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Research paper

The global seroprevalence of *Toxoplasma gondii* in pigs: A systematic review and meta-analysis

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ABSTRACT

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is an important disease with worldwide distribution. Infection can occur from ingesting raw or undercooked infected meat, and among food animal species, pork is known to be one of the main sources of meat-borne infection. Here, we present results of the first systematic review and meta-analysis on the global *T. gondii* seroprevalence in pigs. PubMed/MEDLINE, Scopus, and EMBASE databases were comprehensively searched for relevant studies published between January 1, 1990 and October 25, 2018. We used a random effects model to calculate pooled seroprevalence estimates with 95% confidence intervals (CI) and analyzed data from five continents. We also conducted subgroup and meta-regression analyses to evaluate the effects of geographical and climate variables on pooled seroprevalence rates. Among 1542 publications identified, 148 studies containing 150 datasets were included in the meta-analysis, and comprised 148,092 pigs from 47 countries. The pooled global *T. gondii* seroprevalence in pigs was estimated to be 19% (95%CI, 17–22%; 23,696/148,092), with the lowest seroprevalence in Europe (13%; 10–15%) and highest seroprevalence in Africa (25%; 17–34%) and North America (25%; 19–33%). The seropositivity rates in Asia and South America regions were (21%, 16–26%) and (23%; 17–30%), respectively. A significantly higher *T. gondii* seroprevalence was associated with higher mean annual temperature and lower geographical latitude. The presence of cats on farms was identified as a potential risk factor for *T. gondii* seropositivity (OR, 1.41; 95%CI, 1.00–2.02). Our findings highlight the importance of pigs as a possible source of human *T. gondii* infections.

1. Introduction

Toxoplasmosis is a cosmopolitan zoonotic disease caused by the obligate apicomplexan intracellular parasite *Toxoplasma gondii* (Dubey, 2008). *Toxoplasma* can infect a wide range of warm-blooded vertebrates (Dubey, 2008, 2009; Khademvatan et al., 2013; Rostami et al., 2017) and based on formal reports, over one-third of the human population is seropositive (Foroutan-Rad et al., 2016; Wang et al., 2017). The infection is transmitted by ingestion of sporulated oocysts present in contaminated soil or water directly or on contaminated fruit or vegetables, by ingestion of raw or undercooked meat from infected animals, by vertical transmission from infected mother to fetus, and rarely by blood transfusion or organ transplantation (Dubey, 2008; Fallahi et al., 2018; Foroutan and Majidiani, 2018; Wang et al., 2017).

The seroprevalence of *T. gondii* in humans is different between and within countries depended on geographic regions, social and cultural conditions, climate, and environmental variables (Foroutan-Rad et al., 2016). According to the United States Centers for Disease Control and Prevention (CDC), *T. gondii*, *Salmonella*, and *Listeria* are responsible for over 70% of all mortality due to foodborne illness in the United States

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(Scallan et al., 2011). It has been reported that *T. gondii* alone accounts for approximately 24% of all deaths due to foodborne pathogens (Scallan et al., 2011). *T. gondii* infection in food producing animals has become an important public health issue, as a source for human toxoplasmosis. Although, the safety of meats are constantly under rigorous surveillance, concerns remain regarding the potential risk of transmission of the parasite through consumption of raw/undercooked meat. Transmission of *T. gondii* via meat and meat products depends on cultural practices and hygiene habits and differs by region (Belluco et al., 2016; Guo et al., 2015).

Pigs are susceptible and can acquire *T. gondii* infection in a variety of ways (ingestion of oocysts in feed, water, or the environment or ingestion of infected rodent carcasses), and pork is one of the main sources of human toxoplasmosis in some countries (Belluco et al., 2016; Dubey, 2009; Feitosa et al., 2017; Guo et al., 2015; Hamilton et al., 2015; van der Giessen et al., 2007). Most infections in pigs are subclinical, although infection can cause disease in neonates; unlike some other livestock species, transplacental transmission is not common in pigs (Dubey, 2009).

Historically, bioassay (in mice or cats) has been the gold standard for the definitive diagnosis of *Toxoplasma* infection in food animals, but serological methods are commonly used in epidemiological surveys (Casartelli-Alves et al., 2014; Dubey et al., 2007). The modified agglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA) are the most common serological methods used, while techniques such as the indirect fluorescent antibody test (IFAT), the latex agglutination test (LAT), the direct agglutination test (DAT), the indirect hemagglutination test (IHAT) and western blot are used less frequently (Dubey, 2010). These serological methods have different sensitivity and specificity, and all may differ from results obtained by bioassay. Therefore results using these various methods may not represent a true prevalence rate, and therefore should be considered an apparent prevalence rate of infection (Gardner et al., 2010; Shaapan et al., 2008; Sroka et al., 2008). Despite this limitation, numerous studies have used these methods to investigate the epidemiology of *Toxoplasma* infection in food animals including pigs, sheep and goats (Guo et al., 2015). During recent years, a large number of studies have been published on the seroprevalence of *T. gondii* in pigs, although a gap in knowledge remains for many countries and territories. Considering the public health concern about pork as a source of *T. gondii* infection, summary data on the global prevalence of *T. gondii* infection in pigs would be useful information for public health officials. To our knowledge, there has not been a comprehensive systematic review with meta-analysis regarding the seroprevalence of *T. gondii* infection in pigs. It should be noted that in some studies, one relevant aspect of *T. gondii* seroprevalence in pigs is clustering at the farm level. For example, some farms have high within-farm seroprevalence while other farms do not have infected pigs. Therefore, the main aim of the current systematic review and meta-analysis study was evaluate animal-level *T. gondii* seroprevalence and related risk factors in pigs from a global perspective.

2. Methods

2.1. Search strategy

In the current study, the methodology recommended by the preferred reporting items for systematic reviews and meta-analysis (PRISMA) were applied to report our results (Moher et al., 2015). To assess the seroprevalence of *T. gondii* infection in pigs from a global perspective, the international electronic databases Scopus, PubMed, and EMBASE were searched by two trained authors for relevant studies published from January 1, 1990 until October 25, 2018. A combination of the following search terms were used in our literature searches as follow: ("*Toxoplasma gondii*" OR "*Toxoplasma* infection" OR "Toxoplasmosis") AND ("Pig" OR "Swine") AND ("Prevalence" OR "Seroprevalence" OR "Seroepidemiology"). The bibliographies of identified studies and relevant reviews were manually reviewed to identify other relevant studies that were not initially found through database searching. The main outcome of interest in this meta-analysis was *T. gondii* seroprevalence in pigs; studies with the highest relevance were retrieved and reviewed in depth.

2.2. Study selection and data extraction

After removal of duplicate references and the primary screening based on title and abstract, eligible citations were chosen for full-text download through online resources. Final eligibility and inclusion criteria were appraised by two trained investigators (M. F and A. T) and contradictions or disagreements were resolved through discussion and consensus with a third reviewer (A. R). Subsequently, two authors (M. F and S. E) extracted the required information, and the others (A. T and S. N) rechecked them. Any conflicts of opinion or disagreement was resolved by the lead investigator (A. R). The following inclusion criteria were defined for this systematic review and meta-analysis study: (1) peer-reviewed original articles, brief reports, or letters to the editors without geographical limitation; (2) cross-sectional investigations which estimated the *T. gondii* seroprevalence in pigs; (3) studies published in English; (4) studies published or released online between January 1, 1990 and October 25, 2018; (5) studies for which the full texts were available; (6) reports with information on the total sample size and positive samples; (7) studies that used serological techniques. Each article that did not fulfill the aforementioned inclusion criteria was excluded from further consideration. Finally, a data collection form was prepared in Microsoft Excel software and the following variables were extracted for each study including, the first author's last name, year of publication, continent, country, geographic location (latitude and longitude), relative humidity, mean annual temperature, annual rainfall, serological method used, total sample size, number of positive samples, sex and age of pigs, and the presence or absence of cats on the farm. With respect to age, we divided pigs into two sub-groups ($\leq$12 months and $\geq$13 months); if a single study had animals from both age groups, we extracted the data for both groups.

2.3. Data synthesis and statistical analysis

Meta-analyses were performed as described previously (Rostami et al., 2017, 2018). In this study, we estimated animal-level of *T. gondii* seroprevalence in pigs. First, we estimated the seroprevalence of *T. gondii* (at 95% confidence intervals [CI]) for individual countries and subsequently for continents by synthesizing the seroprevalence rates of all studies from each country or continent. The ratio of positive samples to total samples was defined as seroprevalence and was estimated using the Metaprop command (Nyaga et al., 2014). Heterogeneity between studies was calculated by Cochran *Q* and I^2 statistics and a χ^2 test with a *P* value of < 0.05 and an I^2 statistic with a cut off of 50%, (Higgins et al., 2003) were used to define a statistically significant degree of heterogeneity. In the current study, I^2 was substantial; therefore, we used a random effects model at a 95% CI, to give a more conservative estimate of the country, continent, and global *T. gondii* seroprevalence.

To evaluate the source of heterogeneity among studies, subgroup and meta-regression analyses were performed. We did subgroup analyses based on geographical latitude, longitude, relative humidity, mean annual temperature, annual precipitation, age group of pigs ($\leq$12 months and $\geq$13 months), gender (male and female) and presence or absence of cats on the farms. The seroprevalence rates across individual parameters were compared by an $\chi 2$ test and generation of an odds ratio (OR) and related 95%CI. For this analysis, the subgroup with the lowest prevalence was considered as the reference, and others were compared with it. Moreover, due to different sensitivities and specificities of serological methods, we assumed that our results would be "apparent" seroprevalence rates, and did not represent true

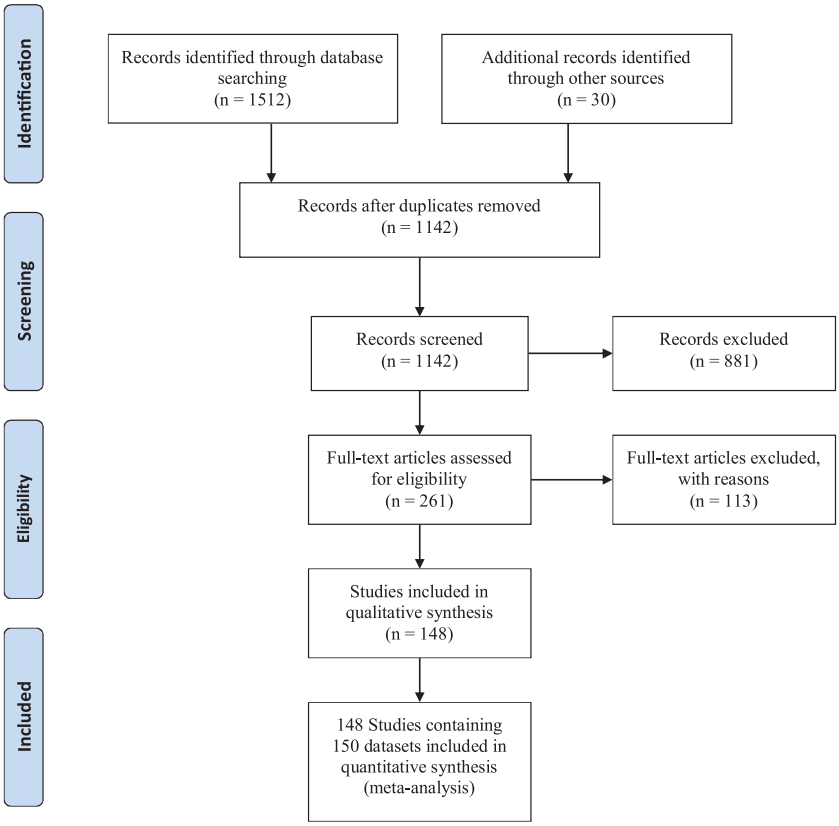

Fig. 1. PRISMA chart of the study selection process showing inclusion and exclusion of studies.

seroprevalence rates. To minimize the biases, we performed a subgroup analysis based on diagnostic methods. We also did meta-regression analyses to evaluate the influence of geographical and climate parameters on the seroprevalence rate of *T. gondii* in pigs. Furthermore, to identify trends of seroprevalence over time, we did a meta-regression analysis based on year of study. Since we evaluated prevalence data, we did not undertake a publication bias assessment (Fakhri et al., 2018; Haghighi et al., 2018; Sabbagh et al., 2018). STATA software version 13 (STATA Corp., College Station, Texas) was used for all statistical analyses, and a P value < .05 was considered significant.

3. Results

3.1. Study characteristics

We identified a total of 1542 records following the initial search of databases; after removing duplicates and/or non-eligible papers, 148 articles containing 150 datasets were eligible to be included in this systematic review and meta-analysis (Fig. 1). These 150 datasets included 148,092 pigs from 47 countries and five continents (28 from Asia [26,431 pigs] (Chandrawathani et al., 2008; Chang et al., 1991, 2013; Devleesschauwer et al., 2013; Du et al., 2012; Fan et al., 2004; Huang et al., 2010; Huong and Dubey, 2007; Ichikawa-Seki et al., 2015; Inoue et al., 2001; Jiang et al., 2014; Liu et al., 2012; Matsuo et al., 2014; Rajamanickam et al., 1990; Roqueplo et al., 2011; Shu et al., 2011; Tao et al., 2011; Thiptara et al., 2006; Tuda et al., 2017; Wang et al., 2016; Wu et al., 2012a, 2017; Wu et al., 2012b; Xu et al., 2015, 2014; Yu et al., 2011; Zhou et al., 2010; Zou et al., 2009), 51 from Europe [58,354 pigs] (Bacci et al., 2015; Balea et al., 2012; Bartova and Sedlak, 2011; Berger-Schoch et al., 2011; Damriyasa and Bauer, 2005; de Sousa et al., 2006; Deksne and Kirjusina, 2013; Djokic et al., 2016; Edelhofer, 1994; Esteves et al., 2014; Felin et al., 2015; Garcia-Bocanegra et al., 2010a, b; Gazzonis et al., 2018; Halova et al., 2013; Hejlíček and Literák, 1993; Hernandez et al., 2014; Herrero et al., 2016; Hirvela-Koski, 1992; Holec-Gasior et al., 2010; Kijlstra et al., 2004; Klun et al., 2006, 2011; Kofoed et al., 2017; Kuruca et al., 2016, 2017; Limon et al., 2017; Lopes et al., 2013; Lunden et al., 2002; Macaluso et al., 2018; Meerburg et al., 2006; Pablos-Tanarro et al., 2018; Papatsiros et al., 2016; Papini et al., 2017; Pastiu et al., 2013; Pipia et al., 2018; Powell et al., 2016; Santoro et al., 2017a; Santoro et al., 2017b; Slany et al., 2016; Sroka et al., 2008, 2011; Sroka et al., 2007; Steinparzer et al., 2015; Turcekova et al., 2013; van der Giessen et al., 2007; van Knapen et al., 1995; Veronesi et al., 2011; Villari et al., 2009; Vostalová et al., 2000; Wallander et al., 2016), 27 from North America [50,325 pigs] (Alvarado-Esquivel et al., 2012, 2011; Alvarado-Esquivel et al., 2014, 2015; Arias et al., 1994; Assadi-Rad et al., 1995; Chikweto et al., 2011; Correa et al., 2008; Davies et al., 1998; Dubey et al., 2002, 2012; Dubey et al., 1991, 1995a; Dubey et al., 1995c; Dzib-Paredes et al., 2016; Gajadhar et al., 1998; Gamble et al., 1999; García-Vázquez et al., 1993; Gebreyes et al., 2008; Hamilton et al., 2015; Hernandez-Cortazar et al., 2016; Hill et al., 2010; Poljak et al., 2008; Saavedra and Ortega, 2004; Sharma et al., 2015; Smith, 1991; Smith et al., 1992), 34 from South America [9,883 pigs] (Azevedo et al., 2010; Basso et al., 2013; Bezerra et al., 2009; Cademartori et al., 2014; Caporali et al., 2005; Carletti et al., 2005; Cavalcante et al., 2006; Clementino Andrade et al., 2013; de Sousa et al., 2014; dos Santos et al., 2005, 2015; Dubey et al., 1992; Feitosa et al., 2017, 2014; Frazao-Teixeira and de Oliveira, 2011; Freitas et al., 2009; Luciano et al., 2011; Magalhaes et al., 2017; Marques-Santos et al., 2017; Millar et al., 2008; Moura et al., 2007; Munoz-Zanzi et al., 2012; Muraro et al., 2010; Pardini et al., 2012; Pezerico et al., 2007; Piassa et al., 2010; Saavedra and Ortega, 2004; Samico-Fernandes et al., 2015, 2017; Samico Fernandes et al., 2012; Suarez-Aranda et al., 2000; Trevisani et al., 2013; Venturini et al., 2004), nine from Africa [3,050 pigs] (Abdel-Hafeez et al., 2015; Arko-Mensah et al., 2000; Bamba et al., 2017, 2016; Gebremedhin et al., 2015; Hove and Dubey, 1999; Hove et al., 2005; Onyiche and Ademola,

Table 1
Global, regional and national pooled seroprevalence of *Toxoplasma gondii* in pigs (results from studies performed in 47 countries).

WHO regions/ country	Number studies	Positive samples/total samples	Pooled prevalence (95% CI)	Weight	Heterogeneity			
					χ^2	df	I^2 (%)	*P* value
Global	**150**	**23,696/148,092**	**19% (17-22%)**	**100**	20975.**69**	**149**	**99.29%**	< **0.001**
Asia	**28**	**6806/26431**	**21% (16-26%)**	**18.69**	**2647.92**	**27**	**98.98%**	< **0.001**
China	17	4612/19997	24% (19-30%)	11.56	1232.95	16	98.70%	< **0.001**
Indonesia	3	50/723	7% (1-15%)	2.01	NE	2	NE	NE
Taiwan	2	1860/3991	47% (45-48%)	1.34	NE	1	NE	NE
Malaysia	2	19/222	6% (3-9%)	1.30	NE	1	NE	NE
Thailand	1	10/14	71% (42-92%)	47.00	NE	1	NE	NE
Vietnam	1	87/742	27% (24-31%)	0.68	NE	0	NE	NE
Nepal	1	160/587	12% (9-14%)	0.68	NE	0	NE	NE
Japan	1	8/155	5% (2-10%)	0.66	NE	0	NE	NE
Europe	**51**	**6431/58354**	**13% (10-15%)**	**34.32**	**4293.7**	**50**	**98.84%**	< **0.001**
Italy	8	864/6225	23% (13-36%)	5.25	624.35	7	98.88%	< **0.001**
Spain	5	1435/8573	19% (13-26%)	3.42	238.49	4	98.32%	< **0.001**
Czech Republic	4	377/4152	10% (1-26%)	2.71	435.86	3	99.31%	< **0.001**
Netherlands	4	475/7560	5% (1-12%)	2.74	390.67	3	99.23%	< **0.001**
Serbia	4	263/1293	25% (12-42%)	2.52	90.55	3	96.69%	< **0.001**
Poland	4	548/3119	18% (13-24%)	2.69	37.42	3	91.98%	< **0.001**
Portugal	3	104/968	11% (6-16%)	2.02	38.1	2	92.8%	< **0.001**
Finland	2	90/3200	3% (2-3%)	1.37	NE	1	NE	NE
Austria	2	434/6065	7% (7-8%)	1.37	NE	1	NE	NE
Sweden	2	97/1779	5% (4-7%)	1.37	NE	1	NE	NE
UK	2	121/2691	4% (4-5%)	1.37	NE	1	NE	NE
Romania	2	1016/4029	25% (24-26%)	0.67	NE	0	NE	NE
Switzerland	1	63/270	23% (18-29%)	1.37	NE	0	NE	NE
Germany	1	140/1500	9% (8-11%)	0.68	NE	0	NE	NE
Latvia	1	34/803	4% (3-6%)	0.67	NE	0	NE	NE
Slovakia	1	21/970	2% (1-3%)	0.67	NE	0	NE	NE
Ireland	1	15/317	5% (3-8%)	0.67	NE	0	NE	NE
France	1	248/3595	7% (6-8%)	0.67	NE	0	NE	NE
Greece	1	26/609	4% (3-6%)	0.67	NE	0	NE	NE
Estonia	1	22/382	6% (4-9%)	0.67	NE	0	NE	NE
Denmark	1	38/254	15% (11-20%)	0.67	NE	0	NE	NE
North America	**27**	**7447/50325**	**25% (19-33%)**	**18.04**	**8199.72**	**26**	**99.68%**	< **0.001**
USA	12	5930/35054	25% (15-36%)	8	1936.35	33	98.30%	< **0.001**
Mexico	7	841/4149	32% (18-48%)	4.65	5197.4	11	99.79%	< **0.001**
West Indies	3	162/541	33% (17-52%)	1.98	524.17	6	98.86%	< **0.001**
Canada	3	421/10291	5% (0-15%)	2.06	NE	2	NE	NE
Costa Rica	1	216/496	44% (39-48%)	0.68	NE	2	NE	NE
Panama	1	93/290	32% (27-38%)	0.67	NE	0	NE	NE
South America	**34**	**2136/9883**	**23% (17-30%)**	**22.33**	**1936.35**	**33**	**98.30%**	< **0.001**
Brazil	27	1386/7974	20% (14-27%)	17.66	1235.89	26	97.90%	< **0.001**
Argentina	3	404/827	48% (39-58%)	0.68	NE	2	NE	NE
Peru	2	99/573	30% (24-36%)	1.3	NE	1	NE	NE
Hawaii	1	247/509	49% (44-53%)	2.01	NE	0	NE	NE
Chile	1	30/340	9% (6-12%)	0.67	NE	0	NE	NE
African region	**9**	**875/3050**	**25% (17-34%)**	**6.02**	**229.64**	**8**	**96.52%**	< **0.001**
Zimbabwe	3	149/882	12% (1-31%)	1.99	NE	2	NE	NE
Burkino Faso	2	209/723	29% (26-32%)	1.35	NE	1	NE	NE
Ghana	1	260/641	41% (37-44%)	0.68	NE	0	NE	NE
Nigeria	1	88/302	29% (24-35%)	0.67	NE	0	NE	NE
Ethiopia	1	129/402	32% (28-37%)	0.68	NE	0	NE	NE
Egypt	1	40/100	40% (30-50%)	0.64	NE	0	NE	NE
Oceania	**1**	**1/49**	**2% (1-4%)**	**0.60**	NE	**0**	NE	NE
New Caledonia	1	1/49	2% (1-4%)	0.60	NE	0	NE	NE

NE: Not existent, UK: United Kingdom, USA: United States; df: degrees of freedom.

2015; Pandey and Van Knapen, 1992), and one from Australia [49 pigs]) (Roqueplo et al., 2011). The countries with the highest number of formal reports were Brazil (27 studies), China (17 studies), the United States (12 studies), Italy (eight studies), and Mexico (seven studies) (Table 1). The serological methods used included the enzyme-linked immunosorbent assay (ELISA, 53 datasets), the modified agglutination test (MAT, 34 datasets), the immunofluorescence assay test (IFAT, 28 datasets), the indirect hemagglutination test (IHA, 13), the direct agglutination test (DAT, seven datasets) and the latex agglutination test (LAT, seven datasets), western blot (WB, five datasets), the Sabin-Feldman dye test (SFT, two datasets) and the complement fixation test (CFT, one dataset). The main study characteristics, sample size, and seropositivity rate of *T. gondii* infection in pigs are presented in Supplementary Table 1.

3.2. Results of meta-analysis

In this systematic review, the global pooled seroprevalence of *T. gondii* infection in pigs was estimated to be 19% (95% CI: 17–22%; 23,696/148,092) (Table 1). In subgroup analysis, the highest pooled seroprevalence was observed in Africa 25% (95% CI: 17–34%; 875/3,050) followed by North America 25% (95% CI: 19–33%; 7,447/50,325), South America 23% (95% CI: 17–30%; 2,136/9,883), Asia 21% (95% CI: 16–26%; 6,806/26,431) and Europe 13% (95% CI: 10–15%; 6431/58,354). Only one study was performed in Australia and reported a seroprevalence of 2% (95% CI: 1–4%) in 49 pigs. *T. gondii*

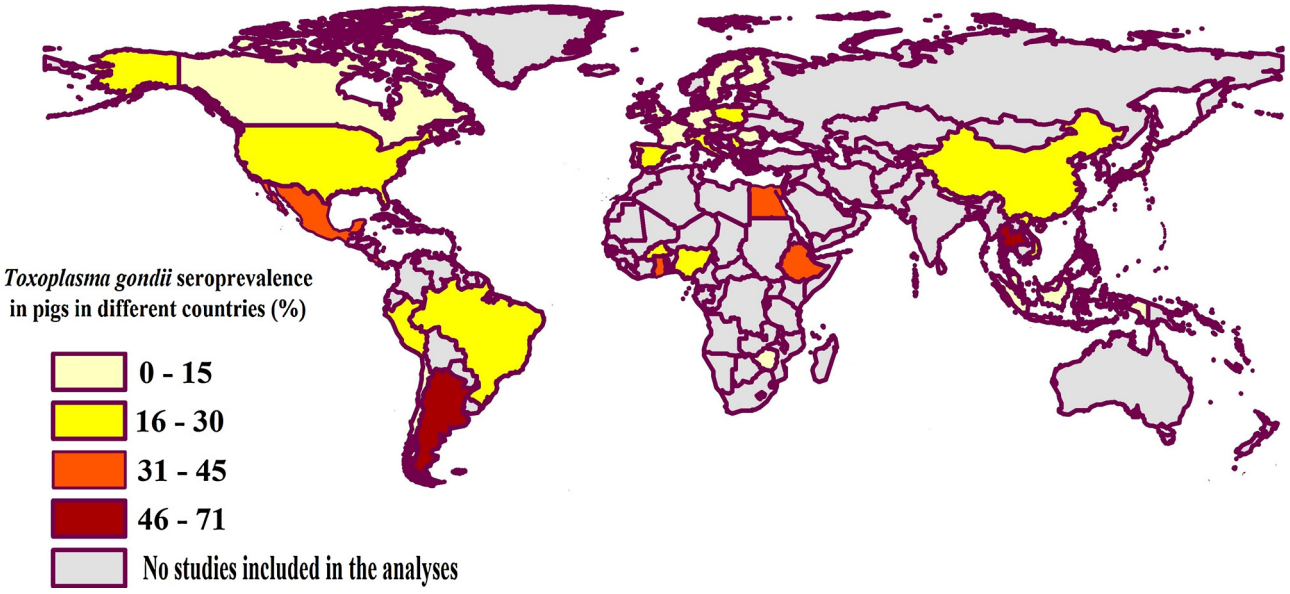

Fig. 2. *Toxoplasma gondii* seroprevalence in pigs from different countries.

seroprevalence in pigs in all 47 individual countries is illustrated in Table 1 and Fig. 2.

In subgroup analysis by geographical latitude, the highest and lowest seroprevalence rates were found at latitudes of 11-20° (30%; 22–39%) and ≥51° (7%; 5–9%), respectively. With respect to geographical longitude, the highest *T. gondii* seroprevalence was estimated at longitudes of 61-80° (40%; 23–58%), while the lowest seroprevalence rate was found at longitudes of ≥ 121° (12%; 6–20%). Considering relative humidity, mean temperature, and precipitation rate the highest seroprevalence rates were observed at 51–75% (21%; 18–25%), 20–30 °C (24%; 18–29%), and 0–250 mm (26%; 22–30%) (Table 2). We compared seroprevalence across individual parameters in the analyzed subgroups using an $\chi 2$ test and univariate analysis. Results of these analyses are presented in Table 2.

Eight papers reported seroprevalence based on age. In subgroup analysis, the pooled *T. gondii* seroprevalence was 18% (95%CI: 10–28%; 295/1,388) and 12% (95% CI: 3–24%; 396/2,765) in pigs aged ≥13 months and ≤12 months, respectively (OR, 1.5; 95% CI, 0.48–4.67), but the difference was not significant. With respect to gender, a slightly higher seroprevalence rate was observed in females (28%; 95%CI: 20–36%; 1,137/4,140) as compared with males (23%; 95%CI: 16–29%; 620/2,644), but the difference was not significant (OR, 1.22; 95%CI, 0.86–1.72). As shown in Table 2, subgroup analysis demonstrated that the seroprevalence rate was significantly higher (OR, 1.41; 95% CI, 1.00–2.02; $P = < 0.001$) for farms where cats were present (24%; 17–31%) compared with farms without cats (17%; 12–22%). In subgroup analyses, we observed approximately equal seroprevalence rates for the main diagnostic methods as follows: ELISA (20%; 95%CI, 15–25), MAT (19%; 95%CI, 14–24), IFAT (20%; 95%CI, 15–25) and IHA (22%; 95%CI, 16–28). Seroprevalence rates using other diagnostic methods are shown in Table 3.

We investigated the effects of geographical and climate variables on *T. gondii* seroprevalence in pigs by separate meta-regression in both linear and non-linear models. A linear model was used first, and if a non-significant result was obtained, we made an attempt to use a non-linear model. If a significant or non-significant result was achieved in both models, we reported only the result of the linear model. According to meta-regression outputs, a significant decreasing trend of seropositivity was observed with elevation in geographical latitude (C = -0.0037; $P <$ 0.001; Fig. 3A). Based on longitude, in the non-linear model, we found that the *T. gondii* seroprevalence increased significantly with increasing longitude (0-90°), (C = 0.038, $P =$ 0.005), while at higher longitudes this value decreased (Fig. 3B). Also, a significant, increasing trend in seroprevalence was associated with higher mean annual temperature (C = 0.005; *P* value = 0.002; Fig. 3C). Meta-regressions on annual precipitation and relative humidity showed increasing trends, but did not identify significant differences in areas with higher annual precipitation (C = 0.00004; $P =$ 0.88; Supplementary Fig. 1A) and higher relative humidity (C = 0.00042, $P =$ 0.97; Supplementary Fig. 1B). Finally, meta-regression on year of publication showed increasing prevalence over time (C = 0.0017, $P =$ 0.36; Fig. 3D), but this increase was not significant.

4. Discussion

Pork is an important source of protein globally and will continue to be so with an increasing world population. It is estimated that the global consumption of pork will grow to around 130 million tons by 2027. Therefore, pork, especially undercooked, raw, or dry cured pork products, could be a major contributor to the transmission of meat borne pathogens including *T. gondii*. For these reasons, a global estimate of *T. gondii* infection would be useful for health policy-makers with respect to food safety.

In this report, we present the first meta-analysis estimating the prevalence of *T. gondii* infection in pigs by country, by continent, and globally. This study included data from 47 countries. Considering that there are approximately 50 Muslim countries where consumption of pork is forbidden, the countries and the sample size of pigs included here could represent a real estimate of the global status of *T. gondii* infection in pigs. The top 10 pork-producing countries are include China, the European Union (countries with highest production: Germany, Spain, Denmark, Netherland and Belgium), the United States, Brazil, Russia, Vietnam, Canada, the Philippines, South Korea and Mexico (available at: https://www.statista.com/statistics/273232/net-pork-production-worldwide-by-country/). Five of the countries in the EU shipped more than 50% of all pork exports in 2017, followed by the United States at 15.2%, Canada at 8.4% and Brazil at 4.9% (available at: http://www.worldstopexports.com/pork-exports-by-country/). A number studies reported here (17) were from China and showed a seroprevalence rate of 24%; only one study was done in Vietnam and there is no studies from the Philippines. Overall, there were 51 studies from the EU, showing a seroprevalence rate of 13%, but some key countries like Germany (one study with a seroprevalence of 9%) and Denmark (one study with a seroprevalence of 15%) had limited data;

Table 2
Sub-group analysis of the seroprevalence of *Toxoplasma gondii* based on geographical location, climate variables. sex and age of swine, and presence or absence of cats.

Variable/sub-groups	Number studies	Positive samples/total samples	Pooled Prevalence (95% CI)	Weight (%)	Heterogeneity				Univariate-analysis
					χ^2	df	I^2 (%)	*P* value	Odds ratio (95% CI)
Latitude									
0-10°	19	1268/4397	27% (20-35%)	12.48	563.07	18	96.80%	< 0.001	3.86 (2.77-5.38)
11-20°	20	1589/5870	30% (22-39%)	13.02	842.15	19	97.74%	< 0.001	4.29 (3.06-6.01
21-30°	31	5718/22071	20% (15-26%)	20.65	3096.27	30	99.03%	< 0.001	2.86 (2.06-3.97)
31-40°	27	8240/51144	19% (14-24%)	18.24	5512.67	26	99.53%	< 0.001	2.71 (1.96-3.75)
41-50°	30	4880/32614	19% (14-24%)	19.98	3727.24	29	99.22%	< 0.001	2.71 (1.96-3.75)
≥51°	23	2001/31996	7% (5-9%)	15.63	1666.14	22	98.68%	< 0.001	Reference
Longitude									
0-20°	45	5538/49506	14% (12-17%)	30.21	3639.11	44	98.79%	< 0.001	1.17 (0.65-2.09)
21-40°	22	2054/12986	18% (13-25%)	14.63	1684.56	21	98.75%	< 0.001	1.50 (0.71-3.16)
41-60°	19	1065/6806	16% (10-23%)	12.53	999.73	18	98.20%	< 0.001	1.33 (0.67-2.67)
61-80°	15	1787/6534	40% (23-58%)	9.83	2711.72	14	99.48%	< 0.001	3.33 (1.63-6.83)
81-100°	17	5733/39626	24% (16-33%)	11.25	5751.76	16	99.72%	< 0.001	2.00 (1.01-3.88)
101-120°	23	6575/26234	22% (16-28%)	15.59	2779.6	22	99.21%	< 0.001	1.43 (0.98-3.41)
≥ 121	9	944/6400	12% (6-20%)	5.97	538.81	8	98.52%		Reference
Humidity									
≤50	4	380/2076	20% (12-29%)	2.71	57.97	3	94.82%	< 0.001	1.82 (0.75-1.84)
51-75	86	16,279/99961	21% (18-25%)	57.49	13685.2	85	99.38%	< 0.001	1.24 (1.04-1.47)
≥76	60	7037/46055	17% (13-21%)	39.82	7171.44	59	99.18%	< 0.001	Reference
Mean Temperature									
20-30	52	5468/20529	24% (18-29%)	34.25	3973.19	51	98.72%	< 0.001	1.71(1.15-2.56)
10-19	70	13439/89931	19% (16-22%)	46.77	9678.27	69	99.29%	< 0.001	1.36 (0.95-1.95)
≤9	28	4789/37632	14% (9-20%)	18.98	5349.04	27	99.50%	< 0.001	Reference
Precipitation									
0-250 mm	26	6092/26640	26% (22-30%)	17.04	1012.7	25	97.53%	< 0.001	2.89 (2.05-4.08)
251-500	37	5464/40372	17% (12-24%)	24.93	1135.92	23	97.98%	< 0.001	1.89 (1.19-3.00)
501-1000	61	9772/69457	17% (14-21%)	40.94	8030.45	60	99.25%	< 0.001	1.89 (1.19-3.00)
1001-2000	24	2336/11293	21% (16-27%)	15.78	8473.98	36	99.58%	< 0.001	2.33 (1.56-3.50)
≥2001	2	32/330	9% (6-13%)	1.32	.	1	.%	.	Reference
Age									
≥13 months	8	295/1388	18% (10-28%)	45.99	99.72	6	93.98%	< 0.001	1.50 (0.48-4.67)
≤12 months	8	396/2765	12% (3-24%)	54.01	484.75	7	98.56%	< 0.001	Reference
Gender									
Female	18	1137/4140	28% (20-36%)	50.41	608.26	17	97.21	< 0.001	1.22 (0.86-1.72)
Male	18	620/2644	23% (16-29%)	49.59	264.71	17	93.58	< 0.001	Reference
Cat presence									
Yes	7	495/2366	24% (17-31%)	54.33	47.41	4	91.56%	< 0.001	1.41 (1.00-2.02)
No	7	355/2299	17% (12-22%)	45.67	14.78	4	72.93%	< 0.001	Reference

df: degrees of freedom.

there was no data from Russia. There were good number of studies for North American countries (27 studies with a seroprevalence of 25%) and Brazil (27 studies with a seroprevalence of 20%). Overall, our study identified some key countries with and without data, and emphasize the need for further studies and more attention to *T. gondii* infection in pigs in these countries.

Our findings showed that the overall global seroprevalence of *T. gondii* infection in pigs was almost 19%. This seroprevalence rate is slightly lower than *T. gondii* seroprevalence in wild boar (23%) reported from our previous study (Rostami et al., 2017), although we found substantial differences in seroprevalence rates between the different continents and countries. Seroprevalence rates for pigs and wild boar were 13% and 26% in Europe, 21% and 13% in Asia, 25% and 32% in North America and 23% and 5% in South America, respectively. Seroprevalence rates for *T. gondii* infection in wild boar in the U.S. and European countries was higher than seroprevalence rates reported from pigs, while in China and Brazil seroprevalence rates in wild boar were lower than rates reported for pigs. These differences could be explained

Table 3
Heterogeneity of seroprevalence of *Toxoplasma* infection in pigs based on the method of detection.

Detection method	Number of studies	Pooled Prevalence (95% CI)	Heterogeneity		
			χ^2	df	I^2 (%)
Enzyme-linked immunosorbent assay (ELISA)	53	20% (15–25%)	10497.5	52	99.5
Modified agglutination test (MAT)	34	19% (14–24%)	4480.7	33	99.3
Immunofluorescence antibody test (IFAT)	28	20% (15–25%)	1902.6	27	98.6
Indirect Hemagglutination (IHA)	13	22% (16–28%)	567.6	12	97.9
Direct agglutination test (DAT)	7	18% (10–27%)	820.7	6	99.3
Latex agglutination test (LAT)	7	10% (5–16%)	94.5	6	93.6
Western blot	5	27% (4–60%)	868.5	4	99.5
Sabin-Feldman dye test (SFT)	2	6% (5–7%)	NA	1	NA
Complement fixation test (CFT)	1	1% (1–1%)	NA	0	NA
Overall	150	19% (17–22%)	20975.7	149	99.30

df, degrees of freedom; **NA**, not applicable.

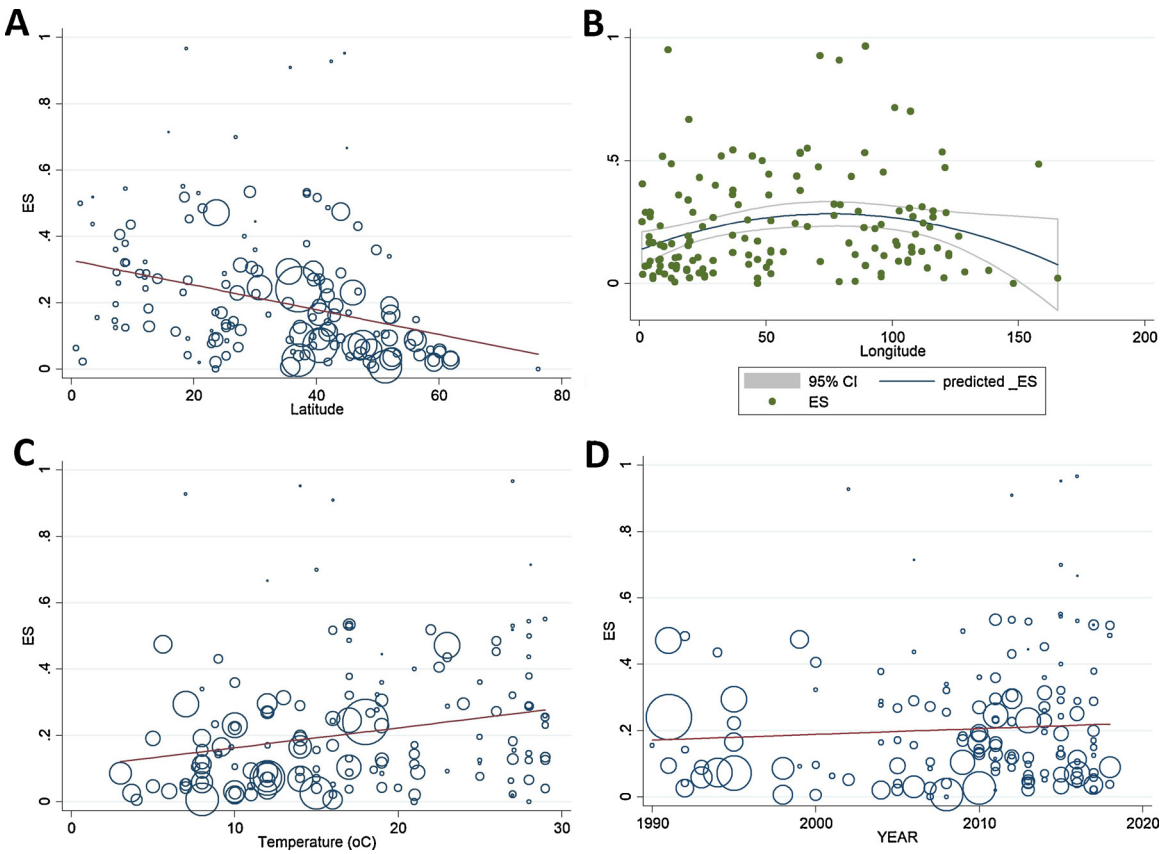

Fig. 3. Results of meta-regression analyses of the effects of geographical latitude (A), longitude (B), mean annual temperature (C), and publication year (D), on the *T. gondii* seroprevalence in pigs. ES: effect size (seroprevalence).

by different diets of pigs and wild boar in these various regions and also different agricultural practices and environmental hygiene.

In the present study, the lowest *T. gondii* seroprevalence in pigs was found in European countries, while seroprevalence rates in Asian, African, North American and South American countries were relatively similar (21–25%). These variations in seroprevalence rates by different geographical areas could be due to several reasons, including climate, animal production systems, and specific control measures. The lower seroprevalence rate in European countries could be explained by this fact that there are more pig farms under intensive management and animal husbandry systems may result in less opportunity for exposure to *T. gondii* in comparison with other countries (Herrero et al., 2016). Several studies have shown that implementation of good management practices such as effective rodent control programs, prevention of access to cats, good hygiene in production facilities, good manufacturing and storage practices for feed, and sourcing of clean, uncontaminated water can lead to a significant decrease in prevalence of *T. gondii* infection in pigs, whereas higher rates of infection are found in farms with poor management or free-range husbandry (Bacci et al., 2015; De Berardinis et al., 2017; Kijlstra and Jongert, 2009; Villari et al., 2009).

In the current meta-analyses, we observed a higher *T. gondii* seroprevalence in pigs aged ≥13 months and in female pigs, but these differences were not significant. *T. gondii* seroprevalence increases with age in humans and many animal hosts (Jittapalapong et al., 2005; Rostami et al., 2017, 2016). An older pig has a longer time for exposure to environmental oocysts, and carcasses of rodents or other animals harboring tissue cysts (Hill et al., 2010; Rostami et al., 2017; Weigel et al., 1995). Considering the presumed lifelong persistence of IgG antibodies against *T. gondii* in infected animals, older hosts are more likely to be seropositive (Rostami et al., 2017). Regarding sex, although it might be an important variable in humans (Jones et al., 2001; Wilking et al., 2016), we do not believe it is significant variable in animals, as under normal conditions, both male and female animals are at equal risk to be exposed to *T. gondii* infection sources. We have shown that the presence of cats on the farm was significantly associated with a higher rate of *T. gondii* seropositivity in pigs. Cats are the definitive host for *T. gondii* and an infected cat can excrete more than 20 million oocysts during a three week infection cycle (Dubey, 1995, 2001). Therfore, cats can be a major contributor to environmental contamination of pig farms (Garcia-Bocanegra et al., 2010b; Ortega-Pacheco et al., 2011).

As reported here, the main serological methods used in the various studies (ELISA, MAT, IFAT and IHA) yielded approximately the same seroprevalence rates. As we mentioned previously, these serological methods have different sensitivities and specificities. Dubey et al. (1995b) showed that sensitivity and specificity were 82.9% and 90.2% for the MAT, 29.4% and 98.3% for IHAT, 45.9% and 96.9% for LAT, and 72.9% and 85.9% for ELISA, when compared with bioassay as the gold standard; the ELISA and MAT had good level of agreement. Several other studies reported good agreement between ELISA and MAT (Gamble et al., 2005; Sroka et al., 2008; Zhu et al., 2012) and it has been shown that IFAT and DAT had moderate agreement with ELISA, MAT and with other serological methods (Garcia et al., 2006; Marca et al., 1996; Seefeldt et al., 1989). These reports demonstrating consistency among the various serological test methods used to detect *T. gondii* infection, suggest that the seroprevalence rates across studies included in this meta-analysis, while classified as apparent seroprevalence rates, could be close to true seroprevalence for *Toxoplasma* infection in pigs.

Our results demonstrated a significant influence of geographical and climate factors on *T. gondii* seroprevalence in pigs. These findings showed significant decreasing and increasing trends in seroprevalence with increases in latitude and temperature, respectively. In fact, these two variables have overlap and are related to the life cycle of *T. gondii*. It is well known that the survival rate of *Toxoplasma* oocysts is higher in

areas with moderate temperatures (15–30 °C) and damp soil, while the survival rate is lower in colder climates (Dubey, 1998; Lindsay et al., 2003; Yan et al., 2016) In agreement with our findings, several other studies reported that areas with high temperatures and moderate rainfall had higher risk of infection with *T. gondii* as compared with regions with cold temperatures (Herrero et al., 2016; Limon et al., 2017; Yan et al., 2016). However, comparisons of seroprevalence rates between areas based on climatic conditions should be made with caution, because there are several confounding factors, notably different management practices (Limon et al., 2017). We also found a slight, although non-significant, increase in global seroprevalence *T. gondii* over time. This increase could be due to increased pork production in some developing countries where modern animal husbandry systems have not yet been adopted (Grace, 2015).

The strengths of this study include the large total sample size, the comprehensive literature search, the rigorous methodology, and several subgroup and meta-regression analyses performed. Moreover, this study has some limitations and the results presented here should be interpreted with regard to these limitations. Limitations include inclusion of reports with limited information on age and sex, some reports with low sample size, high heterogeneity, variations in the sensitivity and specificity of diagnostic methods, and the possibility that our search strategy missed some studies. Due to these limitations, it should be noted that our results may be not reflect a true seroprevalence, and the reported numbers are an apparent seroprevalence. Nevertheless, we believe what we report here is very close to true *T. gondii* seroprevalence in pigs from a global perspective.

In conclusion, our study found that, globally, approximately one fifth of domestic pigs are infected with *T. gondii* infection. Moreover, it was shown that geographical and climate factors have a significant effect on the prevalence rate of infection in pigs. Since pork and pork products are one of the main human food sources, and *T. gondii* seropositivity of pigs correlates with the presence of infective *T. gondii* in pork meat, our findings are important for policy makers in the area of food safety and call for further measures and interventions to control and prevent of *T. gondii* infection in pigs.

Author contributions

All authors conceived the study. M.F., S. N., S.E., A.T. and A. R conducted the searches and collected data. Y. F., S.M.R and A.R analyzed and interpreted the data sets. M. F. A. S., H.R.G and A. R. drafted and edited the manuscript. All authors commented on or edited drafts and approved the final version of the manuscript.

Conflict of interests

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.04.012.

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