichel et al., 2013). No Brasil, os relatos de caprinos soropositivos para neosporose variam de 1,05% a 26,1% (Braz et al., 2018; Lima et al., 2008), e para toxoplasmose de 3,47% a 47,13% (Arraes-Santos et al., 2016; Medeiros et al., 2014). A ocorrência de *T. gondii* em caprinos do estado do Maranhão varia de 4,35% a 36,95% (Moraes et al., 2011; Soares et al., 2010) e de *N. caninum* de 17,39% (Moraes et al., 2011).

Estudos mostram que a origem da água, idade, presença de cães, presença de gatos, sistema de produção, e alguns parâmetros zootécnicos como o intervalo entre partos, histórico de aborto ou problemas reprodutivos, entre outras características, têm sido relacionados com a alta soroprevalência de ambos os parasitas em caprinos (Gazzonis et al., 2016; Moura et al., 2016; Nunes et al., 2013; Topazio et al., 2014).

Considerando a importância econômica e epidemiológica da neosporose e da toxoplasmose como agentes causadores de problemas reprodutivos em caprinos, estudar a soroprevalência e identificar os potenciais fatores de risco no rebanho fornece informações para adoção de medidas zootécnicas de controle.

1.2. Importância da neosporose e toxoplasmose para a produção animal

1.2.1. Neosporose

A neosporose é uma doença causada pelo *N. caninum*, um parasito Apicomplexa que foi descrito pela primeira vez em 1988 (Bjerkås et al., 1984; Dubey et al., 1988). *N. caninum* é um protozoário coccídeo, que tem como hospedeiro definitivo os canídeos e hospedeiros intermediários várias espécies de mamíferos e aves (Dubey et al., 2017). A neosporose é uma das principais causas de abortos em bovinos, caprinos e ovinos, causando prejuízos econômicos significativos em todo o mundo (Dubey and Schares, 2011).

Estima-se que a soroprevalência mundial de neosporose caprina seja de 5,99% (4,38 – 7,83%) e os continentes da América do Sul e Ásia apresentem a maior proporção de animais soropositivos (Rodrigues et al., 2020). No Brasil a soroprevalência de neosporose está amplamente distribuída e estudos mostram que a soropostividade dos rebanhos varia de 1,05% – 26,65% (Lima et al., 2008; Tembue et al., 2011). A variação nos resultados de soroprevalência está associada a múltiplos fatores que vão desde questões climáticas a características de manejo adotadas pelas propriedades.

1.2.2. Toxoplasmose

T. gondii é um protozoário coccídeo do Filo Apicomplexa que pode infectar diversas espécies de mamíferos e aves, sendo seus hospedeiros definitivos os felídeos, como o gato doméstico (*Felis catus*) e outros felinos selvagens (Dubey, 2016).

A toxoplasmose é uma das principais enfermidade causadora de abortos, e mortalidade neonatal em caprinos, causando prejuízos econômicos significativos nos sistemas produtivos (Freyre et al., 1997; Medeiros et al., 2014). A soroprevalência de *T. gondii* em caprinos foi relatada em vários países do mundo. Há estudos em países do continente Asiático, como China (21,23%), Índia (42,47%) e Paquistão (42,83%) (Ahmed et al., 2016; Bachan et al., 2018; Wang et al., 2020). No continente Africano existem trabalhos na Argélia (11,92%), Ilhas Canárias (7,79%) e Tanzânia (19,29%) (Abdallah et al., 2019; Rodríguez-Ponce et al., 2017; Swai and Kaaya, 2012). Na Europa existem relatos de animais soropositivos na Espanha (46,87%), Itália (18,57%) e Grécia (61,66%) (Anastasia et al., 2013; Díaz et al., 2016; Gazzonis et al., 2015). Há relatos também em países das Américas como Estados Unidos (6,81%), México (15,25%),

Costa Rica (62,15%), Argentina (40,79%) (Alvarado-Esquivel et al., 2013; Gos et al., 2017; Villagra-Blanco et al., 2018; Yaglom et al., 2014).

Trabalhos realizados no Brasil mostraram que a soroprevalência de toxoplasmose caprina varia de 3,47% a 47,13% (Arraes-Santos et al., 2016; Medeiros et al., 2014). A heterogeneidade na soroprevalência observadas no mesmo continente ou em continentes distintos estão associadas a vários de motivos, como as condições climáticas, aos tipos de manejo realizado nos animais e a diferentes técnicas de diagnósticos (Medeiros et al., 2014).

1.2.3. Biologia e Controle

N. caninum e *T. gondii* possuem ciclo de vida facultativo, possuindo duas fases: fase assexuada, caracterizada pela multiplicação nos tecidos dos hospedeiros intermediários; e fase sexuada, que ocorre células do epitélio intestinal dos hospedeiros definitivos. Existe três estágios no ciclo de vida desses protozoários: taquizoítos, bradizoítos e oocistos (Dubey, 2016; Dubey et al., 2017).

Os taquizoítos se multiplicam assexuadamente dentro das células do hospedeiro definitivo por um processo chamado endodiogenia, forma de reprodução no qual um parasito da origem a dois parasitos dentro do vacúolo parasitófago. Os bradizoítos são a fase encistada do parasita nos tecidos. Esses cistos teciduais crescem e permanecem intracelular à medida que os bradizoítas se dividem por endodogenia (Dubey, 2016; Dubey et al., 2017).

Os hospedeiros definitivos entram em contato com o parasito através da ingestão de cistos nos tecidos dos hospedeiros intermediários. Nas células do epitélio intestinal dos hospedeiros definitivos ocorre a fase sexuada do parasito, dando origem aos oocistos que sejam eliminados no ambiente através das fezes. Os oocistos não esporulados eliminados no ambiente através das fezes irão passar por um processo de esporogonia, tornam-se esporulados e contaminando o ambiente (Dubey, 2016; Dubey et al., 2017).

A transmissão do *N. caninum* e *T. gondii* ocorre por dois mecanismos, horizontal: através do solo, água e alimento contaminada com oocistos esporulados; e vertical: através da transmissão transplacentária (Dubey, 2016; Dubey et al., 2017). Embora as vias horizontal e vertical sejam as mais conhecidas, transmissão venérea em caprinos por *T. gondii* já foi observada (Wanderley et al., 2015). Além disso, DNA de *T. gondii* já foi encontrado em amostras de leite, sugerindo ser um importante via de transmissão da toxoplasmose para animais e humanos (Bezerra et al., 2015; Ossani et al., 2017).

1.2.4. Fatores de risco e importância zootécnica

O conhecimento dos fatores de risco para soroprevalência de *N. caninum* e *T. gondii* nos rebanhos fornece informações importantes para elaboração e adoção de estratégias zootécnicas de controle (Dubey et al., 2007). A presença de aborto e o fornecimento de carne crua para cães ou gatos nas fazendas aparecem como os principais fatores de risco para neosporose e toxoplasmose em caprinos (Abo-Shehada and Abu-Halaweh, 2010; Fortes et al., 2018; Gharekhani et al., 2018, 2016; Liu et al., 2015; Modolo et al., 2008; Topazio et al., 2014), embora alguns trabalhos também associem o sistema de produção, a origem da água e fatores climáticos com o aumento da soroprevalência de *N. caninum* e *T. gondii* no rebanho (Díaz et al., 2016; Gazzonis et al., 2016; Luo et al., 2016).

No Brasil existem alguns trabalhos que avaliaram a soroprevalência de *N. caninum* e *T. gondii* com possíveis fatores de risco para os caprinos (Modolo et al., 2008; Santos et al., 2013; Topazio et al., 2014). Modolo et al. (2008) relataram que abortos e presença de cães estavam associados com a soroprevalência de *N. caninum* em caprinos. Topazio et al. (2014) observaram que origem da água e a presença de problemas reprodutivos favorecem a soroprevalência de *N. caninum* em caprinos. Santos et al. (2013) relataram que o pastejo de caprinos com outras espécies favoreceu a soroprevalência dos animais. Com relação a *T. gondii*, Pereira et al. (2012) relataram associação da soroprevalência com sistema de produção e manejo reprodutivo. Fortes et al. (2018) observaram que a soroprevalência foi associada com idade, sexo, presença de gatos e pastejo com outras espécies. Rêgo et al. (2016) encontraram associação entre a soroprevalência de *T. gondii* e sexo, sistema de produção e presença de gatos.

Considerando a importância da neosporose e da toxoplasmose na produção animal, principalmente em caprinos, estimativas de soroprevalência e possíveis fatores de risco para *N. caninum* e *T. gondii* em caprinos no estado do Maranhão são necessárias.

1.3. Objetivo

1.3.1. Objetivo geral

Analisar a soroprevalência de neosporose e toxoplasmose associada a fatores de risco nos sistemas produtivos de caprinos no mundo e no estado do Maranhão, Brasil.

1.3.2. Objetivos específicos

Realizar revisão sistemática na literatura e meta-análise de fatores de risco para soroprevalência de *Neospora caninum* em caprinos no mundo;

Realizar revisão sistemática na literatura e meta-análise de fatores de risco para soroprevalência de *Toxoplasma gondii* em caprinos no mundo;

Realizar estudo de soroprevalência de neosporose e toxoplasmose e associar a fatores de risco nos sistemas produtivos de caprinos no estado do Maranhão, Brasil.

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2. CAPÍTULO 2 – A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS OF RISK FACTORS FOR *NEOSPORA CANINUM* SEROPREVALENCE IN GOATS

Este capítulo representa o artigo "**A systematic literature review and meta-analysis of risk factors for *Neospora caninum* seroprevalence in goats**", DOI: https://doi.org/10.1016/j.prevetmed.2020.105176, publicado no periódico Preventive Veterinary Medicine, volume 185, 2020.

A systematic literature review and meta-analysis of risk factors for *Neospora caninum* seroprevalence in goats

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Abstract

This meta-analysis aimed to estimate *N. caninum* seroprevalence in goats worldwide to determine the main risk factors for seropositivity that could be associated with parasite infection in herds. Four electronic databases were searched: PubMed, SciELO, Scopus and the VHL Regional Portal. Firstly, 367 articles were identified. After removing duplicates and non-eligible papers. A total of 36 articles were selected, which contained information concerning 22,234 goats, between 2004–2019. The combined seroprevalence of infection using a meta-analysis of the random effects model was 5.99% (95%, CI 4.38–7.83). The overall estimated *N. caninum* seroprevalence showed high heterogeneity, I^2 = 97%. The present study showed that seropositive goats were 3.07 times more likely to abort (OR 3.07; 95% CI 1.02–9.22) than seronegative animals. The presence of dogs on farms also increased the odds of *N. caninum* seropositivity (OR 1.40; 95% CI 1.01–1.94). In addition, male animals had higher odds of being seropositive to neosporosis than females (OR 1.31; 95% CI 1.00–1.71). *N. caninum* seroprevalence in goats is widely distributed worldwide, with the American continent having a higher proportion of seropositive animals.

Keywords: anti-*Neospora* antibodies, caprine, epidemiological studies, neosporosis, review

2.1. Introduction

Neospora caninum is an Apicomplexa protozoan with worldwide distribution for which canines are the definitive host and homeothermic animals are intermediate hosts (Dubey et al., 2017). Neosporosis is one of the main causes of reproductive failure in ruminants and it has been estimated that the worldwide economic impact is approximately 1.3 billion dollars annually (Dubey et al., 2007; Reichel et al., 2013).

Neosporosis in goats is prevalent in several regions of the world, and seropositivity in a herd is commonly associated with miscarriage and neonatal mortality (Moreno et al., 2012; Mesquita et al., 2013). *N. caninum* seropositivity in goats is widely distributed worldwide and previous studies have shown that the seroprevalence of anti-*N caninum* antibodies ranges between 0.47% and 26.65% (Czopowicz et al., 2011; Tembue et al., 2011).

Sample calculation is an important tool for estimating seroprevalence in herds (Cameron and Baldock, 1998). Furthermore, seroprevalence associate with the potential risk factors is a way of understanding as the parasite maintained and spread among animals in the herd.

Some studies have associated *N. caninum* seropositivity in goats with certain risk factors, i.e. the presence of dogs, reproductive failures on farms and age. In addition, older animals appear to be more susceptible, as they have a longer duration of environmental exposure (Arraes-Santos et al., 2016; Gazzonis et al., 2016; Braz et al., 2018).

As there is no effective vaccine against *N. caninum*, epidemiological studies are essential for adopting biosecurity measures for herds (Dubey et al., 2007). This meta-analysis aimed to estimate the *N. caninum* seroprevalence in goats worldwide and identify the main risk factors for seropositivity that could be associated with parasite infection in herds.

2.2. Material and Methods

459 2.2.1. Literature search strategy

460 This systematic review was structured according to the recommendations of the
461 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et
462 al., 2016). A thorough systematic review was carried out to identify the largest number of
463 scientific articles reporting *N. caninum* seroprevalence in goats. For this, four electronic
464 databases were consulted: PubMed, SciELO, Scopus and the VHL Regional Portal. The
465 combination of terms used in the literary research were: '*Neospora caninum* AND goat',
466 '*Neospora caninum* AND goat AND anti-*Neospora* antibodies', '*Neospora caninum* AND goat
467 AND seroprevalence', '*Neospora caninum* AND goat AND risk factor' and '*Neospora caninum*
468 AND goat AND seroprevalence AND risk factor'. For the selection of articles, there was no
469 restriction on the year of publication and all articles that met the above criteria published until
470 February 2020 were included.

471 The research results were imported into the free web-tool Rayyan for systematic reviews
472 (Ouzzani et al., 2016) where two independent reviewers selected the papers in according to the
473 pre-established criteria. When there was disagreement in the choice of articles, a third reviewer
474 was consulted to resolve the situation. The inclusion criteria were: all articles that reported *N.*
475 *caninum* seroprevalence in goats; that contained, in their methodology, calculation of the
476 sample size of the study to define the seroprevalence found; and published in English,
477 Portuguese or Spanish. Articles that did not meet these prerequisites were excluded. The
478 following were also excluded: studies without a sample size calculation; experimental studies
479 and reviews; studies with other species; studies with other diseases; theses and dissertations.
480 Finally, a search for articles was performed in the reference lists of the included papers in order
481 to find studies not indexed and found through the search items.

482

483 2.2.2. Data selection

484 Firstly, the articles were selected through the title and abstract. Where studies were
485 present in more than one electronic database the duplicate article was deleted. The eligible
486 papers were collected and the following information extracted: author; year of publication; type
487 of study; continent; country; sample size; number of positive samples; diagnostic test;
488 geographic location (latitude and longitude); and possible animal-related risk factors; sex; age

(≤ 1 year old or > 1 year old); abortion; and presence of dogs. The data were organised in Microsoft Excel spreadsheets.

Articles that presented risk factor results only in the form of an odds ratio (OR) and did not have individualised risk factors were excluded from the meta-analysis of risk factors and these studies were only included in the overall meta-analysis of seroprevalence, as they had incomplete information concerning the number of positive animals, total number of the animals in the exposed or unexposed groups, or for evaluating the risk factors at herd level.

Studies that classified age (< 1 year and > 1 year; < 1 year and ≥ 1 year; or in three or more categories that could not be standardised for ≤ 1 year old and > 1 year old) were also excluded from the meta-analysis of risk factors.

Finally, the articles included in the meta-analysis of risk factors were standardised to allow a comparative assessment between the articles considering: age (adult vs young) – adult (> 1 year old) and young (≤ 1 year old); abortion (yes vs no); sex (male vs female); and presence of dogs (yes vs no).

2.2.3. Statistical analysis

The meta-analysis was performed using the R program, version 3.5.2 with RStudio (R Core Team, 2018) using the meta package (Schwarzer, 2007; Schwarzer et al., 2015). The random effects model was used for the meta-analysis (DerSimonian and Laird, 2015). The pooled seroprevalence estimate of *N. caninum* (at 95% confidence intervals) was presented as a percentage ((number of seropositive animals / total of animals tested)*100) and the Freeman–Tukey double arcsine method was used to stabilise the variance using the formula:

$$\sin^{-1}\sqrt{\frac{x}{n+1}} + \sin^{-1}\sqrt{\frac{x+1}{n+1}}$$

Where:

x was the number of seropositive animals;

n was the total of animals tested.

515 The variance was calculated using the formula adapted by Barendregt et al. (2013) from
516 that proposed by Freeman and Tukey (1950):

$$\text{Var} = \frac{1}{n + 0.5}$$

517

518 The risk factors for the studied variables were presented as odds ratios (OR) with a 95%
519 confidence interval. The command metaprop was used to estimate the pooled seroprevalence
520 and the command metabin was used to estimate of the association between seroprevalence and
521 possible risk factors: abortion, age, presence of dogs and sex.

522 Cochran's Q test was used to the heterogeneity between the studies and the I^2 statistic
523 for evaluation of true variation due to heterogeneity (Cochran, 1954; Higgins et al., 2003;
524 Borenstein et al., 2017). The I^2 shows the proportion of the variance, ranging from 0% to 100%
525 and observes the true size effect from all studies in the analysis (Borenstein et al., 2017).
526 Subgroup meta-analysis and meta-regression were used to evaluate possible sources of
527 heterogeneity. Subgroup analysis was done by continent: Africa, Central America, North
528 America, South America, Asia and Europe. The raw prevalence data were used in the meta-
529 regression and the results were presented in decimal numbers. Meta-regression was performed
530 to determine whether the year of publication, longitude and latitude influenced the *N. caninum*
531 seroprevalence in goats through the bubble plot from the metareg command of the R package.

532 Meta-regression was performed by the DerSimonian and Laird model equation:

$$\hat{\tau}^2 = \frac{Q - (K - 1)}{\sum_{k=1}^{K} w_k - \frac{\sum_{k=1}^{K} w_k^2}{\sum_{k=1}^{K} w_k}},$$

533

534 Where:

535 τ^2 was the additive (between-study) component of the variance;

536 k was amount of studies;

Q was the heterogeneity statistic test proposed by Cochran (1954).

w_k was a weighting factor for the *i*-th study, assuming a fixed model:

$$w_k = 1/\hat{\sigma}_k^2$$

Where:

k was amount of studies;

σ was the treatment effect estimate (e.g. a log OR).

Possible publication bias regarding prevalence and risk factors was assessed using Begg's and Egger's tests in combination with a funnel plot and were visualised using funnel plots. Publication bias regarding subgroups and risk factors with at least ten articles was also evaluated (Sterne et al., 2011). In all analyses, a p-value less than 0.05 was considered statistically significant.

2.3. Results

2.3.1. Description of included studies

A PRISMA flow chart was created to briefly illustrate the selected studies included in the meta-analysis (Figure 1). Firstly, 367 articles were pre-selected from the databases. After removing duplicates and non-eligible works, a total of 33 articles were selected. Review of the reference lists yielded three further articles. Finally, a total of 36 articles were eligible for systematic review and meta-analysis (Table 1).

Information concerning 22,234 goats, in these 36 selected articles, were from the period 2004–2019. This was used to determine the *N. caninum* seroprevalence in goats. Data were extracted from 19 locations, which were categorised into six continents, Africa (1), Central America (2), North America (1), South America (2), Asia (5) and Europe (8) (Table 1). The diagnostic methods described included the Enzyme-Linked Immunosorbent Assay (ELISA) (n = 20), Indirect Immunofluorescent Assay (IFAT) (n = 14) and *Neospora* agglutination test (NAT) (n = 2) (Figure 2) (Table 1).

565 2.3.2. Seroprevalence

566 The pooled seroprevalence using the meta-analysis of the random effects model was
567 5.99% (95% CI 4.38–7.83) (Figure 2). The overall estimated *N. caninum* seroprevalence
568 showed a high heterogeneity (I^2 = 97%). Subgroup analysis also showed a high heterogeneity
569 in Asia (I^2 = 92%), Europe (I^2 = 95%) and South America (I^2 = 97%) (Figure 2). In the subgroup
570 meta-analysis, the highest seroprevalence was found in the American continent, with 7.3%
571 (95% CI 5.21–9.71) for Central America, 3.85% (95% CI 1.46–7.21) for North America and
572 8.78% (95% CI 5.85–12.23) for South America. In the other regions, the seroprevalence was
573 3.94% (95% CI 2.12–6.28) in Asia, 1.09% (95% CI 0.36–2.15) for Africa and 4.10% (95% CI
574 1.8–7.25) in Europe (Figure 2).

575 Average neosporosis seroprevalence was reported in 18 countries and ranged from
576 0.47% (Poland) to 11.60% (Brazil) (Figure 3). Seropositivity increased over the time period
577 2004–2019, but the meta-regression analysis was not statistically significant (p = 0.2041)
578 (Figure 4).

579

580 2.3.3. Risk factors

581 The systematic review identified six studies that reported an association between *N.*
582 *caninum* seropositivity and the occurrence of abortion. The meta-analysis confirmed that
583 infected goats are 3.07 times more susceptible to the occurrence of abortion (OR 3.07; 95% CI
584 1.02–9.22; p = 0.0459) than seronegative animals (Figure 5).

585 The age of the animals was collected from six articles and the results of the meta-
586 analysis did not show a significant association between age and *N. caninum* seropositivity in
587 goats (OR 1.14; 95% CI 0.84–1.53; p = 0.3996) (Figure 5).

588 Data on the association between *N. caninum* seropositive goats and the presence of dogs
589 were extracted from four studies. The meta-analysis showed that the presence of dogs on farms
590 increased the risk of *N. caninum* seropositivity in goats (OR 1.40; 95% CI 1.01–1.94; p =
591 0.0462) (Figure 5).

592 Twelve studies considered sex as a risk factor for *N. caninum* seropositivity in goats.
593 The meta-analysis showed that male animals were more neosporosis seropositive than females
594 (OR 1.31; 95% CI 1.00–1.71; p = 0.0478) (Figure 5).

595 According to the meta-regression, there was a significant relationship between
596 geographic latitude and seroprevalence (p = 0.0032) (Figure 6), although countries from higher
597 latitudes had fewer reported seroprevalence studies. However, there was no effect of longitude
598 on seroprevalence (p = 0.1994).

599 No apparent asymmetry in the funnel plot was observed and the absence of evidence of
600 suspected publication bias was supported by Egger's statistical test in relation to sex as a risk
601 factor, which was not significant (p = 0.6186) (Figure 7). Similarly, there was no statistically
602 significant effect of suspected publication bias for overall combined seroprevalence (p =
603 0.6589) (Figure 8) or for combined seroprevalence in South America (p = 0.4061) (Figure 9).

604

605 ## 2.4. Discussion

606

607 This systematic review and meta-analysis evaluated 22,234 goats of different breeds, of
608 both sexes and various ages from 18 countries. The results showed that there was a higher
609 proportion of seropositive animals in the Americas than in other regions of the world.

610 The estimated seroprevalence in the Americas was 7.66%; among which are the
611 following highlights: Brazil had a satisfactory number of indexed studies (14 studies); two other
612 indexed studies were from Argentina; with only one indexed study each from Mexico, Costa
613 Rica and Granada. Some American countries such as the USA, Mexico, Peru, Argentina and
614 Bolivia, which have goat populations greater than one million (FAO, 2018) presented indexed
615 studies with limited or absent seroprevalence data.

616 Europe's seroprevalence was 4.1%, however, there were few indexed studies from
617 traditional countries in goat farming and with a considerable goat population such as Turkey,
618 Greece and Spain, while indexed studies from France and Russia presented limited or absent
619 seroprevalence data.

620 In Asia, eight indexed studies with an average seroprevalence of 3.94% were found.
621 Only two indexed studies were from China, the country with the largest goat population in the
622 world (FAO, 2018). Other countries that also have the large goat population such as Iran had
623 indexed articles containing limited data regarding seroprevalence information and India,
624 Pakistan and Nepal had not presented indexed studies containing seroprevalence data.

Africa has more than 40% of the world goat population (FAO, 2018), but it was the most deficient continent in indexed studies on neosporosis seroprevalence in goats, with just one indexed article from the Canary Islands. In this meta-analysis, we identified that many countries with a representative goat population did not have indexed studies with data on *N. caninum* seroprevalence in goats within the four main databases or when they had indexed articles, they had limited data on seroprevalence. This absence of information does not mean that these countries are areas free of *N. caninum* seropositivity in goats. Probably, the goat population may be infected and seroprevalence data were not in indexed articles, thus limiting researchers' access to this information. Failure to access data concerning seroprevalence generates the impossibility of estimating seroprevalence and risk factors from these countries in this study. We emphasise the need for more indexed studies concerning neosporosis seroprevalence in goats in these countries, mainly in the African continent and if possible, considering the main risk factors found in this meta-analysis.

There was an increase in neosporosis seroprevalence in goats over the years (Figure 4); however, without a statistically significant difference ($p = 0.2041$). Therefore, this variation may be related to an increase in the number of studies carried out in recent years. The highest seroprevalences were found in Central and South American countries compared to countries in other continents (Figure 2), while a statistically significant effect was observed in relation to latitude ($p = 0.032$). According to Dubey (2007), high temperatures may favour the survival and sporulation of oocysts in the environment, increasing the risk of eventual infection. These variations in seroprevalence, in different continents and mainly in relation to latitude, may be related to factors such as climatic conditions, differences in the nutritional and health management of animals, in addition to the adoption of biosafety measures or there may still be some differences due to the use of different techniques in serological diagnosis (Dubey et al., 2007).

The low neosporosis seroprevalence in goats in European and Asian countries may be related to climatic factors, production systems, the use of local breeds, better hygienic–sanitary conditions and nutritional management practices (Al-Majali et al., 2008; Jung et al., 2014; Díaz et al., 2016; Gazzonis et al., 2016; Luo et al., 2016; Villagra-Blanco et al., 2017).

The great variation in seroprevalence in South America (1.05% to 26.65%) has also been reported in other articles (Lima et al., 2008; Tembue et al., 2011). These results may be

656 related to goat production systems, which are extensive production systems with a low
657 technological level. The higher seroprevalence in the Brazilian goat population has been
658 reported mainly in relation to family production systems in which the nutritional and sanitary
659 management is precarious, in addition to concomitant risk factors, such as the presence of dogs.
660 (Uzêda et al., 2007; Modolo et al., 2008; Tembue et al., 2011; Costa et al., 2012; Santos et al.,
661 2013; IBGE, 2017; Braz et. al., 2018).

662 The high heterogeneity of seroprevalence estimated between countries and continents
663 indicates that there is clear evidence that *N. caninum* seroprevalence in goats varies from one
664 population to another and may be related to the particular characteristics of each region, such
665 as climatic characteristics, production systems of each population from where the study
666 originated.

667 Previous studies have already shown that there is a relationship between abortion and
668 *N. caninum* seropositivity in goats (Mesquita et al., 2013; Unzaga et al., 2014; Porto et al.,
669 2016). In this meta-analysis an association between *N. caninum* seroprevalence in goats wherein
670 seropositive goats had more than three times the risk of abortion compared to seronegative goats
671 (p=0.0478) was observed (Figure 5). This result corroborates that found by Gharekhani et al.
672 (2018) and Varaschin et al. (2011), in which seropositive goats had almost four times greater
673 risk of abortion compared to seronegative goats.

674 The spread of oocysts in the environment by infected dogs is an important risk factor
675 for neosporosis (Dijkstra et al., 2002; Bartova and Sedlak, 2012). In the present study, the
676 presence of dogs was shown to be significantly associated with neosporosis seroprevalence in
677 goats (p = 0.0462). These finding corroborate the results of Abo-Shehada and Abu-Halaweh
678 (2010) and Liu et al. (2015) who reported that the presence of cats and dogs, the access of dogs
679 to the pasture, water and feed sources of ruminants, and the hygiene on farms increased the
680 seroprevalence in the herd. Consequently, it is of vital importance to control the dog population
681 as a tool to reduce the spread of the parasite in the environment and hence, reduce
682 seroprevalence (Dubey et al., 2007).

683 Sex, when analysed as a risk factor (OR 1.31; 95% CI 1–1.72; p = 0.0478), indicates
684 that males had greater neosporosis seropositivity than females; this difference was also reported
685 by Moore et al. (2007) and Sun et al. (2020). No publication bias effect was evaluated in the
686 studies that used sex as a risk factor for *N. caninum* seroprevalence in goats (Figure 7), and the

687 explanations for the greater *N. caninum* seropositivity for males were not elucidated in the
688 literature. Several studies did not report a significant association between seroprevalence and
689 sex (Arraes-Santos et al., 2016; Díaz et al., 2016; Gazzonis et al., 2016; Luo et al., 2016;
690 Gharekhani et al., 2018) or when showing a statistical significance, the studies did not describe
691 the possible related causes (Faria et al., 2007). Additional studies are needed to clarify the real
692 mechanisms and factors involved in the relationship between seropositivity and sex.

693 In this meta-analysis, there was no significant association between age and neosporosis
694 seroprevalence in goats ($p = 0.3996$) (Figure 5). This is supported by previous studies
695 demonstrating that there were no statistical differences in relation to seroprevalence between
696 different ages (Moore et al., 2007; Iovu et al., 2012; Gazzonis et al., 2016; Braz et al., 2018).
697 Although age was not found to have a statistically significant effect on seroprevalence, these
698 studies suggest that the infection route is different between young and adult animals. In adult
699 animals, the literature reports that horizontal transmission is probably the most common route
700 of infection as the environments can be contaminated with sporulated oocysts, and the adult
701 animals are exposed to contaminated feed or water for longer periods (Tembue et al., 2011; Sun
702 et al., 2020). The vertical route is the main route of transmission in young animals, which is
703 speculated as being due to the shorter time of exposure to environments with sporulated oocysts,
704 but the possibility of horizontal transmission due to the immunity of young animals should not
705 yet be fully effective. Therefore, age should be considered a risk factor.

706 There was no effect of publication bias in the data analysed and extracted from indexed
707 articles, as shown in the funnel graphs of overall seroprevalence and seroprevalence in South
708 America (Figure 8; Figure 9). However, additional research has not been carried out into other
709 national and regional databases from countries without studies indexed in the main databases,
710 and this may be a limitation of the present study.

711 Among the variables evaluated, there is a need for further studies concerning
712 seroprevalence and risk factors for *N. caninum* in goats, exercising caution when assessing
713 latitude, as some studies attribute a reduction in seroprevalence to climatic conditions (Díaz et
714 al., 2016; Luo et al., 2016; Villagra-Blanco et al., 2017). Possibly, there is a combined effect of
715 risk factors such as the presence of dogs, sex of animals evaluated, management practices and
716 abortions, as well as particular climatic issues in higher latitudes.

In addition to the risk factors discussed in this meta-analysis, the high level of heterogeneity among all prevalence studies may be related to variables that have been associated with increased *N. caninum* seroprevalence in goats, such as the types of productive systems, grazing together with other ruminant species and the presence of wild animals, such as birds, rodents, canines and felines (Huang et al., 2004; Al-Majali et al., 2008; Santos et al., 2013; Gazzonis et al., 2016; Barros et al., 2018).

The extensive search for studies carried out in four databases of worldwide importance in order not to omit important studies on the topic, including only studies that describe the calculation of sample size in their methodology, in addition to investigating possible causes of heterogeneity through meta-regression and subgroup analysis (Crowther et al., 2010), allowing a better understanding of the variations between studies, were strengths of the present study. According to Crowther et al. (2010), comprehensive research generally improves the quality of a review and the inclusion of articles with similar study designs can reduce research bias. As a weakness, we can mention the lack of information on seroprevalence in countries with a large population of goats. We emphasise that searches were not carried out in regional databases of countries with no prevalence or in the grey literature.

2.5. Conclusion

N. caninum seroprevalence in goats is widely distributed throughout the world, in which the American continent presents a higher proportion of seropositive goats. Seropositive goats showed an association with abortion, presence of dogs and sex. Studies concerning seroprevalence and risk factors for caprine neosporosis were reported in a few articles, also missing articles from countries of the world's largest caprine population, both into the main database researched. The largest number of indexed studies were reported from South America, specifically Brazil, which allowed the estimated seroprevalence in that country to be more accurate.

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Declarations of interest

None.

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2.6. List of Figures

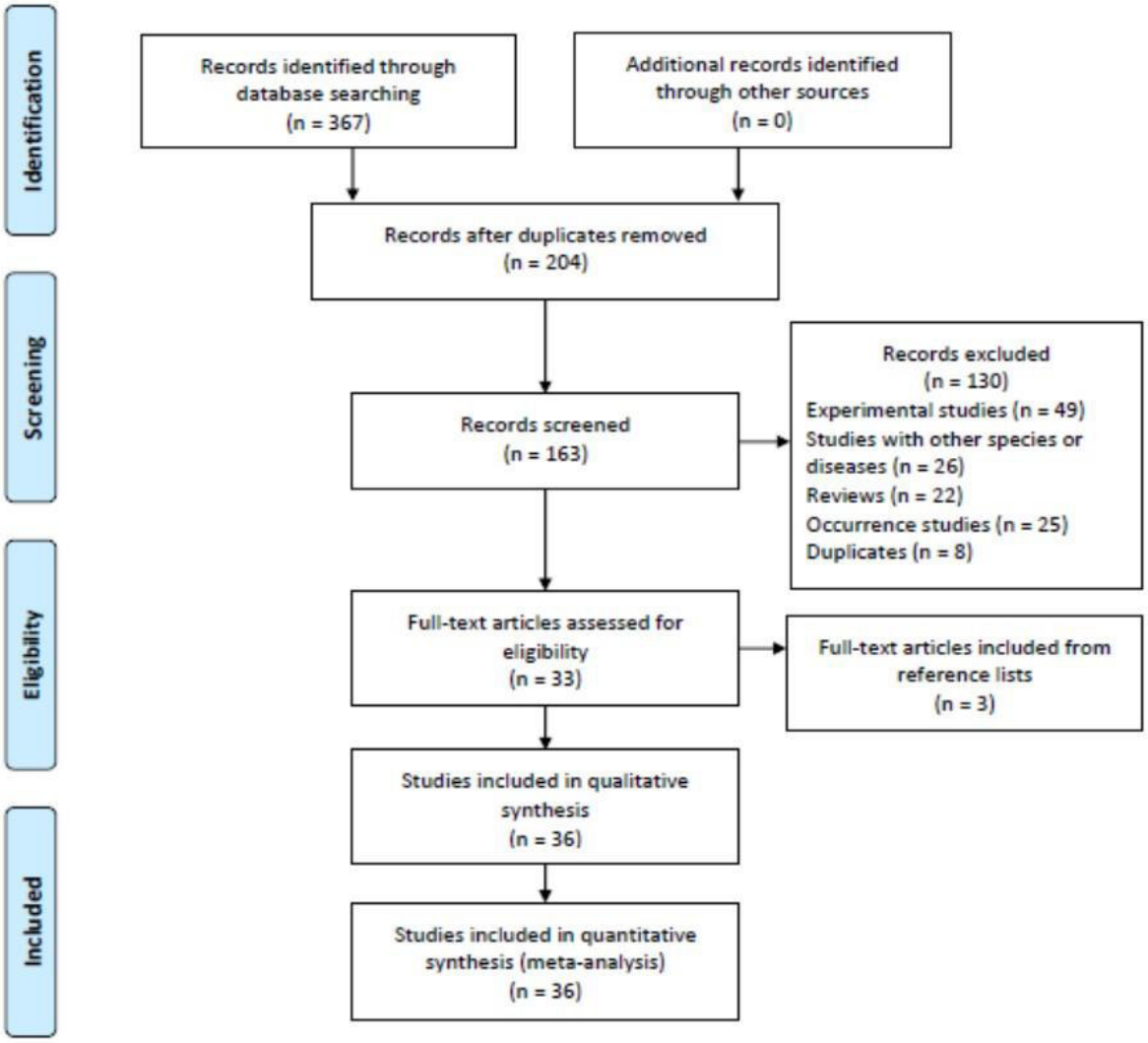


Figure 1. PRISMA flowchart search strategy to select studies concerning *N. caninum* seroprevalence in goats.

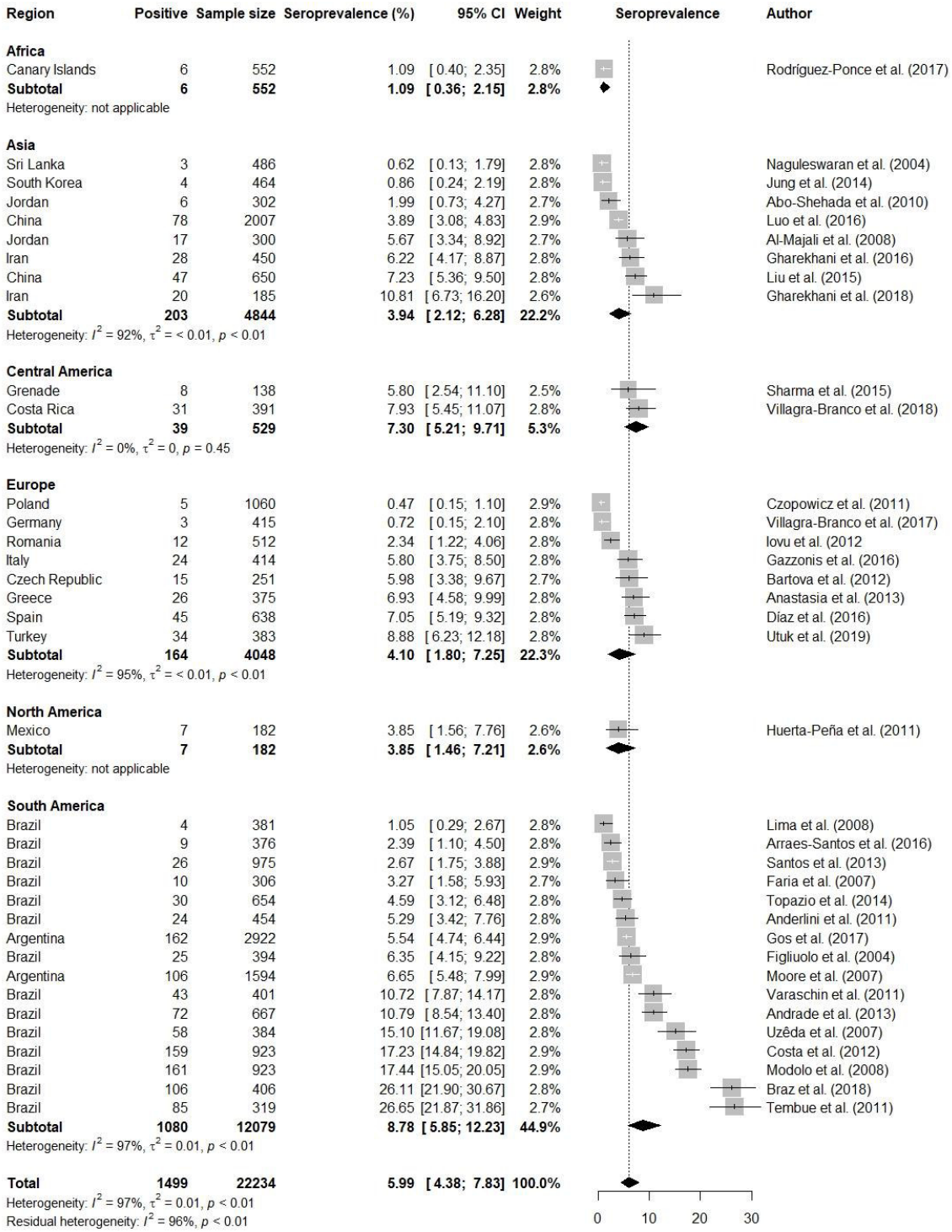
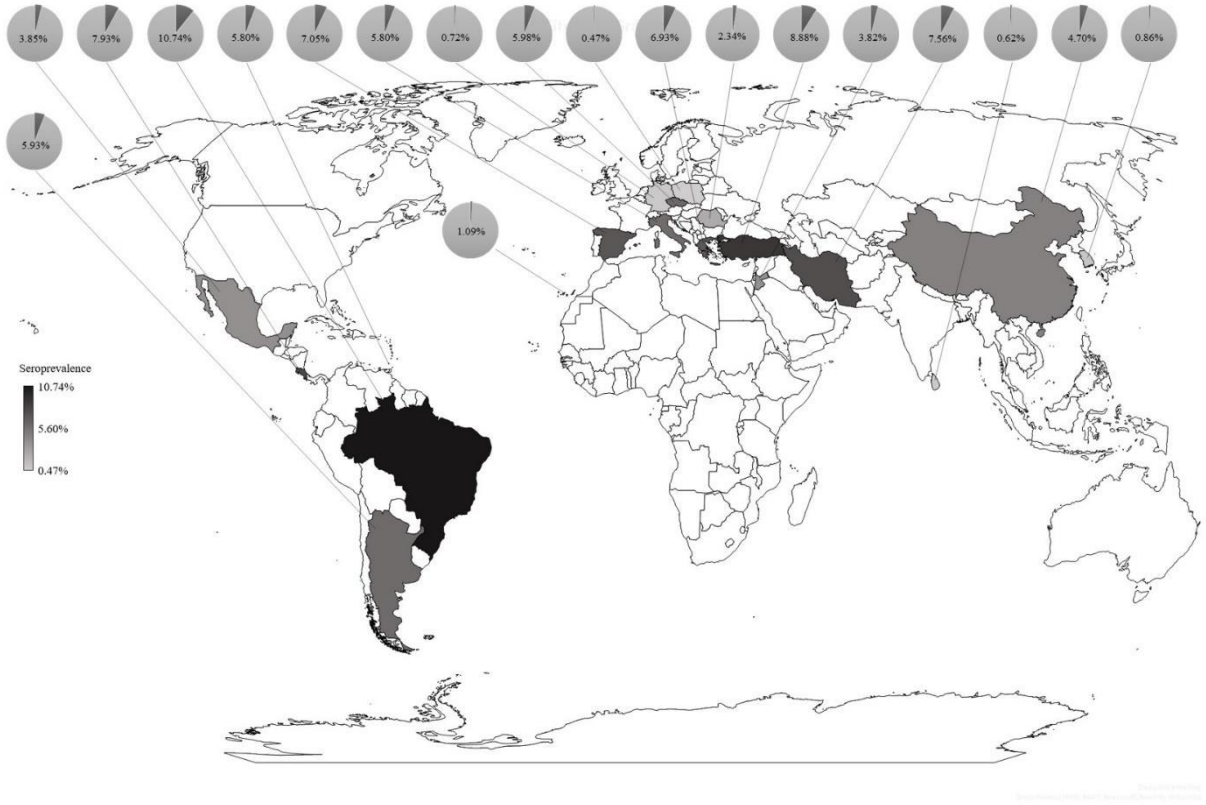


Figure 2. Forest plot comparison of *N. caninum* seropositivity in goats from 36 studies. The black dot point is the estimate and the horizontal line is the 95% CL for seroprevalence plotted for each study. Each column shows the discriminated studies according to the region/country; number of seropositive animals; sample size; seroprevalence %; 95% CL; study weight in the overall meta-analysis; seroprevalence in forest plot; authors. The black diamond at the bottom of each continent is the estimated average *N. caninum* seroprevalence in goats.



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981 Figure 3. World map showing the *N. caninum* seroprevalence in goats from the various
982 countries. The pie graphs show the seroprevalence rates for *N. caninum* estimated according to
983 the articles published in the indexing databases from each country.

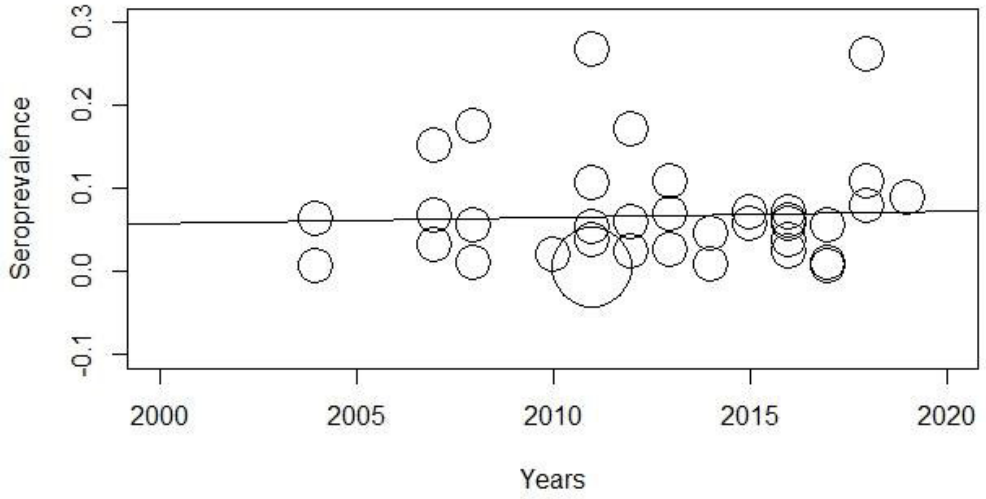


Figure 4. Meta-regression plot of study publication year against *N. caninum* seroprevalence in goats (percentage of seropositivity on the y-axis) (n = 36 studies, p = 0.2041). The circles represent the individual studies. The continuous line represents the regression line. The year of publication is plotted on the horizontal axis. The prevalence of *N. caninum* in plotted numbers is plotted on the vertical axis.

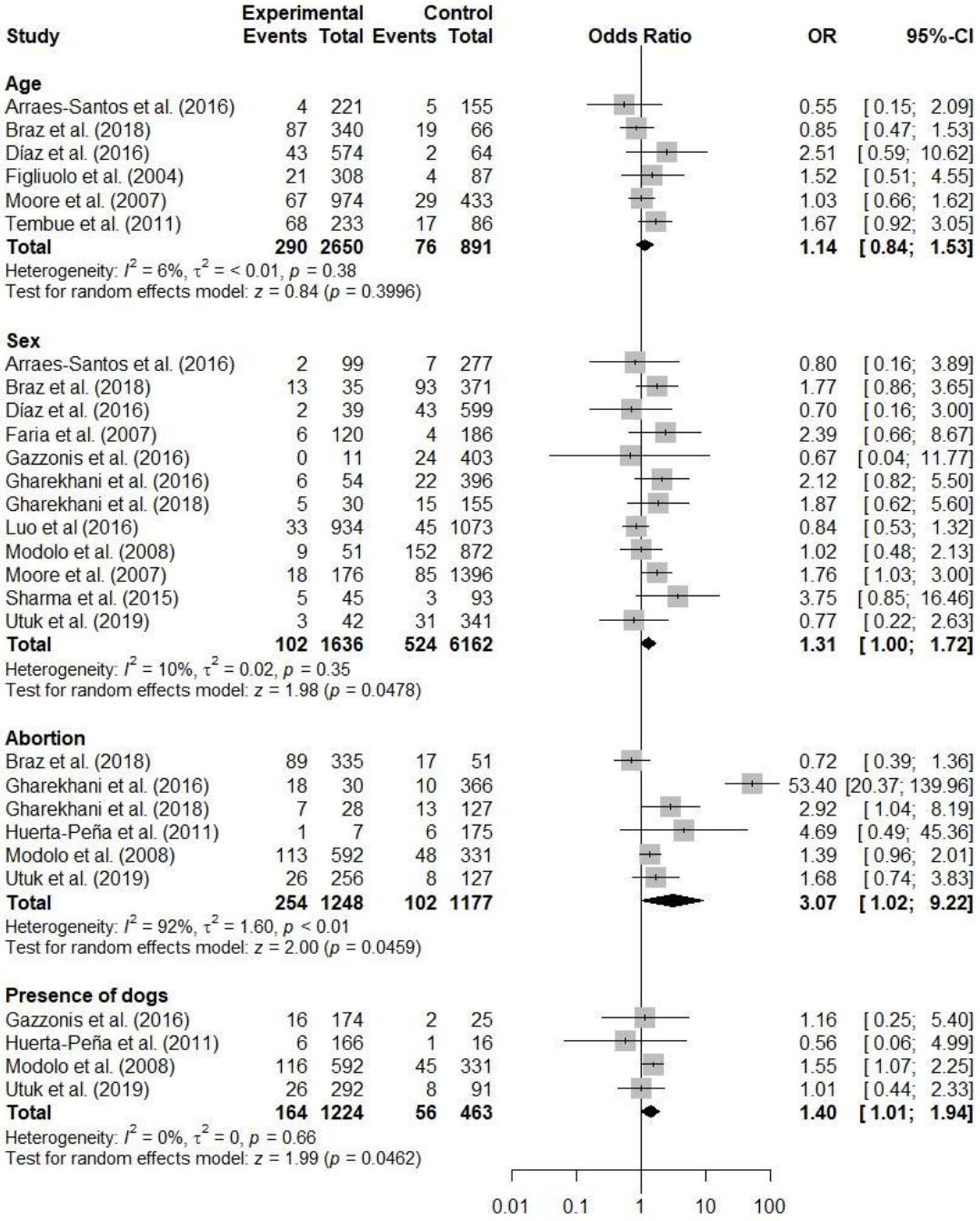
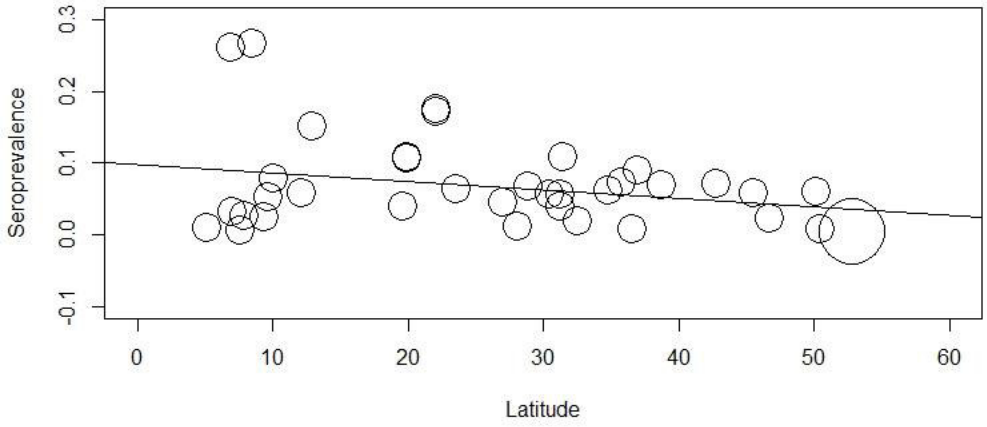


Figure 5. Forest plot comparison of *N. caninum* seroprevalence in goats from 28 studies. The black dot point is the estimate and the horizontal line is the 95% CL for seroprevalence plotted from each study. Each column shows the discriminated studies according to the authors/risk factors: age (adult vs young, respectively ≤ 1 year and > 1 year); sex (male vs female); abortion history (yes vs no), presence of dogs (yes vs no); experimental control with number of seropositive animals and total of animals from each risk factor divided into the risk factor parameters; odds ratio; OR – study weight in the overall meta-analysis; 95% CL. The black diamond at the bottom of each risk factor is the estimated average *N. caninum* seroprevalence.



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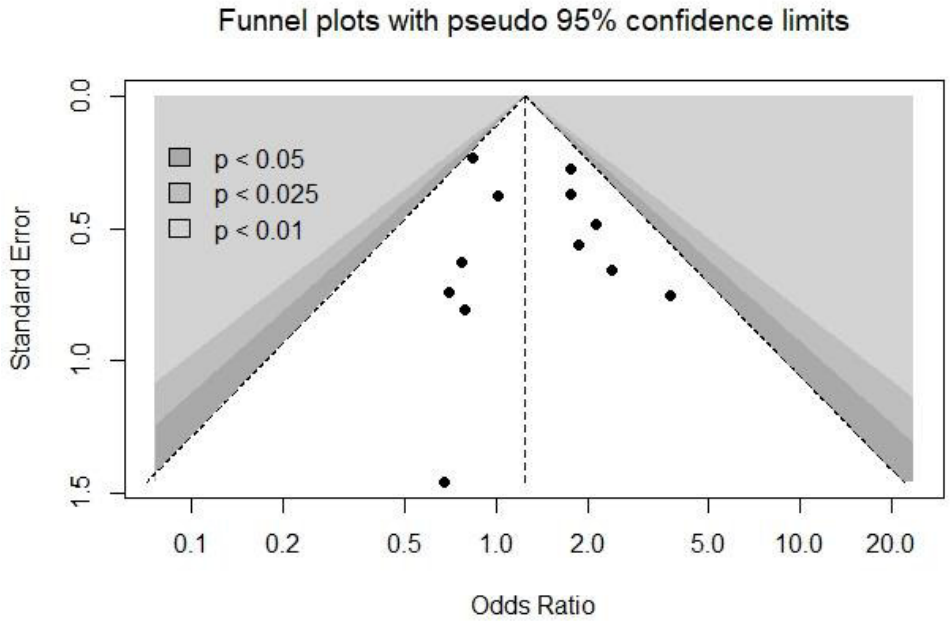
1000 Figure 6. Meta-regression plot of latitude of the studied region against the *N. caninum*
1001 seroprevalence in goats (percentage of seropositivity on the y-axis) (n = 36 studies, p = 0.0032).
1002 The circles represent the individual studies. The continuous line represents the regression line.
1003 The latitude is plotted on the horizontal axis. The prevalence of *N. caninum* in plotted numbers
1004 is plotted on the vertical axis.



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1006 Figure 7. Funnel plot measuring the odds ratio of *N. caninum* seroprevalence in goats with 95%
1007 CL using the Freeman–Tukey double arcsine method to stabilise the variance differences on
1008 the x-axis, standard error on the y-axis from the 12 studies that classified the sex as a risk factor
1009 for *N. caninum* seroprevalence in goats. Individual studies are represented by black dots. The
1010 dashed external lines indicate the triangular region where 95% CL of the studies are expected,
1011 and the central vertical line is the axis of the general effect.

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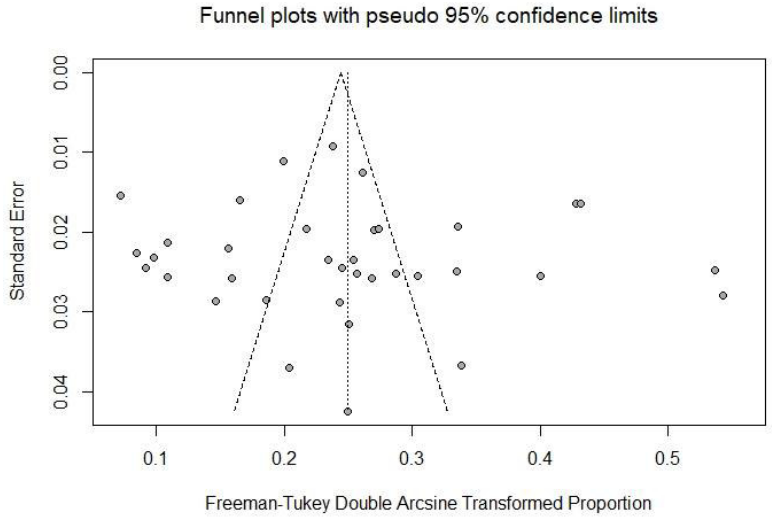

Figure 8. Funnel plot measuring the odds ratio of *N. caninum* seroprevalence in goats with 95% CL which was transformed using the Freeman–Tukey double arcsine method to stabilise the variance differences on the x-axis, standard error on the y-axis of the animals (in relation to the total sample) from 36 studies of *N. caninum* seroprevalence in goats. Individual studies are represented by black dots. The dashed external lines indicate the triangular region where 95% CL of the studies are expected, and the central vertical line is the axis of the general effect. All values were $p > 0.05$ (Egger's test), indicating that there is no evidence of significant publication bias.

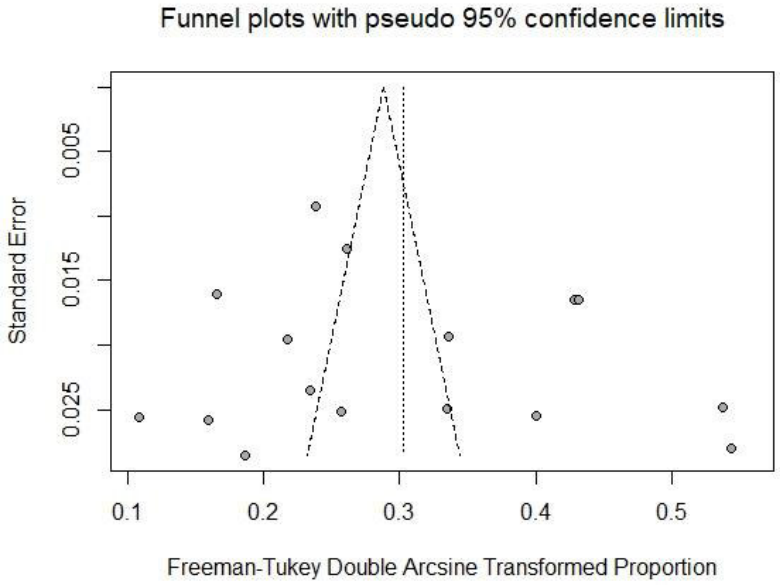

Figure 9. Funnel plot measuring the odds ratio of *N. caninum* seroprevalence in goats with 95% CL which was transformed using the Freeman–Tukey double arcsine method to stabilise the variance differences on the x-axis, standard error on the y-axis from 16 studies of *N. caninum* seroprevalence in goats in South America. Individual studies are represented by black dots. The dashed external lines indicate the triangular region where 95% CL of the studies are expected, and the central vertical line is the axis of the general effect. All values were $p > 0.05$ (Egger's test), indicating that there is no evidence of significant publication bias.

2.7. List of Tables

Table 1. Summary of extracted data from all included studies.

Author	Study type	Region	Country	Sample size	Positive	Seroprevalence	Diagnostic test	Cut-off	Risk factors
Rodríguez-Ponce et al. (2017)	Cross-sectional	Africa	Canary Islands	552	6	1.09%	ELISA		
Naguleswaran et al. (2004)	Cross-sectional	Asia	Sri Lanka	486	3	0.62%	ELISA		
Jung et al. (2014)	Cross-sectional	Asia	South Korea	464	4	0.86%	ELISA		
Abo-Shehada et al. (2010)	Cross-sectional	Asia	Jordan	302	6	1.99%	ELISA		
Luo et al. (2016)	Cross-sectional	Asia	China	2007	78	3.89%	ELISA		Sex
Al-Majali et al. (2008)	Cross-sectional	Asia	Jordan	300	17	5.67%	ELISA		
Gharekhani et al. (2016)	Cross-sectional	Asia	Iran	450	28	6.22%	ELISA		Abortion, sex
Liu et al. (2015)	Cross-sectional	Asia	China	650	47	7.23%	ELISA		
Gharekhani et al. (2018)	Cross-sectional	Asia	Iran	185	20	10.81%	ELISA		Abortion, sex
Sharma et al. (2015)	Cross-sectional	Central America	Grenade	138	8	5.80%	ELISA		Sex
Villagra-Branco et al. (2018)	Cross-sectional	Central America	Costa Rica	391	31	7.93%	ELISA		
Czopowicz et al. (2011)	Cross-sectional	Europe	Poland	1060	5	0.47%	ELISA		
Villagra-Branco et al. (2017)	Cross-sectional	Europe	Germany	415	3	0.72%	ELISA		
Iovu et al. (2012	Cross-sectional	Europe	Romania	512	12	2.34%	ELISA		
Gazzonis et al. (2016)	Cross-sectional	Europe	Italy	414	24	5.80%	ELISA		Presence of dogs, sex
Bartova et al. (2012)	Cross-sectional	Europe	Czech Republic	251	15	5.98%	ELISA		
Anastasia et al. (2013)	Cross-sectional	Europe	Greece	375	26	6.93%	ELISA		
Díaz et al. (2016)	Cross-sectional	Europe	Spain	638	45	7.05%	ELISA		Age, sex
Utuk et al. (2019)	Cross-sectional	Europe	Turkey	383	34	8.88%	ELISA		Abortion, presence of dogs, sex
Huerta-Peña et al. (2011)	Cross-sectional	North America	Mexico	182	7	3.85%	ELISA		Abortion, presence of dogs
Lima et al. (2008)	Cross-sectional	South America	Brazil	381	4	1.05%	IFAT	1:50	

Arraes-Santos et al. (2016)	Cross-sectional	South America	Brazil	376	9	2.39%	IFAT	1:50	Age, sex
Santos et al. (2013)	Cross-sectional	South America	Brazil	975	26	2.67%	IFAT	1:50	
Faria et al. (2007)	Cross-sectional	South America	Brazil	306	10	3.27%	IFAT	1:50	Sex
Topazio et al. (2014)	Cross-sectional	South America	Brazil	654	30	4.59%	IFAT	1:50	
Anderlini et al. (2011)	Cross-sectional	South America	Brazil	454	24	5.29%	IFAT	1:50	
Gos et al. (2017)	Cross-sectional	South America	Argentina	2922	162	5.54%	IFAT	1:100	
Figliuolo et al. (2004)	Cross-sectional	South America	Brazil	394	25	6.35%	IFAT	1:50	Age
Moore et al. (2007)	Cross-sectional	South America	Argentina	1594	106	6.65%	IFAT	1:50	Age, sex
Varaschin et al. (2011)	Cross-sectional	South America	Brazil	401	43	10.72%	IFAT	1:50	
Andrade et al. (2013)	Cross-sectional	South America	Brazil	667	72	10.79%	IFAT	1:50	
Uzêda et al. (2007)	Cross-sectional	South America	Brazil	384	58	15.10%	IFAT	1:100	
Costa et al. (2012)	Cross-sectional	South America	Brazil	923	159	17.23%	NAT	1:25	
Modolo et al. (2008)	Cross-sectional	South America	Brazil	923	161	17.44%	NAT	1:25	Abortion, presence of dogs, sex
Braz et al. (2018)	Cross-sectional	South America	Brazil	406	106	26.11%	IFAT	1:50	Abortion, age, sex
Tembue et al. (2011)	Cross-sectional	South America	Brazil	319	85	26.65%	IFAT	1:50	Age

1033

3. CAPÍTULO 3 – A SYSTEMATIC LITERATURE REVIEW AND META-ANALYSIS OF TOXOPLASMA GONDII SEROPREVALENCE IN GOATS PRODUCTION

A systematic literature review and meta-analysis of *Toxoplasma gondii* seroprevalence in goats production

Abstract

Seroprevalence of *T. gondii* in goats worldwide were estimated through meta-analysis which aimed to evaluate the main risk factors in the herds that were associated to parasite infection. Seven electronic databases were searched: EMBASE, PubMed, SciELO, ScienceDirect, Scopus, VHL Regional Portal and Web of Science. Our search resulted in a total of 2,032 articles, and 75 reports published from 2000 to 2020 were selected. We used a random effects model to calculate pooled seroprevalence estimates with 95% confidence intervals (CI). We also conducted subgroup and meta-regression analyses to evaluate the effects of geographical and year of publication on pooled seroprevalence rates. A total of 55,317 goats were evaluated, of which 14,480 were seropositive for *T. gondii*. The pooled general seroprevalence of goats infected with *T. gondii* was 27.49% (95% CI 24.15 - 30.95; I^2 = 99%), with the lowest seroprevalence in Asia (20.74%; 95% CI 16.45 - 25.39) and highest seroprevalence in Central America (62.15%; 95% CI 57.28 - 66.90) and Europe (31.53%; 95% CI 21.71 - 42.26). The seropositivity rates in Africa and South America regions were (29.41%; 95% CI 19.11 - 40.89) and (29.76%; 95% CI 25.84 - 33.83), respectively. Our results suggest that the increase in *T. gondii* seroprevalence in goats is associated with the presence of cats (OR 2.22; 95% CI 1.30 - 3.82), goats greater than one year of age (OR 1.77; 95% CI 1, 37 - 2.29), females (OR 1.43; 95% CI 1.23 - 1.65), rearing system (extensive vs. intensive) (OR 4.82; 95% CI 1.96 - 11, 84) and rearing system (semi-intensive vs. intensive) (OR 1.48; 95% CI 1.48 - 6.13). Global seroprevalence, seroprevalence on the Asian continent and seroprevalence on the European continent have been associated with geographical longitude. Our results suggest that toxoplasmosis is prevalent in goats worldwide. Therefore, it is necessary to monitor the seroprevalence of *T. gondii* in goats and the intake of raw or undercooked meat and unpasteurized milk must be avoided to reduce human exposure to *T. gondii*.

Keywords: anti-Toxoplasma antibodies, caprine, epidemiological studies, meta-analysis, toxoplasmosis

3.1. Introduction

Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, obligate intracellular protozoan of the Phylum Apicomplexa (Dubey, 2010). Toxoplasmosis in livestock, most markedly in sheep and goats, is responsible for significant economic loss associated with abortion, stillbirth and neonatal losses (Dubey et al., 2020; Moreno et al., 2012; Unzaga et al., 2014).

In humans, toxoplasmosis tends to be asymptomatic or manifest with symptoms common to other pathologies, however, in some situations, the infection can become quite severe, especially in patients whose immunity has been reduced by malignant diseases and anti-tumor therapy or by immunodeficiency acquired (AIDS) (Dubey, 2010).

Felids are considered definitive hosts of *T. gondii*, while birds and several species of mammals, such as goats, sheep, pigs and humans, are intermediate hosts (Dubey, 2010). Infection in humans and animals occurs through ingestion of water or food contaminated with sporulated oocysts or by consumption of raw or undercooked meat containing tissue cysts (Dubey, 2010).

The occurrence of toxoplasmosis in goats has been reported in several countries (Fortes et al., 2018; Jiménez-Martín et al., 2020; Luo et al., 2016; Martínez-Rodriguez et al., 2020; Pagmadulam et al., 2020; Rodríguez-Ponce et al., 2017), and the main risk factors associated with the disease were age, the presence of cats and the extensive production system (Ahmad et al., 2015; Bachan et al., 2018; Fortes et al., 2018; Gazzonis et al., 2015; Rêgo et al., 2016). The products (meat and milk) and by-products of goats are consumed in various parts of the world and unpasteurized meat and milk from these animals can be a way of transmitting *T. gondii* to humans (Guo et al., 2016; Tonouhewa et al., 2017).

Cross-sectional studies are often used to estimate seroprevalence and associations with risk factors. Using an adequate sample size provides more reliable and statistically valid results (Hajian-Tilaki, 2011; Suresh and Chandrashekara, 2012), because it increases the power of the study, that is, the ability of a test to correctly reject the null hypothesis (Verma and Verma, 2020). However, many studies that estimate *T. gondii* seroprevalence in goats do not calculate the minimum sample size, resulting in smaller sample sizes, less reliable estimates, and lower study power (Verma and Verma, 2020).

Considering the economic and public health importance of toxoplasmosis, this systematic review and meta-analysis aimed to estimate the worldwide seroprevalence of *T. gondii* in goats and to assess potential risk factors.

3.2. Material and methods

3.2.1. Search strategy and selection criteria

Preferred Report Items for Systematic Reviews and Meta-analyses (PRISMA) determined the steps of article selection to structure the systematic review (Moher et al., 2016; Page et al., 2021). The largest number of scientific articles reporting seroprevalence of *T. gondii* in goats were identified by a complete systematic review. Seven electronic databases were consulted to recruit the articles: EMBASE, PubMed, SciELO, ScienceDirect, Scopus, VHL Regional Portal and Web of Science to

The articles were searched using the keywords, index items or words in the title included the following terms in combination: ((*Toxoplasma gondii* OR toxoplasmosis) AND (goat OR caprine) AND (occurrence OR prevalence OR seroprevalence OR infection)); and ((*Toxoplasma gondii* OR toxoplasmosis) AND (goat OR caprine) AND (occurrence OR prevalence OR seroprevalence OR infection) AND (risk factors OR risk factor)). Articles were restricted to year of publication between 2000 and October 2020. Articles identified as duplicates, i.e., present in more than one database, were removed.

The research results were imported into the free web-tool Rayyan for systematic reviews (Ouzzani et al., 2016). The inclusion criteria were: observational or cross-sectional articles that reported seroprevalence of *T. gondii* in goats; observational or cross-sectional articles that associated seroprevalence of *T. gondii* in goats with risk factors; articles published in English, Portuguese or Spanish. Articles were excluded when not meet these prerequisites. Also excluded were: review studies; studies with other species; studies with other diseases; theses and dissertations. Two reviewers independently screened the articles using these criteria, and when there was disagreement in the choice of articles, a third reviewer was consulted to decide about the inclusion or exclusion of the article.

Only articles that calculated the minimum sample size to define seroprevalence were used in the statistical analysis of the meta-analysis of seroprevalence and risk factors (Rodrigues et al., 2020).

3.2.2. Data extraction

Initially, the articles were selected by means of the title and abstract. Articles identified as duplicates, i.e., present in more than one database, were removed. The eligible articles were collected, and the following information extracted: author; year of publication; type of study; continent; country; sample size; number of positive samples; diagnostic test; geographical location (altitude, latitude and longitude); and possible risk factors related to animals; sex; age (> 1 or <1 year;> 1 or ≤1 year; ≥1 or <1 year); rearing system; presence of other species; breed; and presence of cats. The data were organized using Microsoft Excel® spreadsheets.

Articles that presented results of the analysis of risk factors only in the form of odds ratio (OR) presented incomplete information, therefore, they were not included in the risk factor meta-analysis and included only in the general meta-analysis of seroprevalence. In addition, articles that assessed risk factors at the herd level were excluded.

Finally, the articles included in the meta-analysis of risk factors were standardized to allow a comparative assessment between the articles considering: age (adult vs. young) - adult (> 1 year; ≥1 year) and young (<1 year; ≤ 1 year); sex (male vs. female); rearing system (extensive vs. intensive; semi-intensive vs. extensive; semi-intensive vs. extensive); presence of other species (yes vs. no); breed (dairy vs. meat); and presence of cats (yes vs. no).

The production systems were characterized as follows: extensive system: low stocking rate; animals spend most of their time on natural pastures; absence of supplementation; absence of periodic sanitary management; low production rates; semi-intensive system: stocking rate higher than extensive system; improved or cultivated pastures; use of moderate amounts of concentrates, especially during the dry period of the year; little or no periodic sanitary management; low production rates; intensive system: animals are fed in confinement; it involves a large initial investment of money; control in the quality and quantity of the feed offered; greater sanitary management; increased growth performance, feed efficiency and

reproductive potential (Budai et al., 2013; Costa et al., 2008; Karthik et al., 2021; Kochewad et al., 2017).

3.2.3. Quality assessment

The quality of each study was assessed based on previously published criteria (Li et al., 2020; Liu et al., 2009; Speich et al., 2016). These criteria were derived from the Grading of Recommendations Assessment, Development and Evaluation method (Atkins et al., 2004). Each study was classified by sample size, the method of diagnosis of *T. gondii* exposure or infection and whether it contained information about the risk factors addressed in the present study (age, sex, rearing system, presence of other species, breed and presence of cats) (Table 2).

Publications with a total score of 6 - 5 points were considered of high quality, 4 - 3 points were of moderate quality, while 2 - 0 points were considered of low quality. No publication was excluded based on the result of the quality assessment (Table 2).

3.2.4. Statistical analysis

The meta-analysis was performed using the R program, version 3.5.2 with RStudio (R Core Team, 2018), using the "meta" package (Schwarzer, 2007; Schwarzer et al., 2015). The random effects model was used for the meta-analysis (DerSimonian and Laird, 2015). The pooled seroprevalence estimate of *T. gondii* (at 95 % confidence intervals) was presented as a percentage ((number of seropositive animals / total of animals tested)*100) and the Freeman–Tukey double arcsine method was used to stabilise the variance (Freeman and Tukey, 1950).

The risk factors for the variables studied were presented as odds ratios (OR) with a 95% confidence interval. The "metaprop" command was used to estimate seroprevalence and the "metabin" command was used to estimate the association between seroprevalence and possible risk factors: age, sex, rearing system, presence of other species, breed and presence of cats.

The Baujat chart was used to detect sources of heterogeneity (Baujat et al., 2002). The horizontal axis represents the contribution of each study to the general statistics of the Cochran Q test for heterogeneity, and the vertical axis represents the influence of each study. The most heterogeneous studies appear in the upper right area of the graph.

The heterogeneity of the results was analyzed using a Q test based on χ^2 and the I^2 statistic to assess the real variation due to heterogeneity (Borenstein et al., 2017; Cochran, 1954; Higgins et al., 2003). I^2 shows the proportion of the variance, ranging from 0% to 100%, and observes the effect of the real size of all studies in the analysis (Borenstein et al., 2017).

Subgroup meta-analysis and meta-regression were used to assess possible sources of heterogeneity. Subgroup analysis was done by continent: Africa, Central America, North America, South America, Asia and Europe. The raw prevalence data were used in the meta-regression and the results were presented in decimal numbers. Meta-regression using the DerSimonian and Laird model was performed to determine whether the year of publication, altitude, longitude and latitude influenced the seroprevalence of *T. gondii* in goats using the bubble graph of the "metareg" command.

Potential publication bias was assessed by visually inspecting funnel charts and objectively using the Egger test. The publication bias in the analysis of subgroups and risk factors with at least ten articles was also assessed (Sterne et al., 2011). The "trim-and-fill" method was used to estimate the number of studies potentially missing due to publication bias (Shi et al., 2019). In all analyzes, a value of $p < 0.05$ was considered statistically significant.

3.3. Results

3.3.1. Results of the systematic review

Using the selection criteria described, a total of 2,032 articles were identified. After excluding duplicate publications, 1,277 articles remained. Articles that did not meet the inclusion criteria as previously described, were excluded. These criteria were articles that evaluated the seroprevalence of *T. gondii* in goats; articles that associated the seroprevalence of *T. gondii* in goats with risk factors; articles published in English, Portuguese or Spanish; and

articles that calculated the minimum sample size. Finally, 75 were included in the statistical analysis (Figure 10).

The serological tests used in studies included in the meta-analysis, involved seven tests, including commercial kits and home-made protocols of Enzyme Linked Immunosorbent Assay (ELISA, n = 30) (Supplementary material 1), Indirect Hemagglutination Test (IHA, n = 6), Direct Agglutination Test (DAT, n = 3), Modified Agglutination (MAT, n = 5), Indirect Agglutination Test (IAT, n = 1), Indirect Immunofluorescence Test (IFAT, n = 25), and Latex Agglutination Test (LAT, n = 5) (Supplementary material 2) (Table 3).

According to the quality criteria, seven articles were of high quality, 49 articles were considered of moderate quality and the remaining 19 studies were of low quality (Table 3). The types of sampling reported in each study are also in this table.

3.3.2. Seroprevalence

Of the 75 articles, a total of 55,317 goats from 32 countries and five continents were evaluated between the years 2000 to 2020 for exposure to *T. gondii* (Figure 11, Figure 12). Based on the meta-analysis of the random effects model, we estimated that the global seroprevalence of *T. gondii* in goats was 27.49% (95% CI 24.15 - 30.95), with wide heterogeneity in seroprevalence between studies ($I^2 = 99\%$) (Figure 11). The Baujat plot shows the study by Luo et al. (2016) appearing in the upper right area, suggesting a considerable influence and contribution to the general heterogeneity (Figure 13A). Herd seroprevalence was reported in 30 of the 75 papers analyzed, with a total of 75.37% (1322/1754; 95% CI 73.28 - 77.37) of seropositive herds (Table 3).

In the subgroup analysis, there was wide heterogeneity between studies in all subgroups. The highest seroprevalence was estimated in Central America (62.15%; 95% CI 57.28 - 66.90), followed by Europe (31.53%; 95% CI 21.71 - 42.26), South America (29.76%; 95% CI 25.84 - 33.83), Africa (29.41%; 95% CI 19.11 - 40.89) and Asia (20.74%; 95% CI 16.45 - 25.59) (Figure 11, Figure 12). The Baujat plot for each continent is shown in the figures (Figure 13).

3.3.3. Risk factors

Our results showed that the higher *T. gondii* seroprevalence in goats is associated with the presence of cats (OR 2.22; 95% CI 1.30 - 3.82), age greater than one year (OR 1.77; 95% CI 1, 37 - 2.29), females (OR 1.43; 95% CI 1.23 - 1.65), rearing system (extensive vs. intensive) (OR 4.82; 95% CI 1.96 - 11, 84) and rearing system (semi-intensive vs. intensive) (OR 1.48; 95% CI 1.48 - 6.13) (Figure 14, Figure 15, Figure 16, Figure 17A, Figure 17B). There was no significant association for rearing system semi-intensive vs. extensive, breed and presence of other species (Figure 17C, Figure 18A, Figure 18B).

3.3.4. Publication bias

The results of the Egger test indicated that the publication bias was statistically significant for global seroprevalence ($p = 0.0334$) and seroprevalence in the European continent ($p = 0.0410$). A sensitivity analysis using the "trim-and-fill" method was performed and the adjustment resulted in a funnel plot without publication bias for global seroprevalence ($p = 0.6102$) and European continent seroprevalence ($p = 0.6644$) (Figure 19A, Figure 19D).

The Egger test was not significant, and the funnel plot did not show asymmetry for seroprevalence in the African continent ($p = 0.8167$), Asian ($p = 0.090$), South American ($p = 0.3193$) and for the possible risk factors for age ($p = 0.5132$), rearing system (semi-intensive vs. extensive) ($p = 0.3996$), presence of cats ($p = 0.4888$) and sex ($p = 0, 2863$) (Figure 19B, Figure 19C, Figure 19E, Figure 20A, Figure 20B, Figure 20C, Figure 20D).

In the meta-regression analysis, the global seroprevalence ($p = 0.0167$; $r^2 = 2.29\%$), the seroprevalence of the Asian continent ($p = 0.0010$; $r^2 = 37.96\%$) and the seroprevalence in the European continent ($p = 0.0074$; $r^2 = 33.57\%$) were associated with geographical longitude (Figure 21A, Figure 21B, Figure 21C). On the other hand, there was no significant association between longitude and seroprevalence in Africa ($p = 0.4779$; $r^2 = 9.26\%$) and in South America ($p = 0.8700$). Latitude, year of publication and altitude were not statistically associated with seroprevalence in the meta-regression analysis.

3.4. Discussion

3.4.1. Seroprevalence

This is the first meta-analysis study that estimates the global seroprevalence of *T. gondii* in goats and provides concise data for the adoption of control measures and decision-making in unique health on toxoplasmosis in goats worldwide.

Based on our results 14,480 goats out of a total of 55,317 were seropositive for *T. gondii*, resulting in an estimated global seroprevalence of 27.49% (95% CI 24.15 - 30.95) (Figure 11).

The highest rate of seropositive goats for *T. gondii* was estimated in Central America, in the Costa Rica region (62.15%; 95% CI 57.28 - 66.90) (Figure 11). However, it is noteworthy only one article was used in the estimate and, in addition, countries with a significant goat population, such as Haiti and Cuba (FAOSTAT, 2019), did not present studies indicating seroprevalence data. Therefore, it is not possible to state that this is the real seroprevalence of *T. gondii* in goats for this continent.

The seroprevalence of *T. gondii* in goats on the European continent was 31.53% (CI 95% 21.71 – 42.26). The meta-regression demonstrated an increasing linear trend of seroprevalence in countries of east ($p = 0.0074$) (Figure 21C), possibly influenced by the high seroprevalence of Greece, Romania and Serbia (Anastasia et al., 2013; Djokić et al., 2014; Iovu et al., 2012). In addition, the studies that evaluated the seropositivity of *T. gondii* in goats in Europe reported that adult goats (OR 21.04; 95% CI 5 – 88.1), river water source (OR 2.49; 95% CI 1.71 – 3.63), supplementation (OR 3.88; 95% CI 1.81 – 8.29), semi-intensive rearing system (OR 5.35; 95% CI 2.33 – 12.28), presence of cats (OR 1.80; 95% CI 1.49 – 2.16) and reproductive failure (OR 1.99; 95% CI 1.54 – 2.55) were important risk factors for the parasite (Gazzonis et al., 2015; Iovu et al., 2012; Jiménez-Martín et al., 2020; Tzanidakis et al., 2012). Therefore, control measures aimed at improving the water source, preventing access of cats between animals and monitoring reproductive failures in the herd may be a way to reduce *T. gondii* exposure to goats in Europe.

The estimated seroprevalence of *T. gondii* in goats in Africa was 29.41% (Figure 11). Although it's continent accounts for more than 50% of the world's goat population (FAOSTAT,

2019), few articles were found on the seroprevalence of toxoplasmosis in goats in Africa, using the databases described in this study. The Egger test did not identify publication bias for seroprevalence in Africa, and no association was observed in the meta-regression analysis (Figure 19B).

In the African continent, seroprevalence was influenced by studies carried out in South Africa, Benin, Ethiopia and Zimbabwe, in which *T. gondii* seropositivity in goats was primarily associated with adult goats, extensive rearing and favorable climatic conditions for sporulation and survival of oocysts (Dubey, 2010; Hove et al., 2005; Tagwireyi et al., 2019; Teshale et al., 2007; Tonouhewa et al., 2019). Studies indicate that ambient temperatures from 25°C to 35°C are optimal for the development of the sporogony process, a phase in which the oocysts become infective. On the other hand, temperatures above or below the aforementioned range reduce or make this process unfeasible (Dubey, 1998; Dubey et al., 1970). After the sporulation process, sporulated oocysts remain preserved and viable for long periods at low temperatures (Dubey, 1998; Lindsay et al., 2002).

In South America, seroprevalence was 29.76% (Figure 11). Reports of *T. gondii* seropositivity in goats ranged from 14.48% to 47.13%, and almost all studies were conducted in Brazil (Figure 11). Countries with a goat population greater than 1 million animals, like Bolivia, Peru and Venezuela, do not possess data on the seroprevalence of *T. gondii* in goats (FAOSTAT, 2019).

In this meta-analysis, it was identified that there are several reports of toxoplasmosis in goats in different Brazilian states, with a high rate of seropositive animals in the Brazil states: Paraná, Minas Gerais, Rio Grande do Norte and Piauí (Carneiro et al., 2009; Medeiros et al., 2014; Rêgo et al., 2016; Reis et al., 2007). The high seroprevalence of *T. gondii* in goats is often related to the animals' exposure to risk factors. In the case of Brazil, for example, previous studies have shown that the animal production system is related to seroprevalence in which the highest prevalence is when animals are reared in an extensive system (OR 9,17; CI 95% 1,94 – 56,61), presence of cats (OR 6,56; CI 95% 3,58 – 12,01) and adults caprines (OR 2,66; CI 95% 1,59 – 4,48) were the main factors that favor the exposure of goats to the parasite (Anderlini et al., 2011; Figliuolo et al., 2004; Fortes et al., 2018; Garcia et al., 2012; Modolo et al., 2008; Moura et al., 2016; Neto et al., 2008).

In Asia, the rate of seropositive goats was 20.74% (Figure 11). Most of the articles that evaluated the seroprevalence of *T. gondii* in goats on the Asian continent were carried out in China, Iran and Pakistan, while countries with a large goat population, like India, Bangladesh, Mongolia, Indonesia, Nepal, Myanmar and Turkey, presented indexed studies with limited seroprevalence data or absent (FAOSTAT, 2019).

We did not identify publication bias in the Egger test (p = 0.090), but there was a statistically significant reduction in seroprevalence with the increase in longitude in Asia (p = 0.0010) (Figure 21B). We observed that the high seroprevalence reported in some regions of Iran and Pakistan was associated with adult goats (OR 5.41; CI 95% 1.69 – 17.29), females (OR 1.85; CI 95% 1.06 – 3.23), presence of cats (OR 2.08; CI 95% 1.05 – 4.12), extensive rearing (OR 2.19; CI 95% 1.07 – 4.50) and mild and wet climate (Ahmad et al., 2015; Ahmed et al., 2016; Izadyar et al., 2019), factors that have historically been considered risky for *T. gondii*.

On the other hand, studies conducted in China and South Korea, Asian countries with low seroprevalence, were conducted in herds managed intensively or semi-intensively and in regions with lower annual average temperature, a condition that negatively influences the sporulation and survival of oocysts in the environment (Jung et al., 2014; Wang et al., 2011; Xu et al., 2014).

3.4.2. Risk factors

Our results demonstrated an association between age and risk of being seropositive, with goats greater than one year of age being at higher risk (OR 1.77; 95% CI 1, 37 - 2.29) (Figure 15). In fact, older goats had more time of exposure to sporulated oocysts in the environment throughout their lives (Bachan et al., 2018; Fortes et al., 2018; Jittapalapong et al., 2005; Tilahun et al., 2018; Zewdu et al., 2013).

There was an association between presence of cats and risk of being seropositive (OR 2.22; 95% CI 1.30 - 3.82) (Figure 14). Felines are important in the epidemiology of toxoplasmosis, because when infected with *T. gondii* they eliminate oocysts from the environment (Dubey, 2010). Other studies have also associated the presence of cats with a more

elevated rate of seropositive goats (Bawm et al., 2016; Fortes et al., 2018; Lúcio et al., 2016; Modolo et al., 2008; Neto et al., 2008).

Extensive (OR 4.82; 95% CI 1.96 - 11, 84) and semi-intensive (OR 1.48; 95% CI 1.48 - 6.13) rearing systems were associated with a more elevated rate of goats seropositive compared to intensive systems (Figure 17A, Figure 17B). The *T. gondii* oocysts disseminations in the environment, occurs by rain and snow, by vectors as flies, cockroaches and earthworms and by intermediate hosts (Afonso et al., 2008; Dubey, 2010). Dubey et al. (2010) and Iemmi et al. (2020) verified that wild and migratory birds have great potential for the spread of *T. gondii* oocysts in the environment, behaving like intermediate hosts of *T. gondii* or vectors when transporting intact oocysts that were ingested in the environment (Dubey, 2010; Dubey et al., 2020).

In addition, extensive systems are typically observed in regions with precarious socioeconomic conditions and are adopted by small family farms with inadequate hygienic standards, the presence of cats and, consequently, high dissemination of sporulated oocysts in the environment (Ahmad et al., 2015; Anderlini et al., 2011; Garcia et al., 2012; Gazzonis et al., 2015; Lúcio et al., 2016; Neto et al., 2008; Rêgo et al., 2016). On the other hand, when we evaluate semi-intensive vs. extensive systems there was no significant difference (OR 1.17; 95% CI 0.69 - 2.00) (Figure 17C). Probably because the semi-intensive and extensive systems have similar characteristics, that is, feeding based on natural or improved pastures, inadequate hygiene standards and low growth performance, feeding efficiency and reproductive potential, therefore, they do not differ statistically.

We observed greater seropositivity for *T. gondii* in females than in males (OR 1.43; 95% CI 1.23 - 1.65) (Figure 16). The higher seropositivity in females may be because females stay longer in the herd for reproduction and milk production, while males are slaughtered at an earlier age (Bachan et al., 2018; Fortes et al., 2018). In addition, hormonal factors and physiological status may also be responsible for the higher seroprevalence of females (Roberts et al., 2001).

We did not find an association between breed and *T. gondii* seroprevalence in goats (Figure 18A). Likewise, we found no association between the presence of other species (sheep, cattle, swine and poultry) and the seroprevalence of *T. gondii* in goats (Figure 18B). There was

definitely a limitation in our estimates due to the limited number of studies that investigated the breed and presence of other species as potential risk factors.

3.4.3. Heterogeneity and Limitations

Our results show a wide heterogeneity in seroprevalence among eligible articles, possibly caused by differences in sample size, geographic regions, climatic conditions, socioeconomic factors, ways of handling animals and the presence of potential risk factors. Another essential aspect that may have influenced heterogeneity is the diagnostic methods and the cutoff point selected in the studies to define a goat as seropositive. Opel et al., (1991), defined the titer of 1:64 as the cutoff point for IFAT in goats and is a reference study about it. Dubey and Towle (1986), reviewing serological surveys of 320 abstracts from the world literature, considered that titers below 1:16 in sheep were likely to be nonspecific reactions. Little information is available on the comparison of different serological tests in the detection of specific antibodies to *T. gondii* in gosts (Dubey, 2010), a fact that occurs to date. Therefore, it is still not possible to clearly define, through this review, the definition of cut-off points for each serological test, except IFAT (Supplementary material 2). These differences can lead to false negative or false positive results, may invalidate the serological estimates of the studies (Rostami et al., 2018; Zhang et al., 2016).

However, there are some limitations in the present study that we must highlight. For example, published information on seroprevalence of toxoplasmosis in goats was unavailable in many parts of the world. Subsequent searches were not carried out on regional databases to search for articles from countries with missing information. The articles included were carried out in different periods, and not all studies evaluated the risk factors discussed in the present study.

3.5. Conclusion

The present study showed that *T. gondii* seroprevalence in goats was almost one-third of the world goat population and understanding these data can support epidemiological authorities develop strategies to prevent toxoplasmosis in goats.

Elevated rates of seropositive goats have been reported in countries where cats are present between the herd and the semi-intensive or extensive rearing system. There is significant heterogeneity between studies, and more studies are needed to elucidate this result.

Age, sex, presence of cats and rearing system are important risk factors for toxoplasmosis in goats and must be considered in the control of this parasite.

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Declarations of interest

None.

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3.6. List of Figures

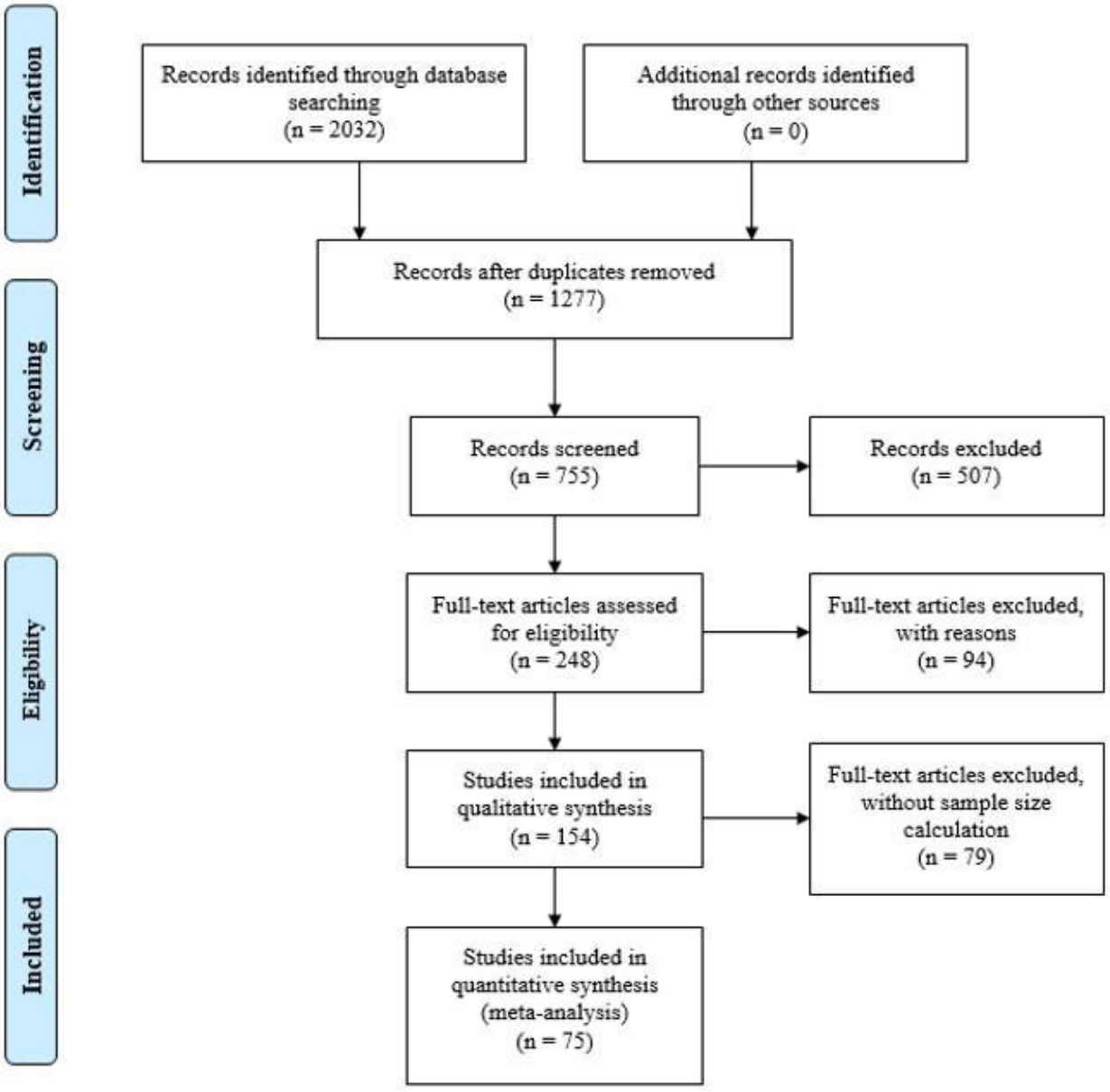


Figure 10. PRISMA flowchart describing included/excluded studies on the global seroprevalence of *T. gondii* in goats (Moher et al., 2009).

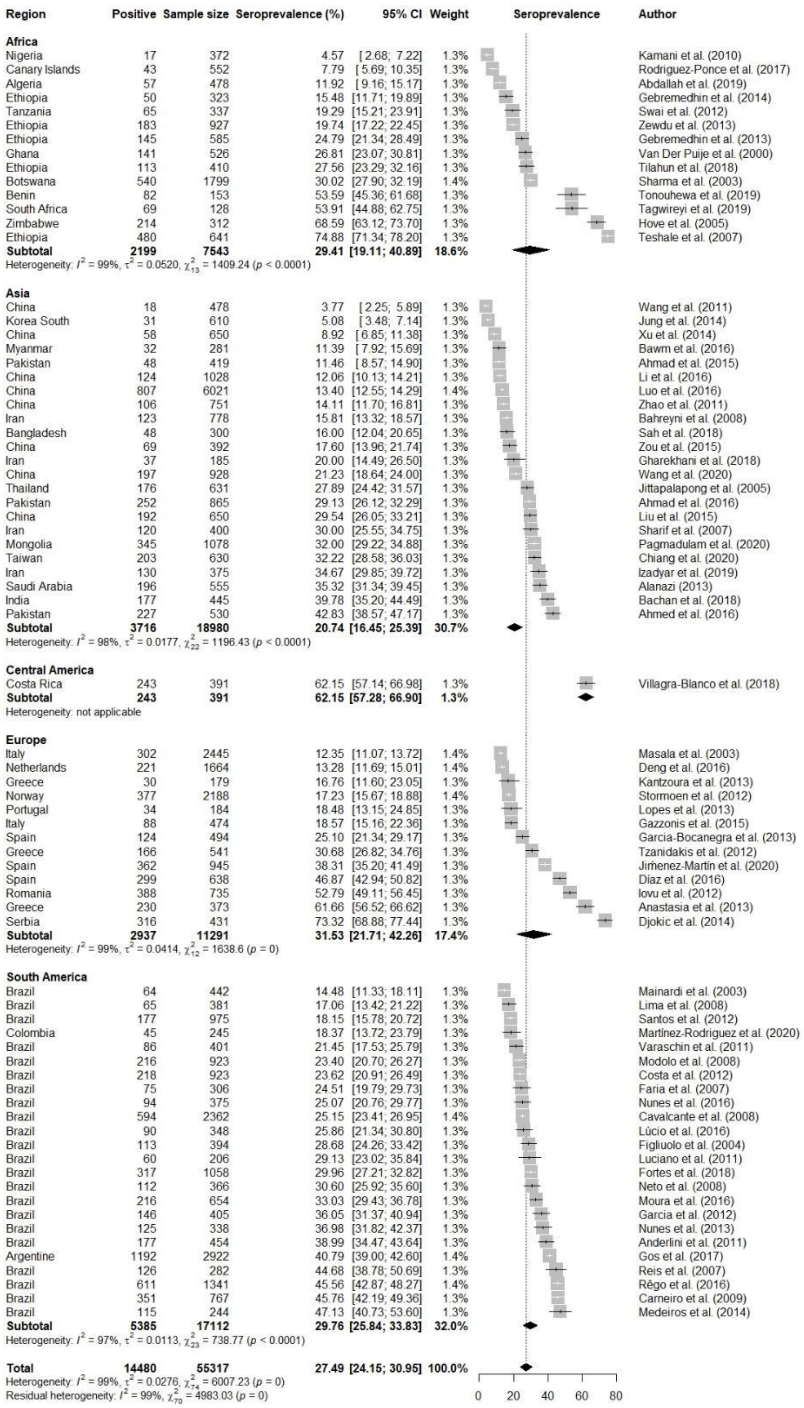
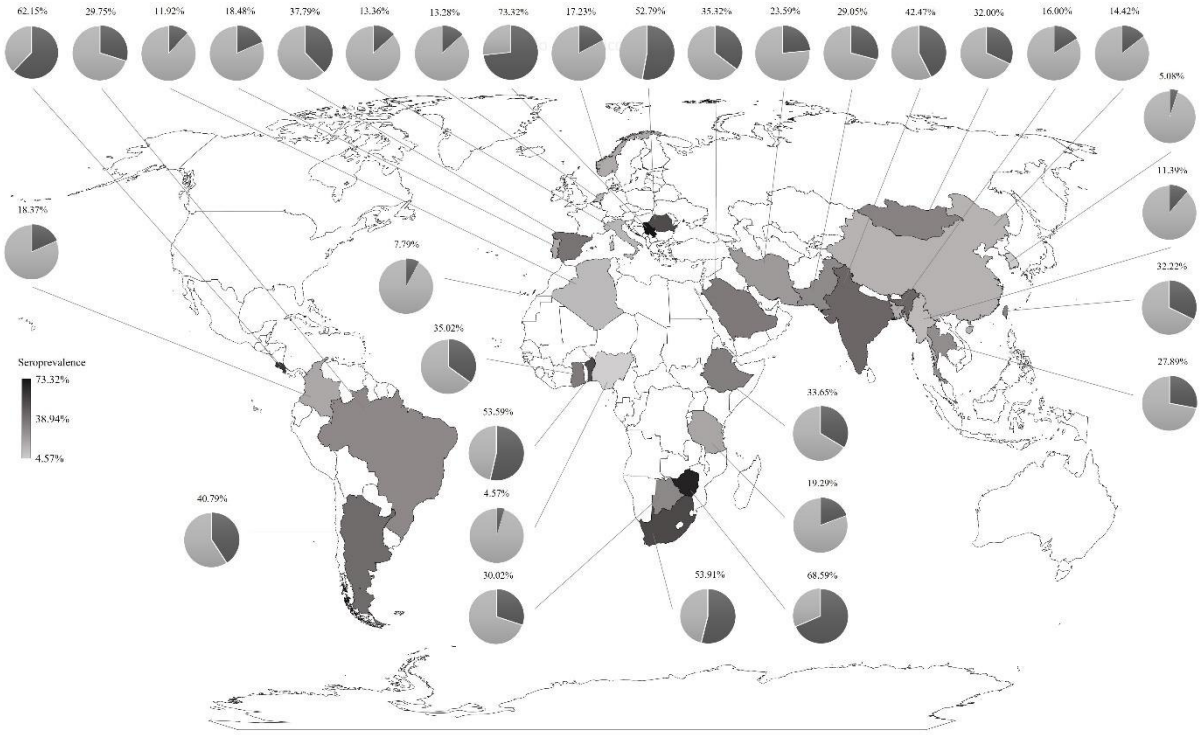


Figure 11. Forest plot of *T. gondii* seroprevalence in goats. The black dot is the estimate and the horizontal line is the 95% CI for the seroprevalence plotted for each study. Each column shows the studies broken down by region / country; number of seropositive animals; sample size; seroprevalence%; 95% CI; study weight in the general meta-analysis; seroprevalence in a forest plot; authors. The black diamond at the bottom of each continent is the estimated average seroprevalence of *T. gondii* in goats.



1859

1860 Figure 12. World map showing the seroprevalence of *T. gondii* in goats from several countries.
1861 The pie charts show the seroprevalence rates for *T. gondii* estimated according to the articles
1862 published in the indexing bases of each country.

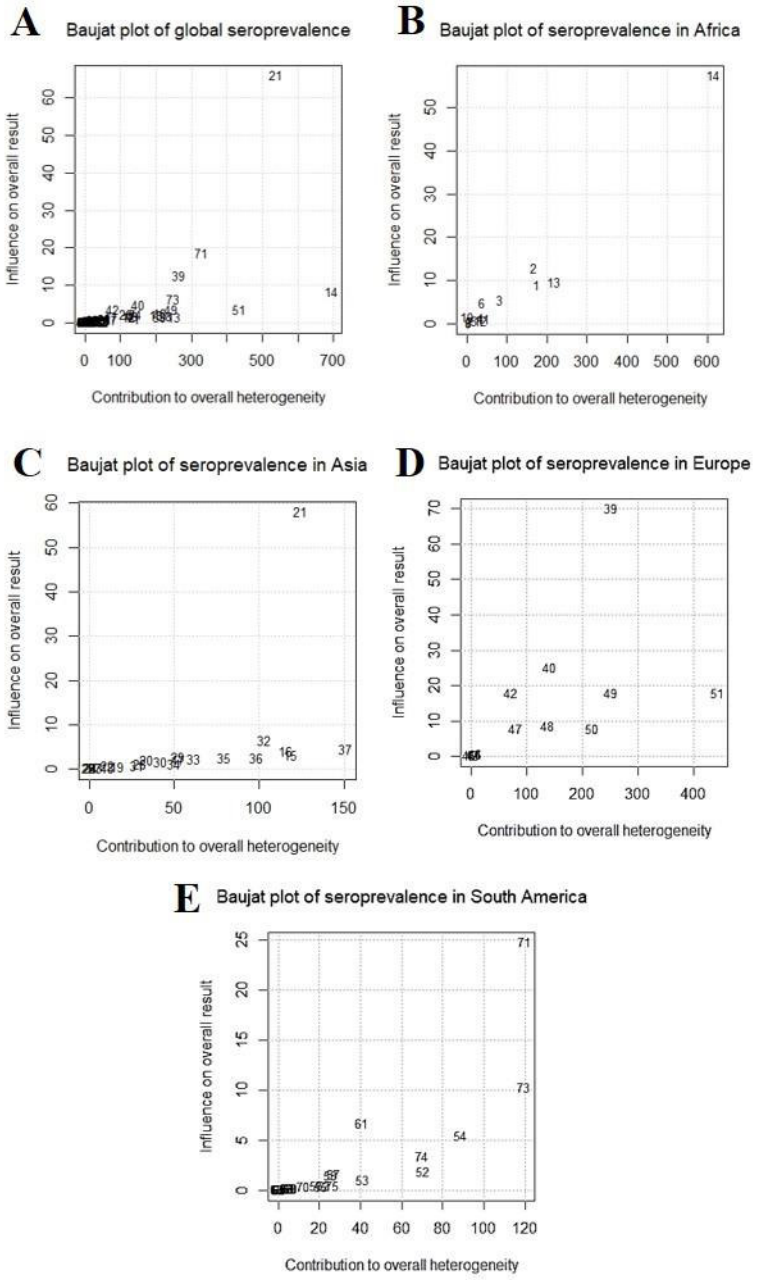


Figure 13. Baujat plot showing the contribution of each study to the general statistics of the Q test for heterogeneity. (A) General seroprevalence. (B) Seroprevalence in Africa. (C) Seroprevalence in Asia. (D) Seroprevalence in Europe. (E) Seroprevalence in South America. Each number represents a study, shown in Table 2. The studies in the upper right corner have the greatest influence on the results and have the greatest contribution to heterogeneity.

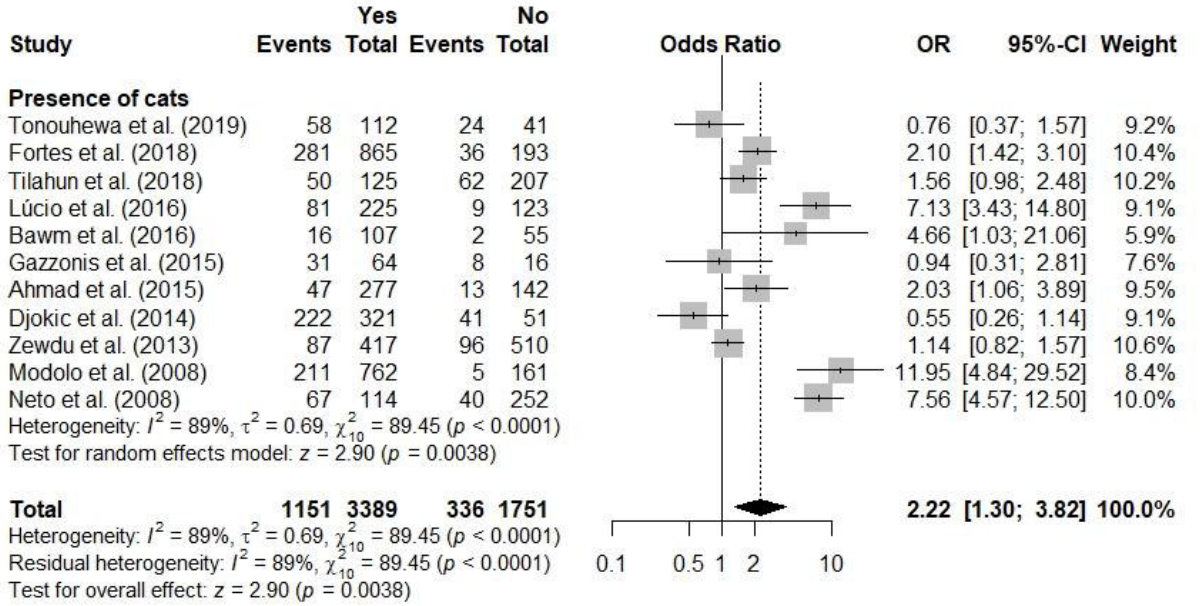


Figure 14. Forest plot of Odds Ratio for the presence of cats. The size of the square corresponds to the weight of the study. Error bars represent 95% confidence intervals. The diamond represents the estimated odds ratio.

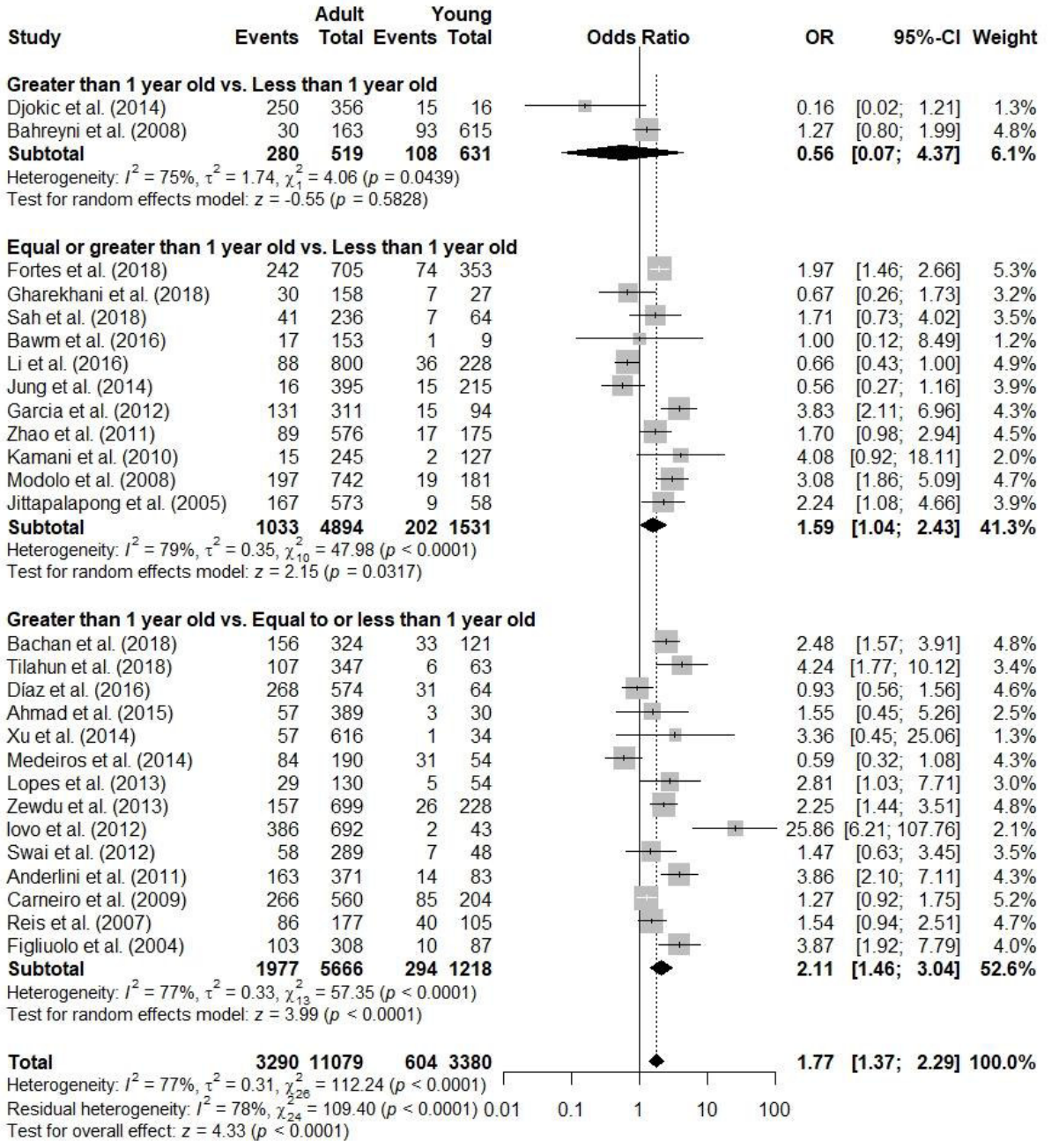


Figure 15. Forest plot of Odds Ratio for age. The size of the square corresponds to the weight of the study. Error bars represent 95% confidence intervals. The diamond represents the estimated odds ratio.

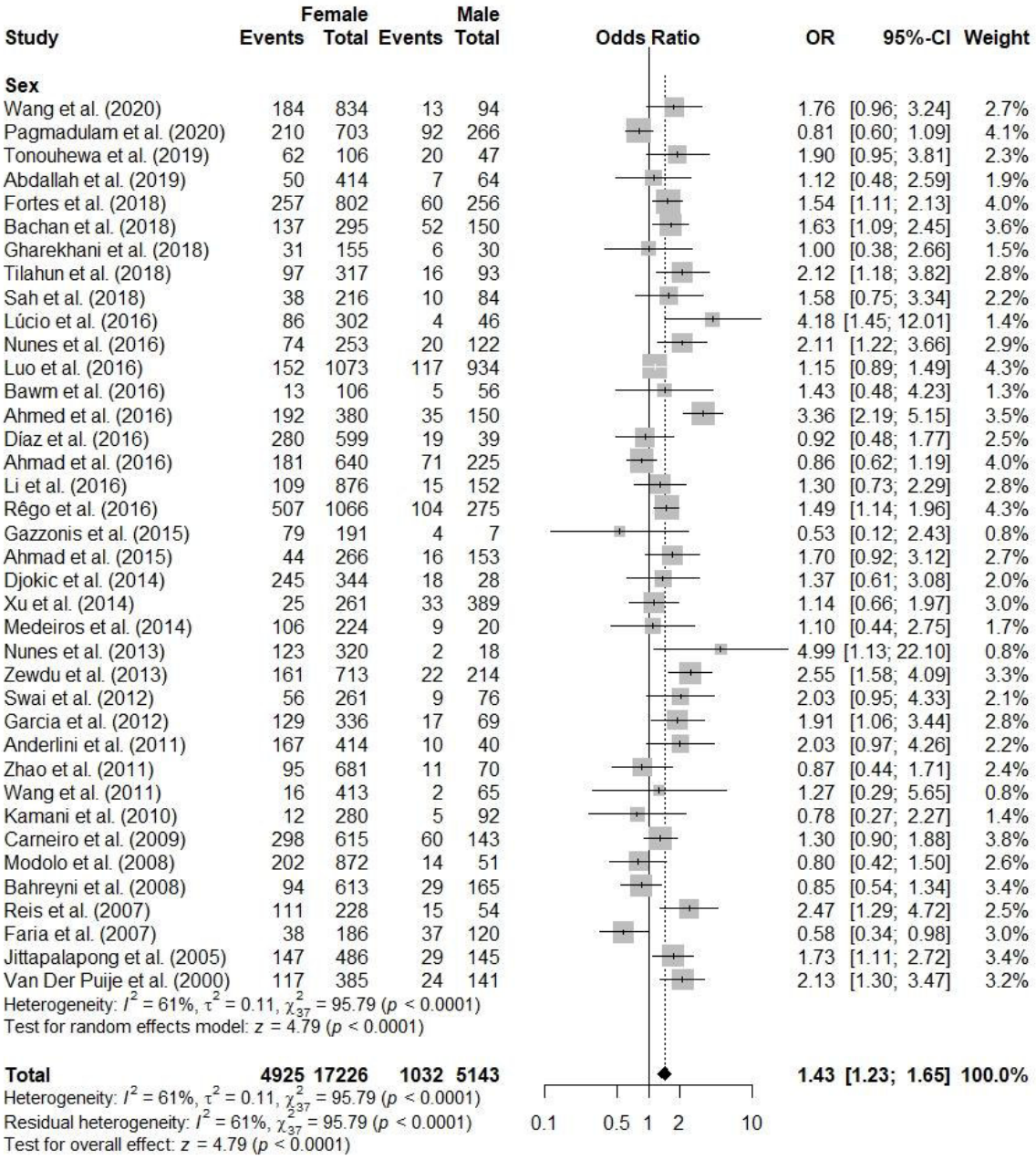


Figure 16. Forest plot of Odds Ratio for sex. The size of the square corresponds to the weight of the study. Error bars represent 95% confidence intervals. The diamond represents the estimated odds ratio.

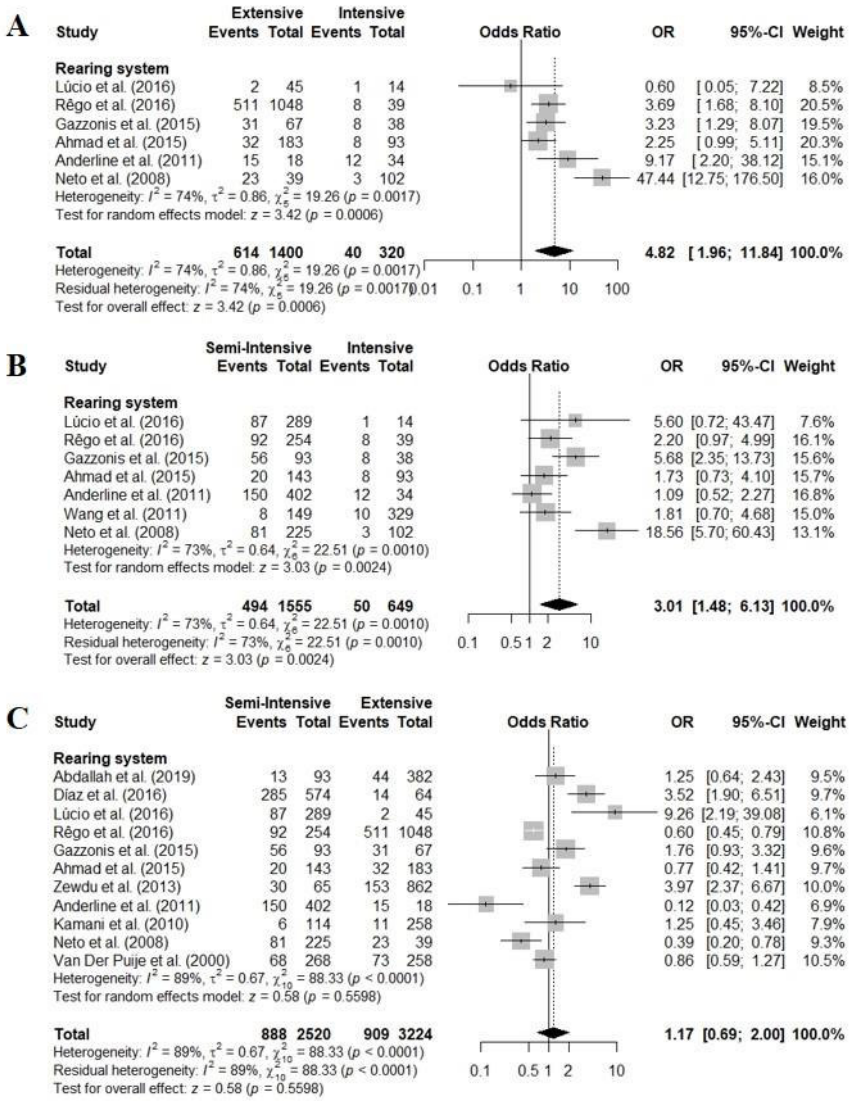


Figure 17. Odds Ratio forest plot for the rearing system. (A) Odds Ratio Forest plot for extensive vs. intensive. (B) Forest plot of Odds Ratio for semi-intensive vs. intensive. (C) Forest plot of Odds Ratio for semi-intensive vs. extensive. The size of the square corresponds to the weight of the study. Error bars represent 95% confidence intervals. The diamond represents the estimated Odds Ratio.

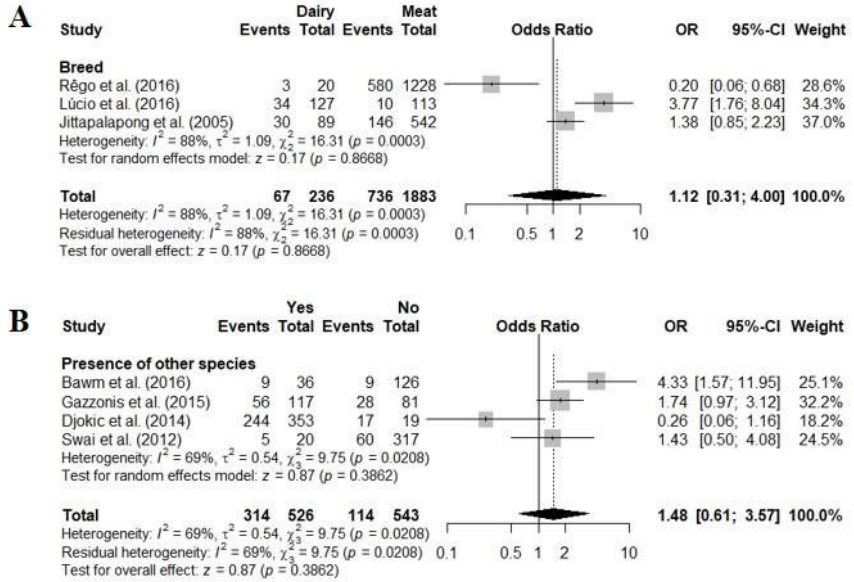
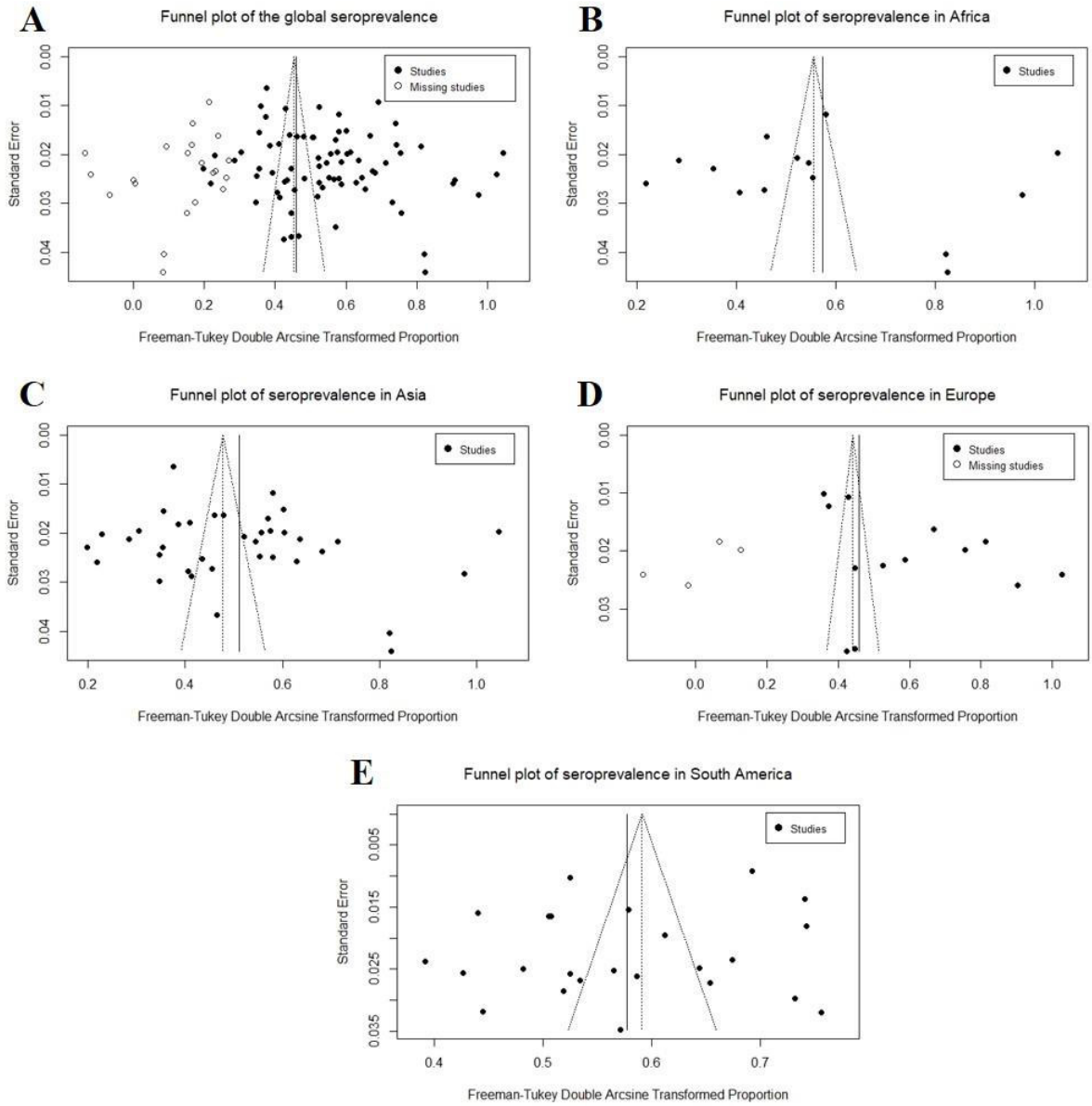


Figure 18. Odds Ratio forest plot for breed and presence of other species. (A) Forest plot of Odds Ratio for breed. (B) Forest portion of Odds Ratio presence of other species. The size of the square corresponds to the weight of the study. Error bars represent 95% confidence intervals. The diamond represents the estimated Odds Ratio.



1893

1894 Figure 19. Funnel plot for analysis of publication bias. (A) Funnel plot general seroprevalence.
1895 (B) Funnel plot seroprevalence in Africa. (C) Funnel plot seroprevalence in Asia. (D) Funnel
1896 plot seroprevalence in Europe. (E) Funnel plot seroprevalence in South America. Individual
1897 studies are represented by gray dots. The dashed external lines indicate the triangular region
1898 where 95% CI of the studies are expected, and the dashed central vertical line is the axis of the
1899 fixed effect and the continuous central vertical line is the axis of the random effect.

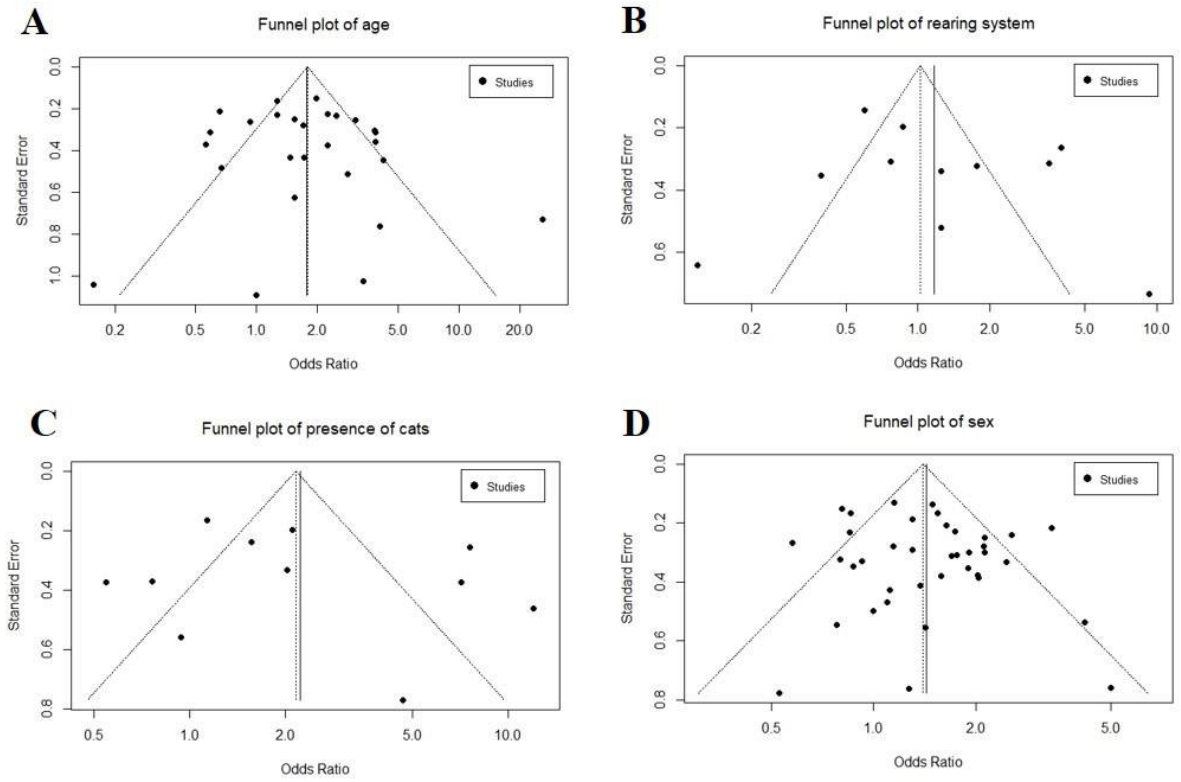
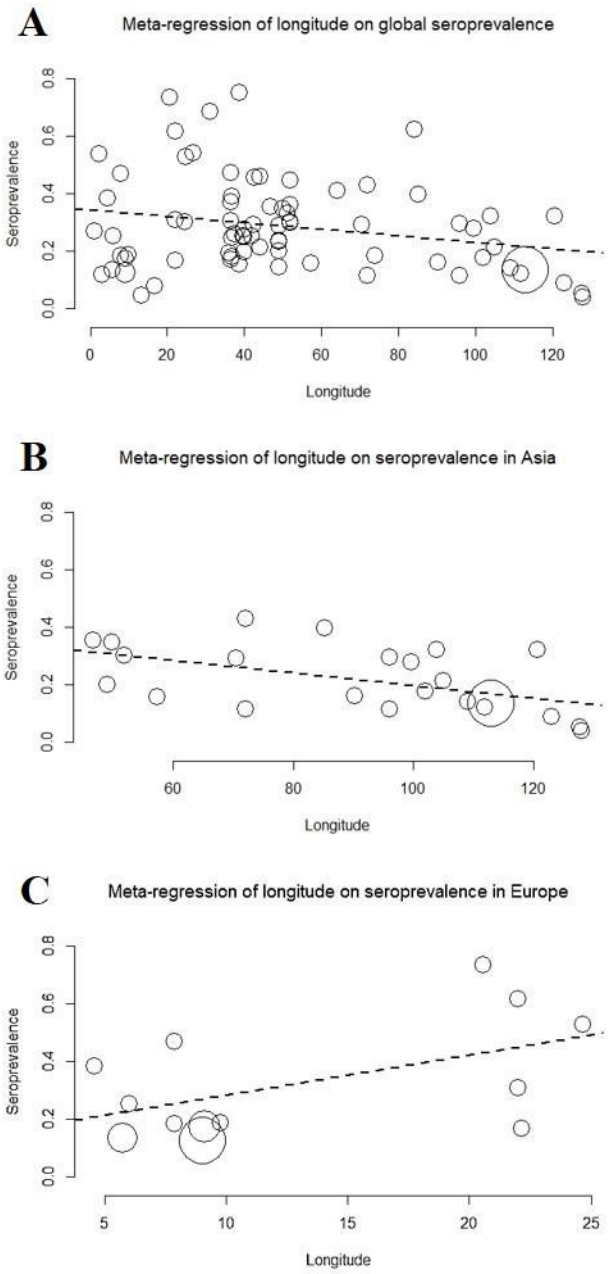


Figure 20. Funnel plot for analysis of publication bias. (A) Funnel plot age. (B) Funnel plot rearing system (semi-intensive vs. extensive). (C) Funnel plot presence of cats. (D) Funnel plot sex. Individual studies are represented by gray dots. The dashed external lines indicate the triangular region where 95% CI of the studies are expected, and the dashed central vertical line is the axis of the fixed effect and the continuous central vertical line is the axis of the random effect.



1907

1908 Figure 21. Meta-regression of the latitude of the studied region against *T. gondii* seroprevalence
1909 in goats. (A) Meta-regression of the latitude of the studied region against the general
1910 seroprevalence of *T. gondii* in goats. (B) Meta-regression of the latitude of the studied region
1911 against *T. gondii* seroprevalence in goats in Asia. (C) Meta-regression of the latitude of the
1912 region studied against the seroprevalence of *T. gondii* in goats in Europe. The circles represent
1913 the individual studies. The continuous line represents the regression line. Latitude is plotted on
1914 the horizontal axis. The seroprevalence of *T. gondii* is plotted on the vertical axis.

3.7. List of Tables

Table 2. Quality criteria for included studies.

Parameter assessed	Score		
	2	**1**	**0**
Sample size	≥501	500-301	≤300
T. gondii Diagnostic test	Provides description of the diagnostic test and using more than one test	Provides description of the diagnostic test or using a test	Ne
Risk fator	Three or more risk factors	One or two risk factors	Ne

Ne: no data evaluated

1918 Table 3. Summary of data extracted from all included studies.

ID	Author	Region	Country	Sampling	Prevalence % (n/total)		Diagnostic test* [cut-off titer]	Risk factors	Study quality
					Animals	Herds			
1	Kamani et al. (2010)	Africa	Nigeria	Multistage and cluster sampling	4.57 (17/372)	-	ELISA[1]	Age, rearing system, sex	Middle
2	Rodriguez-Ponce et al. (2017)	Africa	Canary Islands	Random sampling	7.79 (43/552)	-	ELISA[1]	-	Middle
3	Abdallah et al. (2019)	Africa	Algeria	Random sampling	11.92 (57/478)	87.23 (14/47)	ELISA[1]	Rearing system, sex	Middle
4	Gebremedhin et al. (2014)	Africa	Ethiopia	Random sampling	15.48 (50/323)	-	DAT [1:40]	-	Low
5	Swai et al. (2012)	Africa	Tanzania	Random sampling	19.29 (65/337)	45.10 (46/102)	LAT [1:16]	Age, presence of other species, sex	Middle
6	Zewdu et al. (2013)	Africa	Ethiopia	Cluster random sampling	19.74 (183/927)	58.29 (109/187)	ELISA[1]	Age, presence of cats, rearing system, sex	High
7	Gebremedhin et al. (2013)	Africa	Ethiopia	Cluster random sampling	24.79 (145/585)	59.78 (110/184)	ELISA[1]	-	Middle
8	Van Der Puije et al. (2000)	Africa	Ghana	Random sampling	26.81 (141/526)	-	ELISA[2]/ IFAT [**]	Rearing system, sex	High
9	Tilahun et al. (2018)	Africa	Ethiopia	Random sampling	27.56 (113/410)	55.83 (67/120)	ELISA[1]	Age, presence of cats, sex	Middle
10	Sharma et al. (2003)	Africa	Botswana	-	30.02 (540/1799)	-	IHAT [1:64]	-	Middle
11	Tonouhewa et al. (2019)	Africa	Benin	Random sampling	53.59 (82/153)	-	ELISA[1]	Presence of cats, sex	Low
12	Tagwireyi et al. (2019)	Africa	South Africa	Random sampling	53.91 (69/128)	-	LAT [1:64]	-	Low
13	Hove et al. (2005)	Africa	Zimbabwe	Cluster sampling	68.59 (214/312)	-	IFAT [1:50]	-	Low
14	Teshale et al. (2007)	Africa	Ethiopia	Multistage and cluster sampling	74.88 (480/641)	-	MAT [1:20]	-	Middle
15	Wang et al. (2011)	Asia	China	-	3.77 (18/478)	-	IHAT [1:64]	Rearing system, sex	Middle
16	Jung et al. (2014)	Asia	Korea South	Stratified simple random sampling	5.08 (31/610)	-	ELISA[1]	Age	Middle
17	Xu et al. (2014)	Asia	China	Random sampling	8.92 (58/650)	-	IHAT [1:64]	Age, sex	Middle
18	Bawm et al. (2016)	Asia	Myanmar	Random sampling	11.39 (32/281)	-	LAT [1:64]	Age, presence of cats, presence of other species, sex	Middle
19	Ahmad et al. (2015)	Asia	Pakistan	Cluster random sampling	11.46 (48/419)	-	ELISA[1]	Age, presence of cats, rearing system, sex	Middle
20	Li et al. (2016)	Asia	China	Random sampling	12.06 (124/1028)	-	IHAT [1:64]	Age, sex	Middle
21	Luo et al. (2016)	Asia	China	-	13.40 (807/6021)	-	IAT [1:64]	Sex	Middle
22	Zhao et al. (2011)	Asia	China	Random sampling	14.11 (106/751)	100.00 (9/9)	IHAT [1:64]	Age, sex	Middle

23	Bahrieni et al. (2008)	Asia	Iran	Random sampling	15.81 (123/778)	-	MAT [1:20]	Age, sex	Middle
24	Sah et al. (2018)	Asia	Bangladesh	Random sampling	16.00 (48/300)	-	ELISA[1]	Age, sex	Low
25	Zou et al. (2015)	Asia	China	-	17.60 (69/392)	-	IHAT [1:64]	-	Low
26	Gharekhani et al. (2018)	Asia	Iran	Random sampling	20.00 (37/185)	-	ELISA[1]	Age, sex	Middle
27	Wang et al. (2020)	Asia	China	Random sampling	21.23 (197/928)	-	ELISA[1]	Sex	Middle
28	Jittapalapong et al. (2005)	Asia	Thailand	Random sampling	27.89 (176/631)	-	LAT [1:64]	Age, sex	Middle
29	Ahmad et al. (2016)	Asia	Pakistan	Random sampling	29.13 (252/865)	-	LAT [1:16]	Sex	Middle
30	Liu et al. (2015)	Asia	China	Random sampling	29.54 (192/650)	100.00 (7/7)	IFAT [1:50]/ ELISA[1]	-	Middle
31	Sharif et al. (2007)	Asia	Iran	Random sampling	30.00 (120/400)	-	IFAT [1:16]	-	Low
32	Pagmadulam et al. (2020)	Asia	Mongolia	-	32.00 (345/1078)	-	ELISA[2]	Sex	Middle
33	Chiang et al. (2020)	Asia	Taiwan	Random sampling	32.22 (203/630)	100.00 (42/42)	ELISA[1]	-	Middle
34	Izadyar et al. (2019)	Asia	Iran	Random sampling	34.67 (130/375)	-	ELISA[2]	-	Low
35	Alanazi (2013)	Asia	Saudi Arabia	-	35.32 (196/555)	-	IFAT [1:32]	-	Middle
36	Bachan et al. (2018)	Asia	India	Stratified random sampling	42.47 (189/445)	-	IFAT [1:64]/ ELISA[2]	Age, sex	Middle
37	Ahmed et al. (2016)	Asia	Pakistan	Random sampling	42.83 (227/530	100.00 (20/20)	ELISA[1]	Sex	Middle
38	Villagra-Blanco et al. (2018)	Central America	Costa Rica	Random sampling	62.15 (243/391)	100.00 (13/13)	ELISA[1]	-	Low
39	Masala et al. (2003)	Europe	Italy	Random sampling	12.35 (302/2445)	-	IFAT [**]	-	Middle
40	Deng et al. (2016)	Europe	Netherlands	-	13.28 (221/1664)	61.54 (32/52)	ELISA[1]	-	Middle
41	Kantzoura et al. (2013)	Europe	Greece	Random sampling	16.76 (30/179)	-	ELISA[2]	-	Low
42	Stormoen et al. (2012)	Europe	Norway	Random sampling	17.23 (377/2188)	75.34 (55/73)	DAT [1:40]	-	Middle
43	Lopes et al. (2013)	Europe	Portugal	-	18.48 (34/184)	-	MAT [1:20]	Age	Low
44	Gazzonis et al. (2015)	Europe	Italy	Random sampling	18.57 (88/474)	96.55 (28/29)	IFAT [1:64]	Presence of cats, rearing system, sex	Middle
45	García-Bocanegra et al. (2013)	Europe	Spain	Random sampling	25.10 (124/494)	72.22 (52/72)	ELISA[1]	-	Low
46	Tzanidakis et al. (2012)	Europe	Greece	Random sampling	30.68 (166/541)	98.55 (68/69)	ELISA[2]	-	Middle
47	Jiḿénez-Martín et al. (2020)	Europe	Spain	Random sampling	38.31 (362/945)	93.65 (59/63)	MAT [1:25]	-	Middle

48	Díaz et al. (2016)	Europe	Spain	Random sampling	46.87 (299/638)	74.00 (37/50)	DAT [**]	Age, rearing system, sex	High
49	Iovu et al. (2012)	Europe	Romania	-	52.79 (388/735)	-	ELISA[1]	Age	Middle
50	Anastasia et al. (2013)	Europe	Greece	Random sampling	61.66 (230/373)	100.00 (50/50)	ELISA[2]	-	Low
51	Djokic et al. (2014)	Europe	Serbia	Random sampling	73.32 (316/431)	84.62 (121/143)	MAT [1:25]	Age, presence of cats, presence of other species, sex	Middle
52	Mainardi et al. (2003)	South America	Brazil	-	14.48 (64/442)	-	IFAT [1:16]	-	Low
53	Lima et al. (2008)	South America	Brazil	Random sampling	17.06 (65/381)	92.86 (13/14)	IFAT [1:64]	-	Low
54	Santos et al. (2012)	South America	Brazil	Random sampling	18.15 (177/975)	70.00 (77/110)	IFAT [1:64]	-	Middle
55	Martínez-Rodriguez et al. (2020)	South America	Colombia	Random sampling	18.37 (45/245)	-	ELISA[1]	-	Low
56	Varaschin et al. (2011)	South America	Brazil	Random sampling	21.45 (86/401)	-	IFAT [1:64]	-	Low
57	Modolo et al. (2008)	South America	Brazil	-	23.40 (216/923)	88.24 (15/17)	IFAT [1:16]	Age, presence of cats, sex	High
58	Costa et al. (2012)	South America	Brazil	-	23.62 (218/923)	-	IFAT [1:16]	-	Middle
59	Faria et al. (2007)	South America	Brazil	Random sampling	24.51 (75/306)	-	IFAT [1:64]	Sex	Middle
60	Nunes et al. (2016)	South America	Brazil	Random sampling	25.07 (94/375)	-	ELISA[2]	Sex	Middle
61	Cavalcante et al. (2008)	South America	Brazil	Non-probability sampling	25.15 (594/2362)	-	ELISA[2]	-	Middle
62	Lúcio et al. (2016)	South America	Brazil	Random sampling	25.86 (90/348)	60.00 (9/15)	IFAT [1:64]	Breed, presence of cats, rearing system, sex	Middle
63	Figliuolo et al. (2004)	South America	Brazil	Random sampling	28.68 (113/394)	100.00 (19/19)	IFAT [1:64]	Age	Middle
64	Luciano et al. (2011)	South America	Brazil	Random sampling	29.13 (60/206)	-	IFAT [1:64]	-	Low
65	Fortes et al. (2018)	South America	Brazil	Cluster sampling	29.96 (317/1058)	80.85 (76/94)	IFAT [1:64]/ ELISA[2]/ MAT [1:25]	Age, presence of cats, sex	High
66	Neto et al. (2008)	South America	Brazil	Random sampling	30.60 (112/366)	-	IFAT [1:64]	Presence of cats, rearing system	Middle
67	Moura et al. (2016)	South America	Brazil	Random sampling	33.03 (216/654)	-	IFAT [1:64]	-	Middle
68	Garcia et al. (2012)	South America	Brazil	Random sampling	36.05 (146/405)	100.00 (12/12)	IFAT [1:64]/ ELISA[2]	Age, sex	Middle
69	Nunes et al. (2013)	South America	Brazil	Random sampling	36.98 (125/338)	93.33 (14/15)	ELISA[2]	Sex	Middle
70	Anderlini et al. (2011)	South America	Brazil	Random sampling	38.99 (177/454)	95.83 (23/24)	IFAT [1:64]	Age, rearing system, sex	Middle
71	Gos et al. (2017)	South America	Argentine	Random sampling	40.79 (1192/2922)	95.70 (89/93)	IFAT [1:100]	-	Middle
72	Reis et al. (2007)	South America	Brazil	Random sampling	44.68 (126/282)	-	IFAT [1:64]/ MAT [1:64]	Age, sex	Middle

				stratified by age group					
73	Rêgo et al. (2016)	South America	Brazil	Random sampling	45.56 (611/1341)	-	ELISA[2]	Breed, rearing system, sex	High
74	Carneiro et al. (2009)	South America	Brazil	Non-probability sampling	45.76 (351/767)	-	IFAT [1:64]/ ELISA[2]	Age, sex	High
75	Medeiros et al. (2014)	South America	Brazil	Non-probabilistic sampling	47.13 (115244)	100.00 (9/9)	ELISA[2]	Age, sex	Low

ELISA: Enzyme Linked Immunosorbent Assay; [1]commercial ELISA kit; [2]home-made protocol ELISA; IHA: Indirect Hemagglutination Test; DAT: Direct agglutination Test; MAT: Modified Agglutination Test; IAT: Indirect Agglutination Test; IFAT: Indirect Immunofluorescence Test; LAT: Latex Agglutination Test; *: Information for each ELISA test is detailed in Supplementary material 1; **: No data.

1923 Supplementary material 1. Summary of data extracted from *T. gondii* ELISA tests utilized in all papers analysed.

ID*	AUTHOR	ELISA TEST	ID KIT**/ANTIGEN	CONJUGATE	SUBSTRATE	STOP SOLUTION	OD (NM) ***
1	Kamani et al. (2010)	Commercial kit	Pourquier® ELISA *T. gondii* serum screening version P00710/03	-	-	-	450
2	Rodriguez-Ponce et al. (2017)	Commercial kit	Kit multi-species ID Screen® Toxoplasmosis Indirect, IDVET, Montpellier, France	-	-	-	MR
3	Abdallah et al. (2019)	Commercial kit	Toxoplasmosis Indirect ELISA Multi-species kit (ID Screen, ID.VET. Innovative Diagnostics, Montpellier, France)	-	-	-	450
6	Zewdu et al. (2013)	Commercial kit	ELISA kit (ID VET Innovative Diagnostic, ID Screen®, Montpellier, France)	-	-	-	MR
7	Gebremedhin et al. (2013)	Commercial kit	(P-30 ELISA) kit (ID VET Innovative Diagnostic, ID Screen®, Montpellier, France)	-	-	-	MR
8	Van Der Puije et al. (2000)	Home-made protocol	*T. gondii* STAg[1] - RH strain	Alkaline phosphatase labelled sheep antihuman IgG	p-nitrophenyl phosphate (Sigma 104 phosphatase substrate)	3M NaOH	492
9	Tilahun et al. (2018)	Commercial kit	ELISA multispecies diagnostic kit (ID VET Innovative Diagnostic, ID Screen, Montpellier, France)	-	-	-	MR
11	Tonouhewa et al. (2019)	Commercial kit	ELISA kit from IDvet, targeting P30 surface antigen of T gondii (ID Screen Toxoplasmosis Indirect Multi-Species, ID Vet Innovative Diagnostic, Montpellier, France)	-	-	-	450
16	Jung et al. (2014)	Commercial kit	ELISA kit (IDEXX Laboratories, Westbrook, ME)	-	-	-	MR
19	Ahmad et al. (2015)	Commercial kit	LISA Kit (ID Screen *Toxoplasmosis* Indirect ® (ID-VET Company, France)	-	-	-	MR
24	Sah et al. (2018)	Commercial kit	ELISA kit (ID Screen® Toxoplasmosis Indirect Multi-species, TOXOS-MS ver 1013 GB, Product code: TOXOS-MS, batch:	-	-	-	450

			582, Innovative Diagnostic vet Laboratories, Inc., France)				
26	Gharekhani et al. (2018)	Commercial kit	ELISA kit (ID Screen® Toxoplasmosis indirect multi-species; ID-Vet company, France)	-	-	-	MR
27	Wang et al. (2020)	Commercial kit	ELISA kit from Lanzhou Veterinary Research Institute of Chinese Academy of Agricultural Sciences for goat	-	-	-	450
30	Liu et al. (2015)	Commercial kit	ELISA kits, IDEXX Laboratories,Inc., Westbrook, ME, USA)	-	-	-	MR
32	Pagmadulam et al. (2020)	Home-made protocol	*T. gondii* GRA7 recombinant protein (rTgGRA7)	Horseradish peroxidase-conjugated anti-sheep IgG (Bethyl Laboratories, Montgomery, TX, USA)	ND	ND	415
33	Chiang et al. (2020)	Commercial kit	ELISA kit (ID Screen® Toxoplasmosis Indirect Multi-species, IDvet, rue Louis Pasteur, Grabels, France).	-	-	-	MR
34	Izadyar et al. (2019)	Home-made protocol	*T. gondii* STAg[1] - RH strain	IgG-horseradish peroxidase (HRP) antibody (Razi BioTech, Iran)	3,3′,5,5′-Tetramethylbenzidine (TMB) (Razi BioTech, Iran)	2 M H2SO4	450
36	Bachan et al. (2018)	Home-made protocol	*T. gondii* SAG1 recombinant protein (rSAG1)	HRPO-labeled, rabbit anti-goat IgG (GCC Biotech, India)	O-phenylenediamine dihydrochloride (OPD)	3 N HCl	492
37	Ahmed et al. (2016)	Commercial kit	ELISA Kit (ID Screen Toxoplasmosis Indirect®, ID-VET Company, Montpellier, France)	-	-	-	MR
38	Villagra-Blanco et al. (2018)	Commercial kit	IDScreen® Toxoplasma gondii, Indirect Multispecies ELISAs (ID Vet®, Montpellier, France)	-	-	-	MR
40	Deng et al. (2016)	Commercial kit	ELISA kit (ID ScreenToxoplasmosis Indirect Multi-species; ID.VET Innovative Diagnos-tics, France)	-	-	-	MR
41	Kantzoura et al. (2013)	Home-made protocol	*T. gondii* STAg[1]	Anti-goat IgG (A-4187) alkaline phosphatase conjugate, SIGMA®)	p-nitrophenyl phosphate (SIGMA®)	NaOH 1N	405

45	García-Bocanegra et al. (2013)	Commercial kit	Cmpetitive species-specific ELISA provided by Laboratoire de Pathologie des Petits Ruminants et des Abeilles (CNEVA-LPPRA, Biot., France), from French Food Safety Agency (AFSSA).	-	-	-	MR
46	Tzanidakis et al. (2012)	Home-made protocol	*T. gondii* TaqLSA[2] (TgSAG1)	Rabbit anti-sheep IgG (H + L)-POD and rabbit anti-goat IgG (H + L)-POD (Dianova, Hamburg, Germany)	1% tetra-methyl-benzidine (TMB)	4 N H_2SO_4	450
49	Iovu et al. (2012)	Commercial kit	ELISA kit (Chekit Toxotest Antibody ELISA, Idexx-Bommeli, Switzerland)	-	-	-	MR
50	Anastasia et al. (2013)	Home-made protocol	*T. gondii* STAg[1]	Alkaline phosphatase-conjugated anti-goat IgG A4187 (SIGMA®)	p-nitrophenyl phosphate (SIGMA®)	NaOH 1N	405
55	Martínez-Rodriguez et al. (2020)	Commercial kit	ELISA kit (PrioCHECK® Toxoplasma Ab SR, Prionics, Schlieren-Zurich, Switzerland)	-	-	-	450
60	Nunes et al. (2016)	Home-made protocol	*T. gondii* STAg[1] – RH strain	Peroxidase anti-goat IgG (Bethyl Laboratories, Inc., Montgomery, TX-USA)	O-phenylenediamine dihydrochloride (OPD)	ND	492
61	Cavalcante et al. (2008)	Home-made protocol	*T. gondii* STAg[1] – C4 strain	Rabbit antihuman globulin labelled with alkaline phosphatase (Engvall and Perlmann, 1972)	p-nitrophenylphosphate	NaOH 2M	400
65	Fortes et al. (2018)	Home-made protocol	*T. gondii* STAg[1]	Anti-IgG caprine (Sigma, St. Louis, MO)	ND	ND	ND
68	Garcia et al. (2012)	Home-made protocol	*T. gondii* STAg[1] – RH strain	ND	ND	ND	ND
69	Nunes et al. (2013)	Home-made protocol	*T. gondii* STAg[1] – C4 strain	Rabbit antihuman globulin labelled with alkaline phosphatase (Engvall and Perlmann, 1972)	p-nitrophenylphosphate	NaOH 2M	400

73	Rêgo et al. (2016)	Home-made protocol	*T. gondii* STAg[1] – C4 strain	Rabbit antihuman globulin labelled with alkaline phosphatase (Engvall and Perlmann, 1972)	p-nitrophenylphosphate	NaOH 2M	400
74	Carneiro et al. (2009)	Home-made protocol	*T. gondii* STAg[1] – C4 strain	Rabbit antihuman globulin labelled with alkaline phosphatase (Engvall and Perlmann, 1972)	p-nitrophenylphosphate	NaOH 2M	400
75	Medeiros et al. (2014)	Home-made protocol	*T. gondii* STAg[1] – RH 88 strain	Anti-goat IgG - peroxidase (SIGMA, product A-5420)	ortho-phenylenodiamine	4N-H2 SO4	490

*Referring to table 2 of manuscript; **As described in the article; ***Optical density; [1]STAg: Soluble tachyzoites antigen; [2]TaqLSA: taquizoite lyophilized surface antigen; ND: not described; MR: manufacturer recommendation.

Supplementary material 2. List with cutoff points for *T. gondii* serological tests performed in goats.

Diagnostic test	Cut-off titer	Number of studies	ID (reference table 2)
DAT: Direct agglutination Test	1:40	2	4; 42
	Ni*	1	48
IAT: Indirect Agglutination Test	1:64	1	21
IFAT: Indirect Immunofluorescence Test	1:16	4	31; 52; 57; 58
	1:32	1	35
	1:50	2	13; 30
	1:64	16	36; 44; 53; 54; 56; 59; 62; 63; 64; 64; 66; 67; 68 ;70; 72; 74
	1:100	1	71
	Ni	2	8; 39
IHAT: Indirect Hemagglutination Test	1:64	6	10; 15; 17; 20; 22; 25
LAT: Latex Agglutination Test	1:16	2	5; 29
	1:64	3	12; 18; 28
MAT: Modified Agglutination Test	1:20	3	14; 23; 43
	1:25	3	47; 51; 65
	1:64	1	72

*Ni: not informed

4. CAPÍTULO 4 – SEROPREVALENCE AND RISK FACTORS FOR NEOSPORA CANINUM AND TOXOPLASMA GONDII IN GOATS OF MARANHÃO STATE, BRAZIL

Seroprevalence and risk factors for *Neospora caninum* and *Toxoplasma gondii* in goats of Maranhão state, Brazil

Abstract

We estimated the seroprevalence and possible risk factors for neosporosis and toxoplasmosis in goats in the state of Maranhão, Brazil. In addition, the variables related to these animals and the management of the farm were investigated in terms of the significance of the associations. In total, 383 serum samples from goats, of both sexes and different ages, were collected from 15 farms in four municipalities. The indirect immunofluorescence test was used for antibody detection against *Neospora caninum* and *Toxoplasma gondii*. The overall seroprevalence of *N. caninum* in goats was 26.4% (101/382; IC 95% 22.3–31.1), and 114 out of 383 serum samples were *T. gondii*-seropositive (29.8%, IC 95% 25.4–34.5). In addition, the seroprevalence of coinfection of *T. gondii* and *N. caninum* in goats was 8.6% (33/382; IC 95% 6.2–11.8). The risk factors significantly associated with the seroprevalence of *N. caninum* were age, type of sheepfold floor, rearing system, feeding, pasture area cultivated, cats having access to the feed deposits, worming, slaughter place of the animals, history of abortion, and the presence of dogs and cats. Regarding the seroprevalence of *T. gondii* infection, age, category, presence of other species and purpose of breeding were the risk factors. To our knowledge, this is the first report of the seroprevalence and risk factors for *N. caninum* and *T. gondii* in goats in the state of Maranhão, Brazil, which provides basic data for the implementation of strategies and control measures against neosporosis and toxoplasmosis.

Keywords: Neosporosis, Toxoplasmosis, Small ruminants; Serology, Epidemiology

4.1. Introduction

Parasitic diseases are responsible for substantial economic losses due to decreased food consumption and weight loss (Fthenakis and Papadopoulos, 2018). Sanitary management combined with zootechnical management practices reduces the risk of infection by several diseases, such as neosporosis and toxoplasmosis, which are strongly associated with reproductive issues in small ruminants (Dubey, 2016; Dubey and Schares, 2011).

Neosporosis and toxoplasmosis are cosmopolitan parasitic diseases with the etiological agents *Neospora caninum* and *Toxoplasma gondii*, respectively (Dubey, 2016; Dubey and Schares, 2011). Both agents are intracellular coccidic protozoans that have canines and felines as their definitive hosts and birds and various mammal species as their intermediate hosts, causing economic losses and a reduction in the reproductive rates of the herd (Dubey, 2016; Dubey et al., 2020, 2007; Freyre et al., 1997; Reichel et al., 2013).

The seroprevalence of *N. caninum* in goats worldwide ranges from 0.5 to 26.7%, with the average prevalence in Brazil being 11.6% (Rodrigues et al., 2020). Regarding *T. gondii*, seroprevalence ranges from 3.8 to 74.9%, with an average prevalence in Brazil of 29.8% (in prep). The study of risk factors for the seroprevalence of *N. caninum* and *T. gondii* in herds provides important information for the development and adoption of control strategies (Dubey et al., 2007). The main risk factors for neosporosis and toxoplasmosis in goats are the abortion history and the presence of dogs or cats on farms (Abo-Shehada and Abu-Halaweh, 2010; Fortes et al., 2018; Gharekhani et al., 2018, 2016; Liu et al., 2015; Modolo et al., 2008; Topazio et al., 2014), although some studies have also associated the production system, the origin of the water and the climatic factors with the increase in the seroprevalence of *N. caninum* and *T. gondii* in the herd (Díaz et al., 2016; Gazzonis et al., 2016; Luo et al., 2016). However, there are no studies on seroprevalence and risk factors for neosporosis and toxoplasmosis in goats in the state of Maranhão, Brazil.

Therefore, considering the importance of neosporosis and toxoplasmosis in animal production, especially in goats, and the absence of studies on this region, we estimated the seroprevalence and risk factors for these diseases in goats in goats in eastern Maranhão, Brazil.

4.2. Material and methods

4.2.1. Study area

The state of Maranhão is in the northeastern region of Brazil and covers an area of 331,937 km² (IBGE, 2019). The state is located in a transition region and has three distinct biomes: Amazon, Caatinga and Cerrado (Spinelli-Araujo et al., 2016). The climate is tropical, with an average annual temperature of 27°C and rainfall ranging from 1,500 to 2,500 mm annually.

The study was carried out in 15 goat-breeding farms located in the following municipalities in east Maranhense: Itapecuru Mirim (latitude 03º 23' 33" S and longitude 44º 21' 31" W), Vargem Grande (latitude 03º 32' 35" S and longitude 43º 54' 57" W), Chapadinha (latitude 03º 44' 30" S and longitude 43º 21' 37" W) and Brejo (latitude 03º 41' 04" S and longitude 42º 45' 01" W).

4.2.2. Blood sampling and serological examination

The sample size was calculated to estimate the seroprevalence of *N. caninum* and *T. gondii* in goats, using the EpiInfo 7 program. We considered a prevalence of 50%, an expected error of 5% and a confidence interval of 95%, as well as a population of goats in the East Maranhense region of 36,669 animals (IBGE, 2019). Therefore, the minimum calculated quantity was 380 goat blood samples.

Approximately 5 ml of whole blood was collected from 383 animals of both sexes and different ages. Blood was collected through jugular venipuncture during the period from November 2016 to July 2017. After collection, the serum was obtained via centrifugation at 1,000 G for 3 minutes, labelled and maintained at -18ºC until serological analysis.

During the visit to the farms, parameters related to the management performed, the animals and the property were analyzed. All information was recorded in an epidemiological questionnaire and used to determine the factors associated with the seroprevalence of *N. caninum* and *T. gondii* in goats.

4.2.3. Serological examination

The sera were subjected to an indirect immunofluorescence reaction (RIFI) to detect anti-*N. caninum* antibodies and anti-*T. gondii* of the IgG class, according to the technique described by Conrad et al. (1993) and Camargo (1974). Fluid-labelled anti-goat IgG (SIGMA-CHEMICAL) conjugate (FITC) was used, and tachyzoites from the NC-1 strains (*N. caninum*) were previously fixed on the slides and used as antigens. The cutoff point used was 1:100 (Gos et al., 2017; Uzêda et al., 2007). For serology of *T. gondii*, anti-caprine IgG conjugate (SIGMA F7367) marked with fluorescein (FITC) was used, and tachyzoites from the RH strains (*T. gondii*) were previously fixed on the slides and used as antigens. Positive and negative control samples were included in all tests for comparison purposes. The 1:64 titre was used as the cutoff point (Figliuolo et al., 2004). Positive and negative control samples, from serum bank of previous studies, were included in all tests for comparison purposes.

4.2.4. Statistical analysis

Statistical analysis was performed using the EpiInfo 7 and RStudio version 3.5.2 software. The determination of seroprevalence and risk factors was performed via association between seropositivity for *N. caninum* and *T. gondii* against the information contained in the epidemiological questionnaire.

Spatial analysis using the weighted distance inverse interpolation method was performed to identify the density patterns. For this, the geographical coordinates and the seroprevalence of each herd studied were used. Several packages from the RStudio program were used for processing (dplyr and sf), interpolation (gstat) and visualisation of spatial data (ggplot2). The density themes were estimated in a regular grid, with each cell of this grid expressing the seroprevalence of *N. caninum* or *T. gondii* per 25 square km.

The association between seroprevalence and risk factors was determined by the odds ratio (Odds Ratio, OR), with significance determined for a 95% confidence interval. The association was considered significant when $p < 0.05$. The Chi-Square test with Yates´ correction was used, and when not significant, the Mantel-Haenszel test was applied.

The variables submitted to univariate analysis were regrouped to perform Multivariate Logistic Regression. Variables that showed an association with seroprevalence with a value of

$p \leq 0.20$ were selected, along with some that did not show significant differences but were considered biologically plausible. Pearson´s correlation test was performed to verify multicollinearity. Variables with a correlation coefficient ≥ 80% were included, along with variables considered biologically plausible. The level of significance adopted in the multivariate analysis was 5%. The final model of multivariate logistic regression was verified by the Hosmer and Lemeshow test, with $p \geq 0.05$ indicating an adjusted model.

4.3. Results

4.3.1. Seroprevalence

The seroprevalence for *N. caninum* found was 26.4% (101/382; 95% CI 22.3–31.1) and for *T. gondii*, it was 29.8% (114/383; 95% CI 25.4–34.5). Seropositive animals were found for both parasites in the 15 rural farms visited. The seroprevalence of co-infected goats was 8.6% (33/382; 95% CI 6.2–11.8). Co-infected animals were found in 73.3% (11/15) of the rural farms visited. Seroprevalence of coinfection in the farms ranged from 4 to 26.7% (Table 4).

Via spatial analysis, we observed a hotspot of larger clusters of seropositive animals for *N. caninum* in the northeastern region, in contrast to *T. gondii*, in which the largest clusters of seropositive animals were located in the northern region of the state of Maranhão (Figure 22).

The antibody titres of animals positive for *N. caninum* ranged from 1:100 to 1:800, with 1:100 and 1:200 being observed in 41.6% of the positive animals. The antibody titres of animals positive for *T. gondii* varied from 1:64 to >1:4096 with 1:64 and 1:256 being most commonly observed, i.e., in 28.1 and 29.0%, respectively, of the animals (Table 5).

4.3.2. Risk factors

The risk factors significantly associated with the seroprevalence of *N. caninum* were age, type of sheepfold floor, rearing system, feeding, pasture area cultivated, cats having access to the feed deposits, worming, slaughter place of the animals, history of abortion, the presence of dogs and cats, and regarding the seroprevalence of *T. gondii* infection, age, category, the presence of other species and the purpose of breeding were the risk factors. Additionally, the variables that had an association with coinfection were category, type of sheepfold floor,

worming, the presence of drinking troughs and the purpose of creation (Supplementary material 3).

The final multivariate logistic regression model for *T. gondii* seroprevalence was composed of the variables age, presence of other species, purpose of breeding and supplement. The final logistic regression model for *N. caninum* seroprevalence was composed of the variables presence of drinking troughs, age, presence of cats, type of folded floor and food (Table 6).

4.4. Discussion

This is the first report on the seroprevalence of neosporosis and toxoplasmosis in goats in the state of Maranhão, Brazil. The estimated seroprevalence of these diseases in goats was higher than in several other regions of the world (Arraes-Santos et al., 2016; Díaz et al., 2016; Gharekhani et al., 2016; Gos et al., 2017; Luo et al., 2016; Topazio et al., 2014; Villagra-Blanco et al., 2018), and the seroprevalence of *T. gondii* was similar to that found in some studies in Brazil (Fortes et al., 2018; Luciano et al., 2011) and Asia (Ahmad and Tasawar, 2016; Liu et al., 2015). The variation may be related to the particular characteristics of each region, such as climatic factors, exposure to risk factors and production systems (Rodrigues et al., 2020) and also a different design of studies, diagnosis methodology and populations of goats (Dubey et al., 2020).

We observed that 8.6% (33/382) of the goats were positive for both parasites, indicating that co-infection and cross reactions between the two protozoa are atypical; however, they should not be ruled out. Similarly, Díaz et al. (2016) and Unzaga et al. (2014) reported co-infection by *N. caninum* and *T. gondii* in goats of 6 and 12%, respectively. The detection of cross-reactions between *N. caninum* and *T. gondii* in IFAT may occur, as it is common to have a high concentration of fluorescent antibodies against apical organelles antigens of several apicomplexan parasites, and this is detected as fluorescence in the apical origanelles. Confirmation of positivity requires complete detection of peripheral antibodies, that is, on the entire surface of the parasite (Pare et al. 1995; Dubey, 2016), and serum dilutions ≥1:50 were considered adequate to avoid cross-reactivity between *N. caninum* and *T. gondii* on IFAT in different species, including goats (Opel et al., 1991; Lobato et al. 2006; Silva et al. 2007; Benetti et al. al. 2009). In addition, most of the animals analyzed had titers lower than 1:1024 for *T.*

gondii and 1:400 for *N. caninum*, indicating that a large part of the goats had a chronic phase of infection by both parasites (Dubey and Kirkbride, 1989).

The high risk of infections for *N. caninum* and *T. gondii* in animals aged 3 years or over and in uncastrated goats were detected in this study. The association between age and seroprevalence of *N. caninum* and *T. gondii* in goats has been described previously (Figliuolo et al., 2004; Gharekhani et al., 2016; Iovu et al., 2012; Romanelli et al., 2007; Tembue et al., 2011; Varaschin et al., 2011), and the time of exposure to the parasite is directly proportional to the risk of infection (Dubey et al., 2007).

Several studies have attributed neosporosis as a cause of miscarriages in small ruminants (Howe et al., 2008; Masala et al., 2007; Moreno et al., 2012; Unzaga et al., 2014). In the present study, a significant association with a history of abortion was detected in the properties with *N. caninum*. However, the causes of abortion in goats are numerous and include infectious agents such as *Toxoplasma gondii*, *Chlamydophila abortus*, *Brucella* spp. or even the lack of sanitary or nutritional management of the animals (Ababneh et al., 2014; Abu-Dalbouh et al., 2012; Samadi et al., 2010). In addition, we have detected that herds infected with *N caninum* have no history abortion history when co-infected with *T. gondii*. This may be related to the cross-immunity of these parasites, which has been reported by Gondin et al (2017), but we still need more studies to define the cross-immunity of these parasites.

In the present study, the increased risk of infection by neosporosis and toxoplasmosis seemed to have a direct relationship with the animal husbandry practiced on the properties and the characteristic of the production system. Inadequate animal handling practices, in most cases, favour the spread of parasites within the herd (Rizzo et al., 2017). Additionally, the farms that raise animals for their own consumption and those that slaughter the animals at the farm site were the ones that presented the highest risk of infection with *N. caninum*. The rearing of animals for subsistence, in these study regions, is practiced on farms with low technology and poor sanitary management. Carcass, placental or abortions remains from domestic animals are important route of infection and a risk factor for infection with *N. caninum* (Dubey and Schares, 2011).

We detected a risk of *N. caninum* infections and a risk of 2.0 for co-infection with *T. gondii* in herds with suspended sheepfold, with slatted wood floors. All animal management activities were performed in the sheepfold, which served as parturition, mothering goats and as

a resting place at night, without separation by categories or age. In addition, we verified that the presence of other animals on the property increases the risk of infection for *N. caninum* and *T. gondii* in goats due to access for dogs and cats to food deposits, carcass disposal and contamination of drinking fountains and by intercropping with other farm animals. The interaction among goats with other domestic (cats and dogs), wild (bird and rodents) and livestock, favours the transmission and are important risk factors for spreading both infections (Gazzonis et al., 2015; Dubey, 2016; Bawm et al., 2016; Sá et al., 2017; Barros et al., 2018; Ribeiro et al., 2019)

Goats reared in extensive systems and fed on native pasture with no drinking fountains available had a 1.8 to 2.0 higher risk of infection with *N. caninum*. Extensive management allows animals to come into contact with various sources of contamination (contaminated water/food), increasing the chance of contact with the parasite (Anderlini et al., 2011; Iovu et al., 2012; Snak et al., 2018). In addition, poor sanitary management and the supply of raw meat, carcass remains or abortion to dogs or cats were observed on farms with extensive system.

4.5. Conclusion

The seroprevalence values of *N. caninum* and *T. gondii* in goats in the eastern region of Maranhão were 26.4 and 29.8%, respectively. Risk factors on the farm, such as age of the animals, presence of domestic animals, abortion and sanitary and nutritional management, are the main points to be worked on to reduce the prevalence of protozoa in the herd.

Ethical statement

All procedures were approved by the Ethics Committee of Animal Use at the Federal University of Maranhão.

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Declarations of interest

None.

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4.6. List of Figures

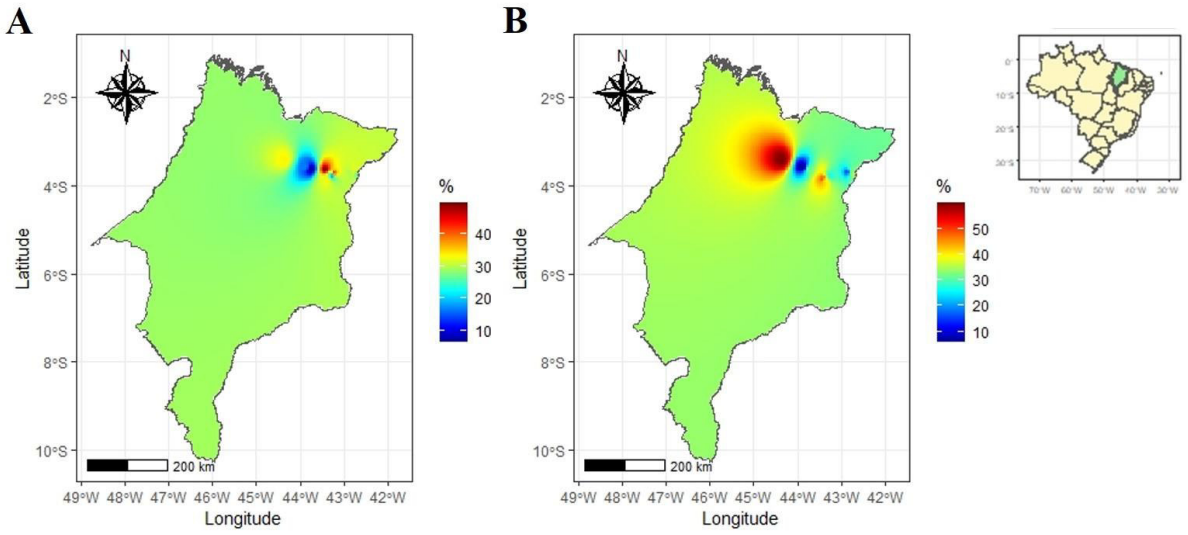

Figure 22. Spatial distribution of goats seropositive for *N. caninum* (A) and *T. gondii* (B). Map shows the seroprevalence using the model of interpolation of the inverse of the weighted distance. The color scale on the map indicates the proportion of positive animals in each location. Panel A: seroprevalence of *N. caninum* in goats. Panel B: seroprevalence of *T. gondii* in goats.

4.7. List of Tables

Table 4. Seroprevalence of *N. caninum* and *T. gondii* in goats on the properties visited.

Farm	*N. caninum*		*T. gondii*		Coinfection	
	Pos[a]/Total (%)	95% CI	Pos[a]/Total (%)	95% CI	Pos[a]/Total (%)	95% CI
1	11/22 (50.0)	28.2-71.8	9/23 (39.1)	19.7-61.5	3/22 (13.6)	2.9-34.9
2	9/25 (36.0)	18.0-57.5	8/25 (32.0)	15.0-53.5	4/25 (16)	4.5-36.1
3	4/14 (28.6)	8.4-58.1	8/14 (57.1)	28.9-82.3	3/14 (21.4)	4.7-50.8
4	3/21 (14.3)	3.1-36.3	5/21 (23.8)	8.2-47.2	0/21 (0.0)	0.0-16.1
5	3/18 (16.7)	3.6-41.4	5/18 (27.9)	9.7-53.5	1/18 (5.6)	0.1-27.3
6	14/27 (51.9)	32.0-71.3	8/27 (29.6)	13.8-50.2	4/27 (14.8)	4.2-33.7
7	7/21 (33.3)	14.6-57.0	10/21 (47.6)	25.7-70.2	5/21 (23.8)	8.2-47.2
8	8/27 (29.6)	13.8-50.2	4/27 (14.8)	4.2-33.7	0/27 (0.0)	0-12.8
9	6/24 (25.0)	9.8-46.7	9/24 (37.5)	18.8-59.4	2/24 (8.3)	1.0-27.0
10	8/28 (28.6)	13.2-48.7	11/28 (39.3)	21.5-59.4	3/28 (10.7)	2.3-28.2
11	7/27 (25.9)	11.1-46.3	6/27 (22.2)	8.6-42.3	0/27 (0.0)	0.0-12.8
12	4/9 (44.4)	13.7-78.8	3/9 (33.3)	7.5-70.1	2/9 (22.2)	2.8-60.0
13	9/54 (16.7)	7.9-29.3	3/54 (5.6)	1.2-15.4	0/54 (0.0)	0.0-6.6
14	3/50 (6.0)	1.3-16.6	16/50 (32.0)	19.5-46.7	2/50 (4.0)	0.5-13.7
15	5/15 (33.3)	11.8-61.6	9/15 (60.0)	32.3-83.7	4/15 (26.7)	7.8-55.1
Total	**101/382 (26.4)**	**22.3-31.1**	**114/383 (29.8)**	**25.4-34.5**	**33/382 (8.6)**	**6.2-11.9**

[a] = Positives; 95% CI = 95% confidence interval.

Table 5. Frequencies of antibody titer against *T. gondii* and *N. caninum* by IFAT in goat sera from Maranhão state, Brazil.

Neospora caninum				*Toxoplasma gondii*			
Titers	**Pos[a]**	**Freq[b] (%)**	**95% CI**	**Titers**	**Pos[a]**	**Freq[b] (%)**	**95% CI**
100	42	41.6	31.9-51.8	**64**	32	28.1	20.1-37.3
200	42	41.6	31.9-51.8	**256**	33	29.0	20.8-38.2
400	16	15.8	9.3-24.5	**1024**	24	21.0	14.0-29.7
800	1	1	0.0-5.4	**4096**	5	4.4	1.4-9.9
				>4096	20	17.5	11.1-25.8
Total	**101**	**100.00**	**22.3-31.1**		**114**	**100.00**	**25.4-34.5**

[a] = Positives; [b] = Frequency; 95% CI = 95% confidence interval.

Table 6. Multivariate logistic regression for seroprevalence of *T. gondii* and *N. caninum* in goats.

Variables	Category	OR	95% CI	P-value
***Toxoplasma gondii*[a]**				
Age	> 3 years	2.4	1.4-4.2	0.002
Presence of other species	Yes	4.6	1.3-16.2	0.019
Purpose of breeding	Family consumption	2.0	1.1-3.5	0.023
Supplementation	Yes	1.6	0.9-2.8	0.010
Intercept				<0.001
***Neospora caninum*[b]**				

Presence of drinking trough	No	1.9	0.8-4.4	0.141
Age	> 3 years	2.1	1.2-3.8	0.011
Presence of cats	Yes	2.6	1.3-5.7	0.011
Type of sheepfold floor	Suspended slat	2.3	1.3-3.8	0.003
Feeding	Native pasture	2.7	1.4-5.1	0.003
Intercept				<0.001

[a] = AIC: 432.9; Pseudo R^2: McFadden 0.09; Teste de Hosmer e Lemeshow: $\chi^2 = 0.725$; p = 0.999
[b] = AIC: 414.8; Pseudo R^2: McFadden 0.09; Teste de Hosmer e Lemeshow: $\chi^2 = 2.190$; p = 0.975
OR = Odds Ratio; 95% CI = 95% confidence interval.

Supplementary material 3. Univariate analysis of risk factors associated with neosporosis and toxoplasmosis in goats from Maranhão State, Brazil.

Variables	Positive/ Total (%)	OR	95% CI	P-value
Neospora caninum				
Age (years)				
> 3	27/69 (39.13)	-	-	-
1 – 3	41/174 (23.56)	2.08	1.09-3.93	0.023
≤ 1	32/137 (23.36)	2.10	1.07-4.12	0.028
Type of sheepfold floor				
Ground	36/180 (20.00)	1.90	1.16-3.13	0.010
Suspended slat	65/202 (32.18)	-	-	-
Rearing system				
Semi-intensive	8/65 (12.31)	2.95	1.33-7.45	0.007
Extensive	93/317 (29.34)	-	-	-
Feeding				
Native pasture	84/263 (31.94)	2.81	1.55-5.34	<0.001
Cultivated pasture	17/119 (14.29)	-	-	-
Pasture area cultivated				
No	43/129 (33.33)	1.68	1.02-2.75	0.040
Yes	58/253 (22.92)	-	-	-
Cats having access to the feed deposits				
No	90/360 (25.00)	2.99	1.13-7.89	0.020
Yes	11/22 (50.00)	-	-	-
Worming				
No	21/48 (43.75)	2.46	1.25-4.80	0.006
Yes	80/334 (23.95)	-	-	-
Place of slaughter of animals				
Inside farm	98/332 (29.52)	6.54	2.03-33.62	0.001
Outside farm	3/50 (6.00)	-	-	-
History of abortion				
No	6/68 (8.82)	4.47	1.85-13.08	<0.001
Yes	95/314 (30.25)	-	-	-
Presence of dogs				

No	7/59 (11.86)	3.04	1.31-8.23	0.009
Yes	94/323 (29.10)	-	-	-
Presence of cats				
No	22/113 (19.47)	1.72	1.01-2.98	0.046[a]
Yes	79/269 (29.37)	-	-	-
Category				
Non castrated male	8/17 (47.06)	-	-	-
Goat kids	32/137 (23.36)	2.89	0.89-9.25	0.07
Castrated male	0/9 (0.00)			
Breeding female	7/35 (20.00)	3.46	0.84-15.07	0.09
Pregnant female	16/52 (30.77)	1.98	0.55-7.04	0.352
Non-pregnant female	38/132 (28.79)	2.19	0.68-6.93	0.209
Presence of other species (poultry / sheep / pigs / cattle)				
No	9/54 (16.67)	1.95	0.89-4.71	0.112
Yes	92/328 (28.05)	-	-	-
Presence of drinking trough				
No	17/48 (35.42)	1.63	0.84-3.08	0.182
Yes	84/334 (25.15)	-	-	-
Breed				
Crossbreed	31/146 (21.23)	1.56	0.94-2.64	0.090
SRD	70/236 (29.66)	-	-	-
Cats or dogs having access to the remains of carcasses				
No	33/153 (21.57)	1.53	0.93-2.57	0.100
Yes	68/229 (29.69)	-	-	-
Toxoplasma gondii				
Age (years)				
> 3	35/69 (50.72)	-	-	-
1 – 3	51/174 (29.31)	2.47	1.34-4.58	0.003
≤ 1	27/137 (19.71)	4.16	2.12-8.29	<0.001
Category				
Non castrated male	6/17 (35.29)	1.31	0.41-4.58	0.810
Goat kids	27/137 (19.71)	2.90	1.63-5.23	<0.001
Castrated male	2/9 (22.22)	2.49	0.45-25.42	0.424
Breeding female	5/35 (14.29)	4.25	1.50-14.94	0.005
Pregnant female	18/52 (34.62)	1.35	0.66-2.81	0.476
Non-pregnant female	55/132 (41.67)	-	-	-
Presence of other species (poultry / sheep / pigs / cattle)				
No	3/54 (5.56)	8.62	2.69-44.16	<0.001
Yes	111/329 (33.74)	-	-	-
Purpose of breeding				
Commercialization	69/275 (25.09)	2.13	1.29-3.50	0.002
Family consumption	45/108 (41.67)	-	-	-
Feeding				

Native pasture	86/264 (32.58)	1.57	0.94-2.68	0.095
Cultivated pasture	28/119 (23.53)	-	-	-
Pasture area cultivated				
No	45/129 (34.88)	1.43	0.88-2.32	0.149
Yes	69/254 (27.17)	-	-	-
Cats having access to herd drinking water				
No	85/307 (27.69)	1.61	0.91-2.81	0.100
Yes	29/76 (38.16)	-	-	-
Fountain water				
Exposed (river, stream, weir, lake)	63/190 (33.16)	1.38	0.87-2.20	0.184
Not exposed (water tanks or similar)	51/193 (26.42)	-	-	-
Presence of drinking trough				
No	19/48 (39.58)	1.65	0.83-3.22	0.155
Yes	95/335 (28.36)	-	-	-
Presence of cats				
No	41/113 (36.28)	1.53	0.93-2.51	0.092
Yes	73/270 (27.04)	-	-	-
Rearing system				
Semi-intensive	25/65 (38.46)	1.61	0.88-2.90	0.125
Extensive	89/318 (27.99)	-	-	-
Supplementation				
No	58/222 (26.13)	1.51	0.95-2.40	0.086
Yes	56/161 (34.78)	-	-	-
Breed				
Crossbreed	36/146 (24.66)	1.50	0.92-2.46	0.109
SRD	78/237 (32.91)	-	-	-
Cats or dogs having access to the remains of carcasses				
No	54/153 (35.29)	1.54	0.97-2.46	0.069
Yes	60/230 (26.09)	-	-	-
Worming				
No	18/48 (37.50)	1.49	0.75-2.80	0.278
Yes	96/335 (28.66)	-	-	-
Co-infection (N. caninum and T. gondii)				
Age (years)				
> 3	16/69 (23.19)	-	-	-
1.5 – 3	9/174 (5.17)	5.49	2.14-14.97	<0.001
≤ 1	8/137 (5.84)	4.83	1.82-13.86	0.001
Category				
Non castrated male	4/17 (23.53)	-	-	-
Goat kids	8/137 (5.84)	4.88	1.14-18.59	0.037
Castrated male	0/9 (0.00)			

Breeding female	0/35 (0.00)			
Pregnant female	6/52 (11.54)	2.33	0.42-11.63	0.411
Non-pregnant female	15/132 (11.36)	2.38	0.50-9.14	0.303
Presence of drinking trough				
No	9/48 (18.75)	2.97	1.13-7.22	0.017
Yes	24/334 (7.19)			
Type of sheepfold floor				
Ground	8/180 (4.44)	3.03	1.28-7.99	0.010
Suspended slat	25/202 (12.38)			
Worming				
No	9/48 (18.75)	2.97	1.13-7.22	0.017
Yes	24/334 (7.19)			
Purpose of breeding				
Commercialization	18/274 (6.57)	2.29	1.03-5.03	0.037
Family consumption	15/108 (13.89)			
Cats having access to herd drinking water				
No	22/306 (7.19)	2.18	1.01-4.73	0.073
Yes	11/76 (14.47)			
Feeding				
Native pasture	27/263 (10.27)	2.15	0.84-6.55	0.137
Cultivated pasture	6/119 (5.04)			
Supplementation				
No	15/222 (6.76)	1.75	0.80-3.86	0.175
Yes	18/160 (11.25)			

(-) = reference; OR = Odds Ratio; 95% CI = 95% confidence interval.

CONSIDERAÇÕES FINAIS

Este é o primeiro estudo que estima a soroprevalência de *N. caninum* e *T. gondii* em caprinos a nível mundial e no estado no Maranhão, Brasil. A soroprevalência de *N. caninum* e *T. gondii* em caprinos encontra-se amplamente distribuída em todo o mundo. No estado do Maranhão, a soroprevalência de neosporose e toxoplasmose em caprinos supera as estimativas mundiais.

Estimativas mundiais mostram que a neosporose em caprinos está associada ao aborto, presença de cães e sexo, enquanto a toxoplasmose está associada à idade, sexo, presença de gatos e sistema de produção. No estado do Maranhão, os principais fatores de risco para neosporose e toxoplasmose foram idade, histórico de aborto, presença de cães e gatos, e deficiência de manejos zootécnicos nutricionais e sanitários nas fazendas.

Considerando a importância do *N. caninum* e *T. gondii* na caprinocultura mundial, medidas de controle devem ser adotadas englobando os fatores de risco abordados no presente estudo. Assim, a compreensão desses dados pode ajudar autoridades epidemiológica desenvolver estratégias zootécnicas de prevenção da neosporose e toxoplasmose em caprinos.