Risk factors for sporadic toxoplasmosis: A systematic review and meta-analysis

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Short Title: Meta-analysis on risk factors for sporadic toxoplasmosis

Abstract

Toxoplasmosis is considered as the most prevalent parasitic zoonotic infection worldwide. The parasitic cycle is mostly known, but the relative contribution of different sources and pathways of transmission was not previously studied by a meta-analysis. A systematic review and a meta-analysis of case-control, cohort, and cross-sectional studies were performed to determine the main risk factors associated with sporadic *Toxoplasma* infection. Suitable scientific articles were identified through a systematic literature search and subjected to a methodological quality assessment. Mixed-effects meta-analysis models were adjusted by population type – children, mixed population, and pregnant women – to appropriate data partitions. 187 primary studies passed the quality assessment stage, investigating risk factors for sporadic infection with *Toxoplasma gondii* conducted between 1983 and 2016. Cases were defined by serology.

The meta-analysis of *Toxoplasma* sporadic infections revealed the significance of transmission by environmental factors such as contact with soil and contact with animals, in particular cats. The consumption of raw or undercooked meat and unwashed vegetables significantly increased the odds of acquiring the disease. Shellfish and raw milk were identified as significant sources of toxoplasmosis. Almost all meat categories were identified as risk factors: pork, poultry, beef, processed meat, lamb, and game meat. Contaminated drinking water may play a role in the acquisition of infection. Moreover, the lack of hygiene in preparing food was identified as a risk factor. A significant risk factor for pregnant women is traveling abroad. Lastly, blood transfusion (in pregnant women) and immunocompromised conditions were found associated with positive serology. The broad definition of exposures and the use of serology for the case definition are the main limitations for the interpretation of the results of this meta-analysis. The transmission pathways require further investigations using longitudinal studies and subtyping approaches.

1. **Introduction**

Toxoplasma gondii, an obligate protozoan parasite of the *Apicomplexa* phylum, is a worldwide parasite that can infect humans and a large range of warm-blooded vertebrates. Three major clonal lineages (type I to III) differ in pathogenicity and prevalence around the world, with genotype II dominating in congenital toxoplasmosis cases in Europe and the USA (Hosseini *et al*., 2019). The disease is generally benign, but some severe or life-threatening effects can occur in children (Dunn et *al*., 1999), when the transmission is congenital, and in immunocompromised patients (Robert-Gangneux and Darde, 2012). Since the conventional designation assumed this clonal population structure, other genotypes have been identified worldwide and termed as "atypical" or "exotic" (Dardé *et al*., 2014). Among these, highly virulent strains circulating mainly in South America have been responsible for severe cases in immunocompetent people (Carme *et al*., 2009). Approximately 30% of the human population is considered infected (Montoya and Liesenfeld, 2004). Serological tests are usually used to detect the infection, with detection of anti-*T gondii* specific IgG and/or IgM antibodies (Montoya, 2002). *T.* gondii is globally distributed and results in a high public health impact. The Global Disease Burden 2015 Study estimated that foodborne toxoplasmosis was responsible for 10.3 million (95% UI 7.40–14.9 million) cases in 2010, and 825,000 DALYs (95% UI 561,000–1.26 million) DALYs (Torgerson *et al*., 2015).

The parasitic cycle of toxoplasmosis is complex. During its primary infection, the cat (or other felines), the definitive host, excretes parasites (oocyst form) in its stool. Excretion in cats is limited in time (about two to three weeks) until immunity is established. Oocysts can contaminate the environment: the soil, water, and therefore shellfish that filter water and plant products directly or via irrigation water. Excreted oocysts are not infectious and become infective after sporulation, after few days in the environment depending on climate conditions, and become infectious with long resistance in environmental conditions (oocysts can survive for long periods, up to years, in a favorable environment). The remarkable resistance of the oocyst wall enables the dissemination of *T. gondii* through watersheds and ecosystems, and long-term persistence in diverse foods such as shellfish and fresh produce (Shapiro *et al*., 2019). Humans and all warm-blooded mammals are infected through the environment or food. Parasites encyst in all tissues, especially striated muscles and the brain. These cysts persist throughout life and can be a source of contamination of new hosts through meat ingestion (carnivorism) (Tenter *et al*., 2000).

T. gondii exposure to humans may have multiple origins, and the prevalence is high (and protective for pregnant women). So, numerous epidemiological studies investigate the main transmission pathways of sporadic *T. gondii* infection by serological studies. A systematic review of outbreaks was recently published (Meireles *et al*., 2015), still a systematic review and a meta-analysis of case-control, cohort, and cross-sectional studies have to be performed to determine the main risk factors associated with sporadic *T. gondii* infection. Characterization of risk factors of *T. gondii* could contribute to identify recommendations for susceptible populations such as pregnant women or immunocompromised patients. The objective of this meta-analysis is to summarize the evidence on risk factors for sporadic *T. gondii* infection regardless of the country of origin from relevant scientific information contained in epidemiological case-control/cohort/cross-sectional studies.

2. Material and methods

The protocol of the systematic review and the meta-analysis model are described in depth in the methodological paper of this issue (Gonzales–Barron *et al*., 2019*)*.

2.1 Systematic review

The literature search was conducted between March 2017 and December 2017 using a combination of keywords related to (1) Toxoplasma OR Toxoplasmosis, (2) "case-control" OR "risk factor" OR cohort (3) infection OR disease, joined by the logical connector AND. Relevant studies were identified from five bibliographic search engines, Science Direct, PubMed, Scielo, ISI Web of Science and Scopus. No restrictions were defined for the year of the study or type of publication. The search was limited to the languages English, French, Portuguese and Spanish (Gonzales–Barron *et al*., 2019).

Each reference record was screened for relevance for inclusion in the meta-analysis study, and subsequently, the methodological quality of the "candidate" studies was assessed using pre-set quality criteria comprising (1) appropriate selection of the controls; (2) adjustment to correct for confounders, (3) comparability between cases and controls, (4) acceptable responses rates for the exposed and control groups; (5) Data analysis appropriate to the study design; (6) provision of Odd ratio (OR) with confidence interval or p-value; or provision of sufficient data to calculate ORs; overall quality of the study (Gonzales-Barron *et al*., 2019). Primary studies that passed the screening for relevance were marked as having a potential for bias if they failed to meet at least one of the methodological quality assessment criteria.

Data from primary studies were extracted using a standardized spreadsheet. Data extracted included the relevant study characteristics (location, time period, population, case definition, design, sample size of the groups, type of model, etc.), the categorized risk factors, the setting, the handling practices and the outcome of the study, odds-ratios (ORs). A data categorization scheme was established to hierarchically group the risk factors into travel, host-specific factors, and pathways of exposure (i.e., person-to-person, animal, environment and, food routes – refer to Gonzales-Barron *et al*., 2019). In addition to the standard risk factors, the class "Hygiene" (e.g. "no handwashing after toilet","poor hygiene habits") was also used. Host-specific factors includes blood transfusion.

Considering the parasitic cycle of *T. gondii*, specific partitions were made to investigate more deeply risk factors. The food class "Other red meats" were stratified into lamb, mutton, goat, boar, and venison/horse meat. The route "contact with cats" was also added within "contact with pets". Sexual transmission was found irrelevant and was excluded from the analysis, as humans are not a reservoir of *Toxoplasma*, nor involved in fecal-oral transmission. The variable "Population" was stratified into mixed (adults, or age not defined), pregnant women, and children (under 16 years old). Susceptible population (HIV infection, AIDS, new-borns, liver diseases, chronic kidney diseases, solid organ transplants, mental illnesses, and neurologic diseases) was excluded at the beginning of the study. The acquisition of *Toxoplasma* could be far before the illness (congenital for new-borns) and immunosuppression is by example, a well-known factor of reactivation of a pre-existing infection. Therefore, it was not relevant to study risk factors of acquiring *Toxoplasma* on such populations. Host-specific factors were reduced to immunosuppressive factors that could be linked to a causal pathway of reactivation. Other factors were found irrelevant and excluded because those factors cannot explain the acquisition of the infection (*i.e.* mental illnesses).

2.2 Data synthesis

The full meta-analytical data was first described using basic statistics. Next, data was partitioned into subsets of categories of risk factors ("data partitions"), such as travel, host-specific factors including transmission pathways related to blood transfusion , animal contact, environmental exposures, and food vehicles. Meta-analysis models were then fitted to each of the data partitions, with subgroup class that depends of data partition. For instance, if the data partition was travel, the meta-regression was taking into account the subgroup classes: inside,

abroad, any (Gonzales-Barron et al., 2019). The meta-analytical models were fitted separately by population type. For some food classes, the effects of handling (i.e., eating raw, undercooked) and setting (i.e., eating out) on the overall OR were assessed by the calculation of the ratio of the mean OR when food is mishandled to the base OR (Gonzales-Barron *et al*., 2019).

The statistical analysis was designed to assess the effect of the geographical region, the study period, and the analysis type (univariate/multivariate) on the final result. The objective of the region-specific meta-analysis was to inform the decision on whether the geographical regions were to be maintained for the subsequent pooling of ORs. Geographical regions (Asia, North America, South America, Africa, Europe, Oceania) were removed from a particular meta-analysis partition when their ORs, for this partition, were different from those associated with the other regions or if only less than 3 ORs represented the region (Gonzales-Barron *et al*., 2019).

All meta-analysis models were substantially weighted random-effects linear regression models.Once a meta-analysis model was fitted, influential diagnostics statistics were applied to remove any influential observation originating from studies marked as having potential-for-bias. Publication bias was assessed by funnel plots and statistical tests (Gonzales-Barron *et al*., 2019) and a statistical test investigating the effect of the study sample size on ORs (Tables 2, 3 and 4) (Gonzalez-Barron *et al*., 2019). Heterogeneity between studies was assessed by different indicators such as the between-study variability (τ^2), the QE test investigating residual heterogeneity, the variance of residuals, and the intra-class correlation I^2 (Gonzales-Barron *et al.*, 2019).

All analyses were produced in the R software (R Development Core Team, 2008) implemented with the *metafor* package (Viechtbauer, 2010).

3. Results

3.1 Descriptive statistics

The quality assessment stage was passed by 213 primary studies investigating risk factors for sporadic infection with *T. gondii,* which were conducted between 1983 and 2016 (80.5% after 2000). Excluding susceptible populations other than pregnant women, and some risk factors (see above), 187 publications were retained for meta-analysis (Figure 1 and Appendix 1). Primary studies investigated risk factors in different types of population, namely children (16 studies), mixed population (98 studies), and pregnant women (76 studies) (some of them also

studied other populations) (Appendix 2). The majority of publications concerns, in descending order, South America (32.5%), Asia (30%), Africa (17.5%), Europe (15%), North America (4%), and Oceania (1%). All publications produced 2050 ORs.

Toxoplasmosis is generally asymptomatic, so only the presence of antibodies indicates past infection. Symptomatic forms are observed mainly in children infected by congenital transmission (infection occurring during pregnancy in mothers), in immunocompromised people by reactivation of their infection, and more rarely in immunocompetent people. In primary studies, the toxoplasmosis infection was diagnosed by routine antibody screening for *T. gondii* IgG and/or IgM antibodies. The target populations considered are the mixed population (930 OR), pregnant women (841 OR), and children (185 OR). During the methodological quality assessment, fourteen studies were marked as being possibly affected by bias (Appendix 2). Potential for selection bias was assigned to thirteen studies whose population groups were believed to have a stronger exposure to *Toxoplasma gondii* infection, such as waste pickers and waste workers (Alvarado-Esquivel *et al*., 2008; Alvarado-Esquivel *et al*., 2010), livestock and abattoir workers (Adeyisum *et al*., 2011; Alvarado-Esquivel *et al*., 2011; Alvarado-Esquivel *et al*., 2014b), agricultural workers or rural people living in poverty (Alvarado-Esquivel *et al*., 2013; Cavalcante *et al*., 2006; Doni *et al*., 2015; Rostami *et al*., 2016), veterinary practitioners (Brandon-Mong *et al*., 2015), inmates (Alvarado-Esquivel *et al*., 2014a; Sari *et al*., 2015), and female patients with miscarriages (Tammam *et al*., 2013). The rationale for assigning a potential-for-bias status to the association measures extracted from Gyang *et al.* (2015) related to the statistical approach employed, which was a log-binomial model producing prevalence ratio estimates. As explained in Gonzales-Barron *et al*. (2019), potentially-biased individual ORs were removed only if their influence on the pooled OR estimates was significant, as assessed by the Cook's distance. The risk factors studied concerned the following routes of exposure: host-specific factors (immunocompromised conditions and blood transfusion) (71 OR), lack of personal hygiene (10 OR), travel (21 OR), environmental transmission (476 OR), contact with animals (pets including cats, farm animals, wildlife (511 OR), and food (867 OR).

3.2 Meta-analysis results

For every data partition, the meta-analyzed risk factors are presented in summary tables only when significant (Tables 1, 2 and 3). Pooled ORs were considered as significant when the lower bound of the 95% CI was equal or greater than 1.Non-significant results for main risk

factors are given in Appendix 3. According to this meta-analysis, travel abroad is a significant risk factor for acquiring positive *Toxoplasma* serology for pregnant women (pooled OR=1.878; 95% CI: 1.284 - 2.746) (Table 1). However, it was non-significant for mixed population, whatever the countries of origin or destination.

Immunocompromised conditions, such as cancer, immunosuppressive treatment or HIV infection, were found to be associated with positive *Toxoplasma* serology in the mixed population (14 publications, pooled OR=2.407; 95% CI: 1.483- 3.909). However, it was non-significant for the pregnant population, with seven publications. Blood transfusion was a significant risk factor for acquiring positive *Toxoplasma* serology in pregnant women (pooled OR=1.785; 95% CI; 1.031 - 3.089) (Forest plot in Figure 2). For the mixed population, blood transfusion did not reach significance (OR=1.368; 95% CI: 0.793 - 2.358). Poor personal hygiene (i.e. “poor hands hygiene”) was jointly analyzed for mixed and children populations, and was found significantly associated with positive *Toxoplasma* serology (pooled OR=2.023; 95% CI: 1.693 - 2.416).

Regarding the animal contact pathways, significant associations were found for contact with animals in the mixed, pregnant, and children populations. Occupational contact (raising animals, or contact with animal products) were significant risk factors in both mixed (pooled OR=2.035) and pregnant populations (pooled OR=1.557). Similarly, contact with farm animals was also found significant in the mixed population (pooled OR=1.482; 95% CI: 1.099 - 1.998). Contact with ~~(wild animals)~~ flies/rodents was found significant for all three subpopulations. However, it is a heterogeneous category rather reflective of a relative lack of hygienic conditions of living, with rats or flies inside or near the house (pooled OR from 1.435 to 1.534) (Figure 3). For all the populations studied (mixed, pregnant, or children population), contact with a pet (Table 1), and in particular with cats (Table 2), was a significant risk factor with pooled ORs between 1.631 and 1.711 (Table 2).

For the environmental pathways, consumption of untreated drinking water, farm environment (“living on farm”), contact with wastewater (including “lack of toilets”) and contact with soil (such as gardening, playground) were significantly associated with positive *Toxoplasma* serology for the three target populations (except in children population for playground) (Table 1). For the mixed population, 22 ORs coming from Africa region were excluded, due to the high level of OR, concerning untreated drinking water (9), farm environment (6), and

playground (7). Of course, considering them is not changing the significativity of results concerning those relevant categories (results not shown).

For the children population,one OR from an Oceanian study was excluded, because it was isolated. Six ORs coming from Africa were also excluded, describing exposure to drinking unboiled water and playground, because of the low values of their OR, in comparison with other regions. For the children population, the contact with soil and garden ("playground") was close to reaching significance (pooled OR=1.138; 95% CI: 0.969 - 1.335).

The meta-analysis on food consumption pathways revealed significant associations with meat for the mixed population (pooled OR=1.761; 95% CI: 1.570 - 1.974) and pregnant women (pooled OR=1.960; 95% CI: 1.472 - 2.610) (Table 1). By contrast, in the children population from six publications from South America and Africa, the overall OR associated with meat was not significant (pooled OR=1.329; 95% CI: 0.891 - 1.985; Appendix 3). For the mixed population, almost all meat matrices were identified as risk factors: pork meat (pooled OR= 2.114; 95% CI: 1.411 - 3.169), poultry (pooled OR=1.623; 95% CI: 1.147 - 2.297), beef (pooled OR=1.635; 95% CI: 1.170 - 2.285), processed meat like salami or sausages (pooled OR=1.365; 95% CI: 1.075 - 1.733), other red meat (goat, mutton or lamb) (pooled OR= 1.897; 95% CI: 1.480 - 2.431) and other types of meat (game or undercooked meat) (pooled OR=1.734; 95% CI: 1.544 - 1.947]) (Table 2). For this population, when it was possible, more precise items could be analyzed such as wild boar meat (pooled OR=2.487; 95% CI: 1.814 - 3.409), lamb meat (pooled OR=2.404; 95% CI: 1.189 - 4.859), goat meat (pooled OR=1.667; 95% CI: 1.212 - 2.294), and other types of red meat (such as venison, ram and horse meat) (pooled OR=1.566; 95% CI: 1.221 - 2.008) (Table 2).

In pregnant women, significant sources were beef (pooled OR=2.052; 95% CI: 1.576 - 2.672), other red meat (goat, mutton or lamb) (pooled OR=1.822; 95% CI: 1.279 - 2.595), poultry (pooled OR=1.514; 95% CI: 1.130 - 2.028), processed meat, like cured meat, sausages and salami (pooled OR=1.532; 95% CI: 1.201 - 1.953), and other meat such as undercooked or raw meat (most often in this category) and game meat (sometimes) (pooled OR=1.592; 95% CI: 1.354 - 1.871) (Table 2). The pork consumption was not found significant for pregnant women with a pooled OR=1.04 (95% CI: 0.780 - 1.387). Within the red meat category (including lamb and mutton/sheep), only the consumption of lamb was found significant (pooled OR=1.832; 95% CI: 1.148 - 2.922; Table 2). In children, meat, dairy products, and

produce consumption were not found significantly associated with positive *Toxoplasma* serology.

The consumption of produce was associated with positive *Toxoplasma* serology for the mixed population (pooled OR=1.872; 95% CI: 1.539 - 2.276) and pregnant women (pooled OR=1.651; 95% CI: 1.267 - 2.151) (Table 1). Within produce, only vegetables were identified as a risk factor for the mixed population (pooled OR=1.866; 95% CI: 1.491 - 2.335) and pregnant women (pooled OR=1.372; 95% CI: 1.198 - 1.571) (Table 2). The consumption of seafood in the mixed population (not studied in pregnant nor in children population) (Table1), in particular shellfish ("mollusks") (Table 2), was found in association with positive *Toxoplasma* serology (pooled OR=1.917; 95% CI: 1.395 - 2.636) (Figure 5). Nevertheless, the fish consumption was not found significant associated with toxoplasmosis.

The consumption of eggs was not found to be a determinant of positive *Toxoplasma* serology either in the mixed or in the pregnant population. Dairy products (mainly raw milk) were significant risk factors for the mixed population (pooled OR=1.563; 95% CI: 1.298 - 1.882) (Figure 4) and pregnant women (pooled OR=1.521; 95% CI: 1.116 - 2.073) (Table 1). The consumption of raw milk or raw milk cheese in comparison with pasteurized dairy products significantly increases the risk by a factor of 1.430 (95% CI: 1.073 - 1.905; Table 3). Regarding the effect of the food practices (Table 3), eating undercooked or raw meats or unwashed produce were identified as well as risk factors. The poor handling of foods (mainly "not washing hands before eating" or "using unwashed kitchen utensils") was also found associated with positive *Toxoplasma* serology in pregnant women (Table 1).

For most of the meta-analytical models reported in Tables 1, 2, and 3 the statistical tests indicated the absence of potential significant publication bias. Exceptions were observed in partitions related to food (Table 1), meat (Table 2), for mixed and pregnant population, and practices of cooking meat (Table 3) (all populations). The funnel plots for food and meat in the mixed population (Figure 6) evidences asymmetry at its basis, in an overestimation manner, and underestimation at the top. The weight given in meta-analysis is lower in study outcomes located at the basis than in those falling at the top of the funnel. The funnel plots for food and meat in pregnant women, as for meat cooking practices, show little asymmetry that may lead to some overestimation (Figure 6). The intra-class correlation I^2 indicates significant low (<25%) to moderate (<50% or around 50%) remaining between-study heterogeneity in all

the data partitions. As Q or QE statistic was still significant, the moderator(s) already considered in the meta-analysis models could not fully explain the between-study heterogeneity (Gonzales-Barron et al., 2019).

4. Discussion

The measurement of seroprevalence is an indicator of *T. gondii* infection, but it does not provide information on the time of infection. Besides, there may be a significant lapse of time between contamination and the collection of information on the exposures of infected persons (most often identified by serological research of IgG) during studies. Consequently, the results are conditioned on the absence of any change in the respondents' exposures over time. Cases could have been identified through serological tests whose performances (sensitivity and/or specificity) are not necessarily equivalent between countries and over time. However, we assume that these meta-analyses are not particularly biased (over or under-estimation) due to those parameters. Furthermore, the origin of the infection (congenital or postnatally-acquired) is not known in the primary studies included in this meta-analysis (case-control or transversal studies). Nevertheless, the relative share of congenital infections in the measurement of seroprevalence in children and adults is supposed to be low.

Considering the high prevalence of *Toxoplasma* infection globally, and the diversity of pathways and sources of exposure to humans, the number of publications and investigated risk factors is large and, most publications are recent. In comparison with the systematic reviews of other foodborne pathogens of this special issue, this is the one with the highest number of included case-control, cohort, and cross-sectional studies. As toxoplasmosis outbreaks are rare, studying sporadic cases is the main way to identify potential sources, and to make specific recommendations to populations at-risk such as pregnant women or immunocompromised individuals. Then, our results are based most often on a considerable number of outcomes and are in agreement with other published evidence.

Blood transfusion was identified as a source of toxoplasmosis in this meta-analysis, which may seem at first surprising, but it has been reported as a risk factor in recent case-control studies and molecular tools allow the detection of *Toxoplasma* DNA in blood samples (Saki *et al*., 2019). Blood transfusion is thought to be a rare source of transmission because the duration of parasitemia during acute infection is brief but transmission by thachyzoites stage is conceivable. Immunocompromised conditions were found to be associated with

Toxoplasma gondii infection in mixed population: this result is in agreement with a recent meta-analysis (Wang *et al.,* 2017). However, its interpretation is not straightforward because the serological methods could be less sensitive and specific in immunocompromised individuals (Wang *et al*., 2017).

As expected, exposure to cats appeared as a significant risk factor, which has long been recognized in many case-control studies (Cook *et al*., 2000). Felids, specifically the domestic cat, have a central role as the source of infective oocysts; the implications of increasing cat populations as a public health risk due to toxoplasmosis have been reviewed in Dabritz and Conrad (2010). One consequence has been increased *T. gondii* oocyst dissemination to the environment, associated with a higher risk of transmission to humans and animals. Significant risk factors as "contact with other animals" are probably due to unhygienic contamination, concerning environmental contamination (e.g. soil on the fur), and "contact with flies or rodents" in children, could reflect indirect contamination. Although this risk factor is not often studied, the role of flies and other pests has been identified as vectors by oocyst transport (Frenkel *et al.* 1995). Environmental transmission by oocyst stage appears to be influential; identified risk factors are contact with wastewater associated with poor hygiene, contact with soil, farm environment that aggregates different risk factors not clearly defined, and consumption of untreated or inadequately treated water.

For occupational exposure, farm animals exposure, and farm environment, more precise definition about the raised animals, e.g., sheep vs. cattle vs. pig farming vs. mixed, or their raising conditions, in future studies, could allow to break down those categories for further meta-analysis investigation. Our results are in agreement with a systematic review of outbreaks that showed that in 38 outbreaks (worldwide), 21% could be attributed to contaminated waters and 26% to contact with contaminated soil (Meireles *et al*.,2015). Contamination of water and soils by oocysts was shown in different studies (Aubert and Villena, 2009; Bahia-Oliveira, 2017; Bahia-Oliveira *et al.*, 2003; Shapiro *et al*., 2019).

With regards to food consumption, undercooked meat, unwashed vegetables, raw milk, and shellfish are risk factors for positive *Toxoplasma* serology. Those results are in accordance with the analysis of published outbreaks, showing that raw or undercooked meat was the origin of 44.7% of the outbreaks and raw vegetables of 5.3% (Meireles *et al*., 2015). Furthermore, our results are in line with the meta-analysis conducted by Belluco *et al*. (2018) to assess the risk of humans developing acute *T. gondii* infection, due to the consumption of

undercooked beef, sheep (or meat), which were also found significant. In their study, consumption of raw/undercooked pork, raw eggs, and unpasteurized milk were non-significant risk factors. Concerning the role of meat, raw or undercooked meats were identified as significant risk factors for toxoplasmosis. Within meats, with except for wild boar, the type of meat found is consistent with the literature. Five publications, all conducted in Mexico, identified wild boar meat as a risk factor (which is a low number of studies compared to other risk factors). Game meat also stands out significantly. *T. gondii* has been detected in sheep and lamb (Dubey, 2009; Halos *et al.,* 2010), cattle (Dubey 1986;1992; Fromont *et al*., 2009; Blaga *et al.*, 2019), pork (Djokic *et al., 2016;* Foroutan *et al*., 2019), wild boar (Roqueplo *et al.,* 2017; Rostami *et al*., 2017) and also other animals like poultry (Tonouhewa, 2017; Guo *et al*., 2016).

The identification of raw milk as a risk factor is based on 16 publications for the mixed population and 27 for pregnant women. A recent review on milk consumption and *Toxoplasma* infection reported mainly goat milk as a source of infection (Boughattas, 2017). Moreover, *T. gondii* has been detected in raw milk from infected animals (Cisak *et al.*, 2017; Vismarra *et al*., 2017). Further studies should investigate the contamination in milk (sheep/cows), and the spatial and temporal variability of contamination in farms and animals could be better explored in the future. Shellfish (oysters, mussels, etc.) are identified from four studies conducted in Taiwan (2), the United States (1), and the United Kingdom (1) only. Shellfish contamination has been recently established (Cong *et al*., 2017; Ghozzi *et al*., 2017; Coupe *et al*., 2018). However, compared with meat-producing and poultry animals, research on seafood species contaminated with *T. gondii* represents a relatively new field of study, and harmonized methods of oocysts detection are lacking (Shapiro *et al.,* 2019).

Lack of hygiene, in particular, the lack of handwashing before eating or preparing a meal, was also found to play a role in the acquisition of *Toxoplasma* infection. Produce, in particular unwashed vegetables, was a significant risk factor which is expected considering the life cycle of the parasite. The contamination of vegetables was explored in few studies, but data are lacking in numerous countries due to the absence of standard detection methods for *T. gondii* oocysts in these matrices (Shapiro *et al*., 2019). The vast range of types of vegetables, not described in the studies, could be better investigated (i.e., leaf or root vegetables).

5. Conclusion

The risk factors identified in this meta-analysis could complement those already established in future sporadic case-control studies quantifying the attributable risk fraction: mollusks (regarding the species), raw milk (goat/cows), vegetables (including the type of vegetable and the preparation i.e., washing), game meat and drinking water. Furthermore, the development of sensitive methods for the detection and isolation of *T. gondii* in these matrices is needed to confirm the causal association revealed in epidemiological studies. In the context of the global market of food and animals, it is essential to obtain information from an international meta-analysis. This meta-analysis can be seen as a tool to identify emerging situations. The transmission pathways need further investigations using longitudinal studies (to avoid memory bias) and subtyping approaches (to differentiate between strain infectivity). Information on the frequency or the duration of exposure could be further investigated. Investigation of sporadic cases of infection remains essential for further understanding of *Toxoplasma gondii* transmission.

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621 **FIGURE CAPTIONS**

622 Figure 1: Flow chart of literature search for case-control,cohort and cross-sectional studies of
623 human sporadic toxoplasmosis

624

625 Figure 2: Forest plot of the association of blood transfusion with positive *Toxoplasma*
626 serology in pregnant women

627

628 Figure 3: Forest plot of the association of flies/rodents with positive *Toxoplasma* serology in
629 the mixed population

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631 Figure 4: Forest plot of the association of consumption of dairy products with positive
632 *Toxoplasma* serology in the mixed population

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634 Figure 5: Forest plot of the association of consumption of molluscs with positive *Toxoplasma*
635 serology in the mixed population

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637 Figure 6: Funnel plots of meta-analyses investigating categorized risk factors (i.e., food in
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645 Table 3. Effect of poor handling on the pooled association between positive *Toxoplasma*
646 serology and consumption of meat, vegetables and dairy

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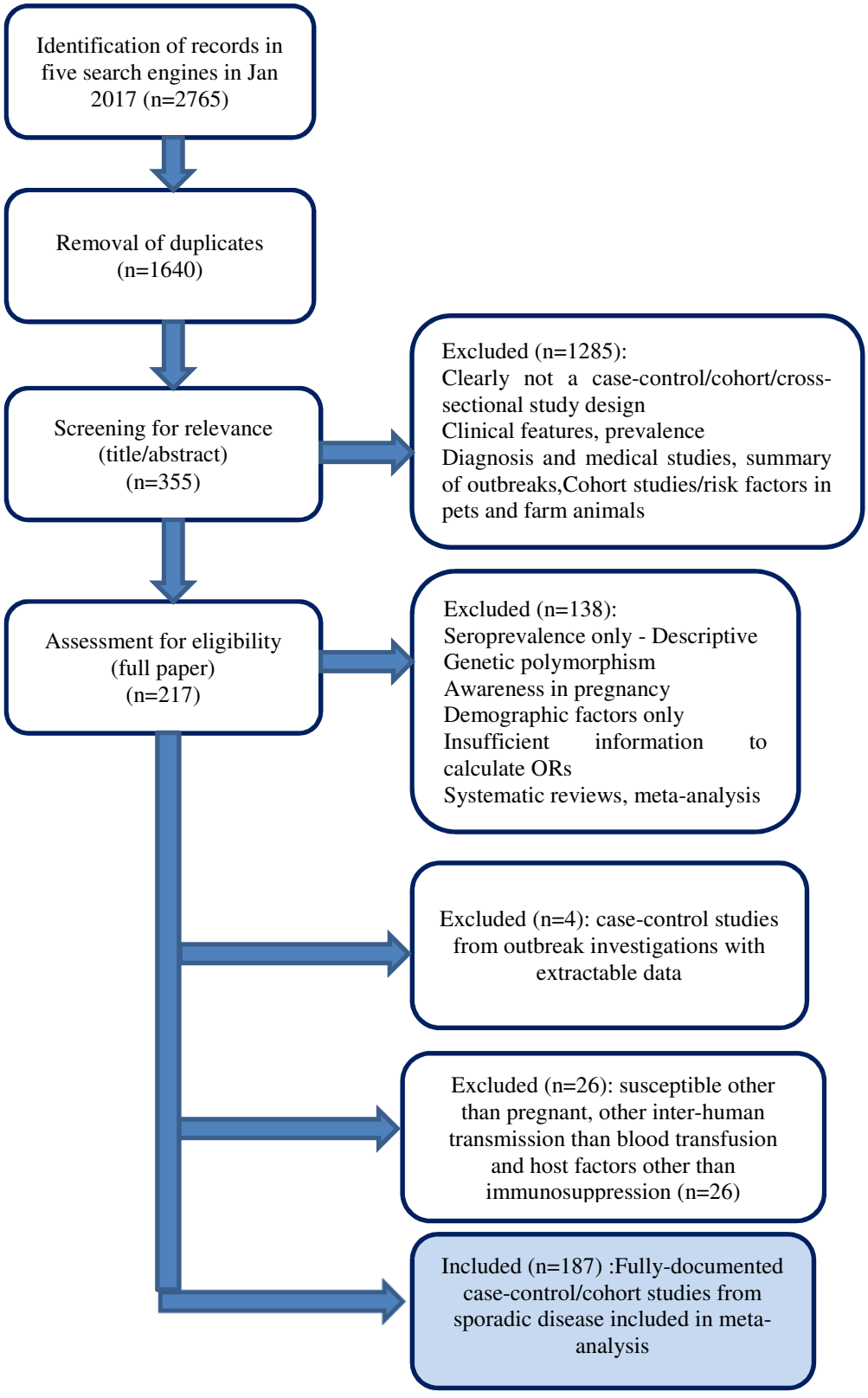

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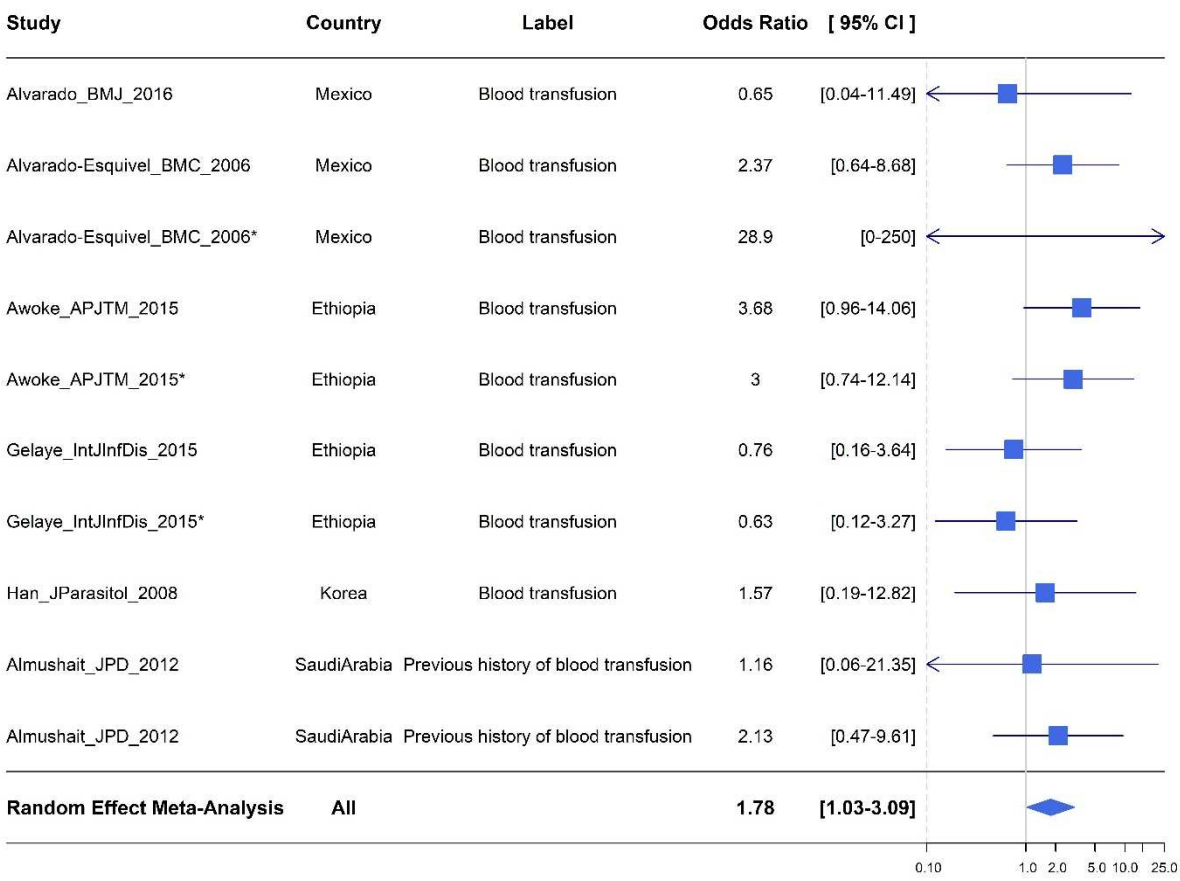


Figure 2: Forest plot of the association of blood transfusion with positive *Toxoplasma* serology in pregnant women. (* adjusted OR as described in Gonzalez-Barron *et al*., 2019)

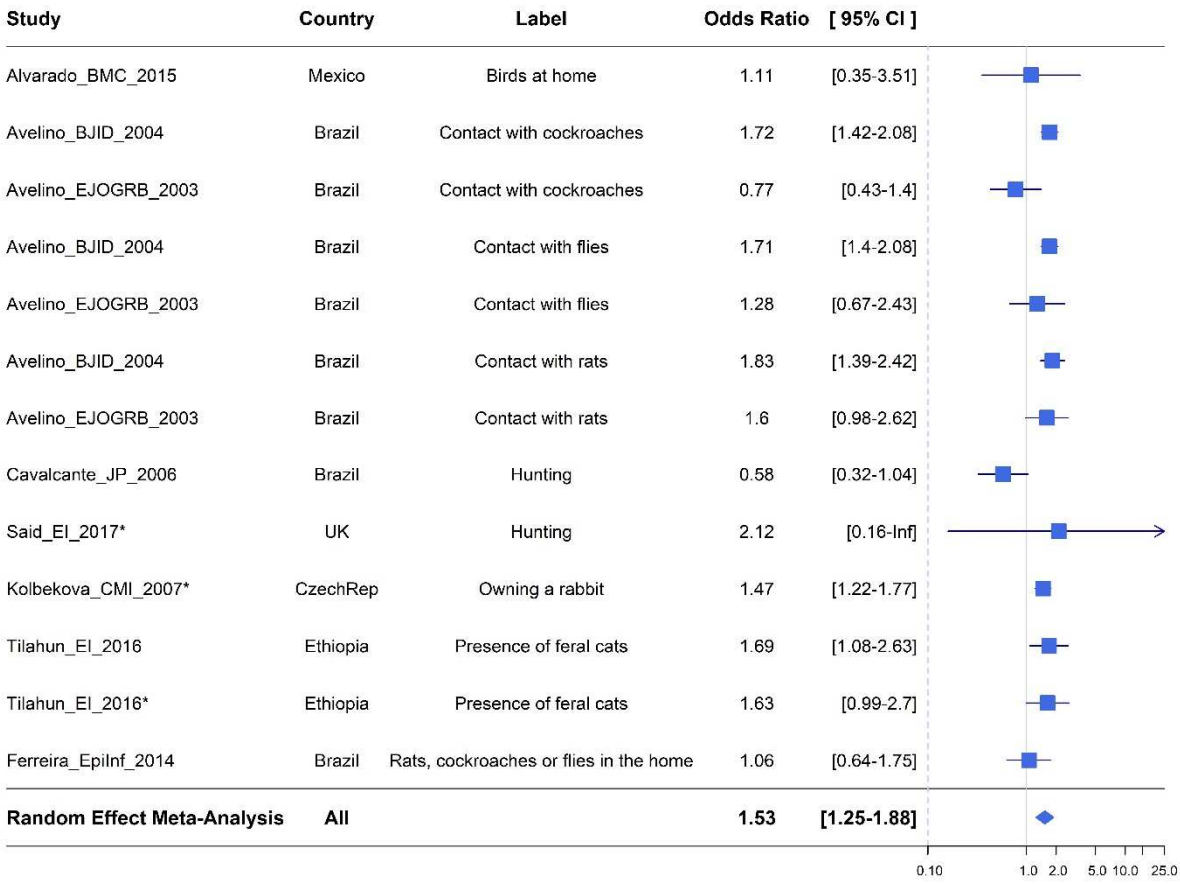


Figure 3: Forest plot of the association of flies/rodents with positive *Toxoplasma* serology in the mixed population. (* adjusted OR as described in Gonzalez-Barron *et al*., 2019)

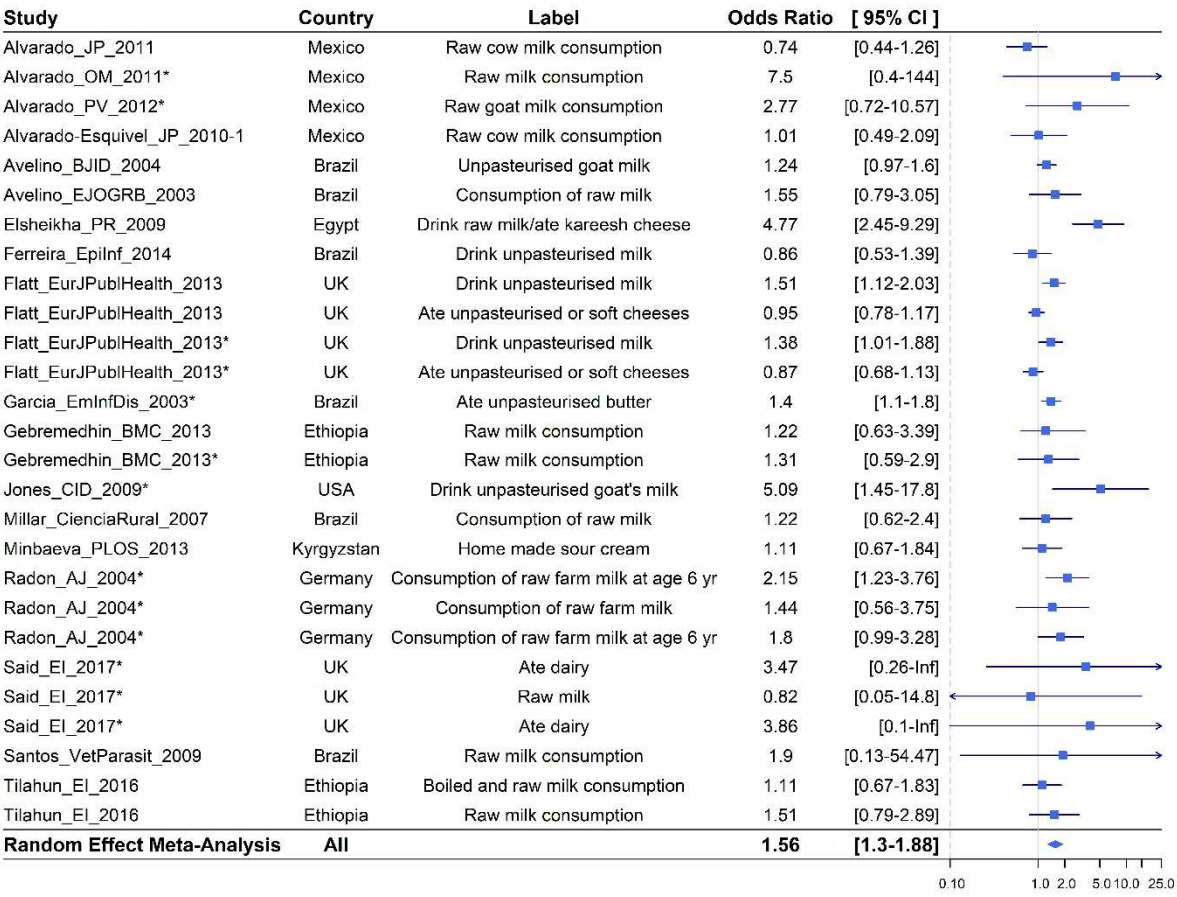


Figure 4: Forest plot of the association of consumption of dairy products with positive *Toxoplasma* serology in the mixed population. (* adjusted OR as described in Gonzalez-Barron *et al*., 2019)

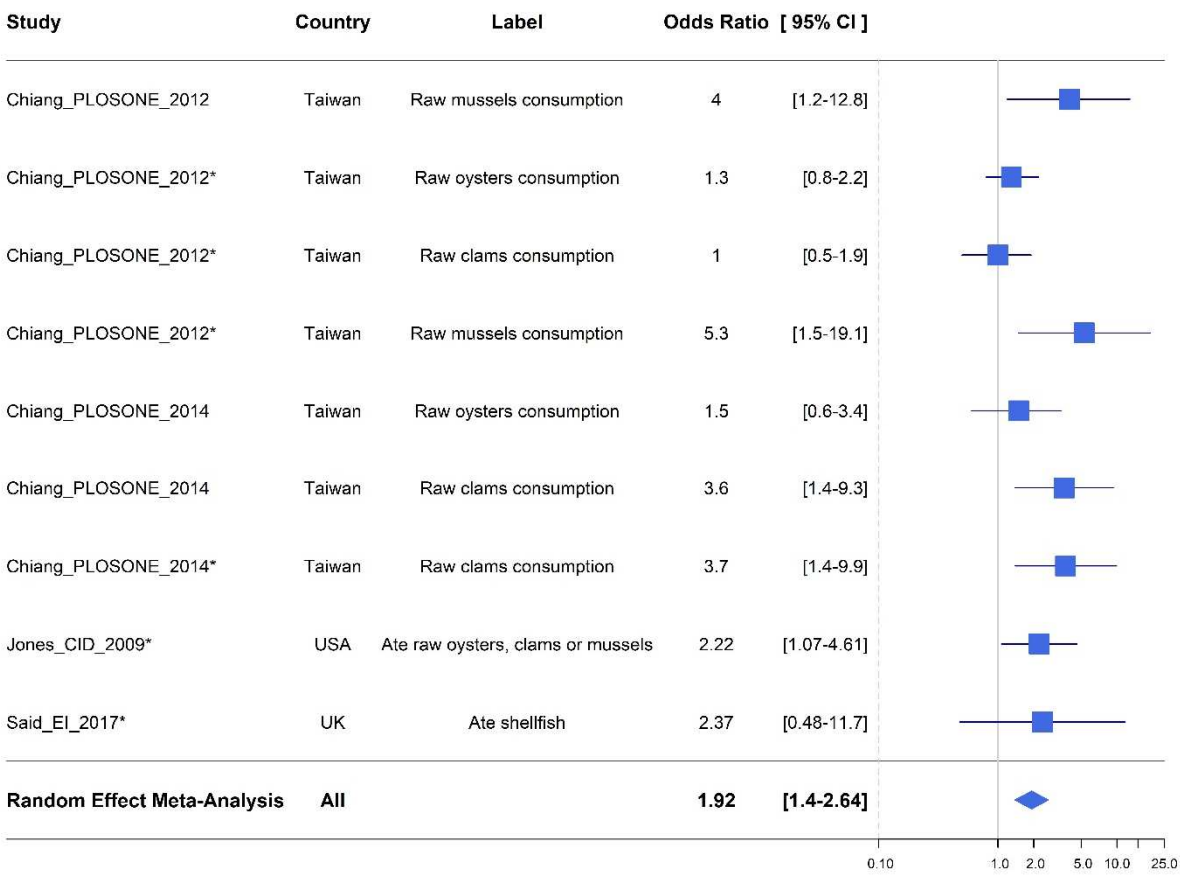
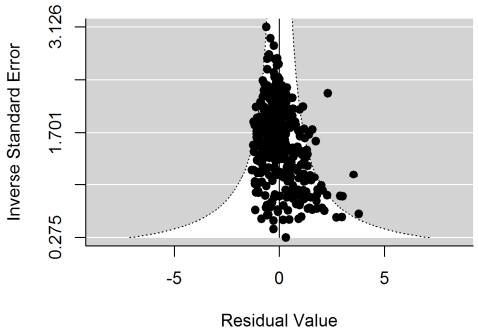
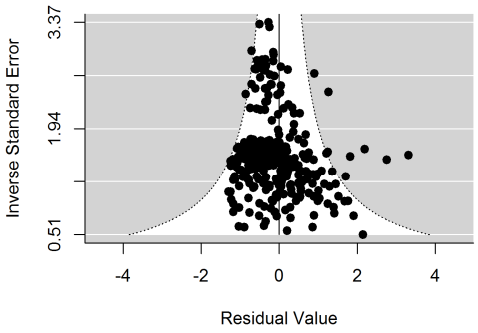


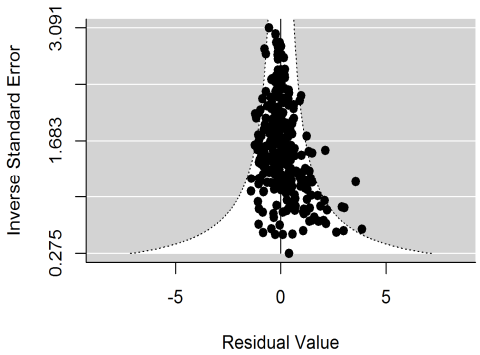
Figure 5: Forest plot of the association of consumption of mollusks with positive *Toxoplasma* serology in the mixed population . (* adjusted OR as described in Gonzalez-Barron *et al*., 2019)



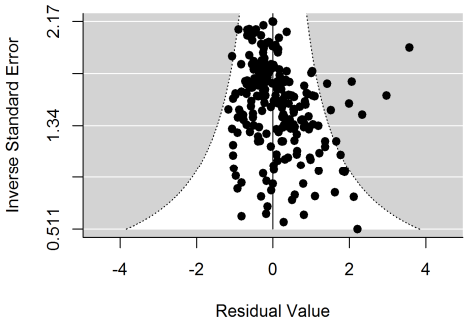
Food in the mixed population



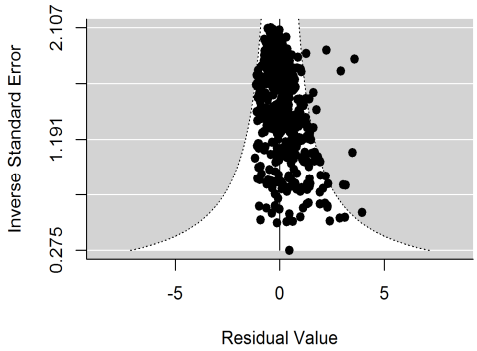
Food in pregnant women



Meat in the mixed population



Meat in pregnant women



Practices of cooking meat (“handling”)

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713 Figure 6: Funnel plots of meta-analyses investigating categorized risk factors (i.e., food in
714 mixed and pregnant population, meat in mixed and pregnant population, and practices of
715 cooking meat)

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720 Table 1. Meta-analysis results on main (significant) risk factors for positive *Toxoplasma* serology

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Population	Geographical area	Risk factor	Pooled OR [95% CI]	N/n*	p-val risk factor	Publication bias p-value	Points removed **	Heterogeneity analysis***
Travel								
Pregnant	All	Abroad	1.878 [1.284 - 2.746]	6/7	0.001	0.754	0	τ^2=0.083 Q(df=6) = 11.08; p-val = 0.086 s^2=0.380 I^2=17.94
Host specific								
Mixed	All	Immuno-compromising conditions	2.407 [1.483 - 3.909]	14/32	0.001	0.188	0	τ^2=0.175 QE(df=41) = 128.1;p-val < .0001 s^2=0.955 I^2=15.451
Pregnant	All	Blood Transfusion	1.785 [1.031 - 3.089]	6/10	0.039	0.063	0	τ^2=0.003 QE(df=23) = 14.51; p-val = 0.911 s^2=0.812 I^2=0.368
Personal Hygiene								
All	All	Poor personal hygiene	2.023 [1.693 - 2.416]	3/9	<.0001	0.743	0	τ^2=0 Q(df=8) = 6.596;p-val = 0.581 s^2=0.241 I^2=0
Animals								
Mixed(at)	All	Farm animals	1.482 [1.099 -1.998]	6/8	0.009	0.176	0	τ^2=0.801 QE(df=218)= 838.7; p-val < .0001 s^2=0.555 I^2=59.1
		Occupational	2.035 [1.641 - 2.522]	20/65	<.0001			
		Pets	1.759 [1.496 - 2.067]	67/137	<.0001			
		flies/rodents	1.534 [1.249 - 1.882]	8/13	<.0001			
Pregnant	All	Occupational	1.557 [1.245 - 1.948]	8/9	0.0001	0.214	0	τ^2=0.163 QE(df=198)= 511.4;p-val < .0001 s^2=0.553 I^2=22.75
		Pets	1.536 [1.374 - 1.717]	71/176	<.0001			
		flies/rodents	1.470 [1.133 - 1.908]	4/6	0.004			
Children	All	Pets	1.634 [1.331 - 2.005]	14/74	<.0001	0.234	0	τ^2=0.539 QE(df=82) = 413.7; p-val < .0001 s^2=0.356 I^2=60.27
		Wild	1.435 [1.052 - 1.959]	3/6	0.023			

Environment								
Mixed	Africa removed (22 ORs)	Untreated drinking water	1.429 [1.217 - 1.677]	32/64	<.0001	0.061	0	τ^2=0.588 QE(df=197)= 709.0;p-val < .0001 s^2=0.359 I^2= 62.03
		Farm environment	1.363 [1.146 - 1.621]	37/62	0.001			
		Playground	1.656 [1.403 - 1.953]	41/66	<.0001			
		Waste water****	1.523 [1.047 - 2.214]	4/6	0.028			
Pregnant	All	Untreated drinking water	1.487 [1.279 - 1.729]	34/54	<.0001	0.187	0	τ^2=1.071 QE(df=189)= 534.4; p-val < .0001 s^2=0.539 I^2=66.50
		Farm environment	1.804 [1.465 - 2.221]	32/52	<.0001			
		Playground	1.462 [1.319 - 1.621]	47/74	<.0001			
		Waste water	1.863 [1.162 - 2.986]	7/11	0.010			
Children	Oceania (1 OR excluded) and Africa excluded (6 OR excluded)	Untreated drinking water	1.403 [1.215 - 1.620]	2/23	<.0001	0.947	0	τ^2=0.178 QE(df=47) = 64.15;p-val = 0.045 s^2=0.203 I^2=46.87
		Farm environment	2.642 [1.768 - 3.946]	3/7	<.0001			
		Waste water	1.802 [1.228 - 2.645]	2/5	0.003			
Food								
Mixed	Oceania excluded (1 OR excluded)	Dairy	1.563 [1.298 -1.882]	18/27	<.0001	0.031	4	τ^2=2.212 QE(df = 396) = 1146, p-val < .0001 ; s^2=0.669 I^2=76.7569
		Meat	1.761 [1.570 - 1.974]	66/287	<.0001			
		Produce	1.872 [1.539 - 2.276]	37/58	<.0001			
		Seafood	1.702 [1.332 - 2.176]	4/12	<.0001			
Pregnant	All	Dairy	1.521 [1.116 - 2.073]	28/44	0.008	0.002	0	τ^2=1.044 QE(df=36) = 1368; p-val < .0001 s^2=0.459 I^2=69.46
		Meat	1.960 [1.472 - 2.610]	65/241	<.0001			
		Produce	1.651 [1.267 - 2.151]	34/64	0.001			
Poor handling								
Pregnant	All	Poor (no handwashing before eating or cooking, no washing knife)	2.000 [1.598 - 2.504]	19/35	<.0001	0.599	0	τ^2=0.378 QE(df=45) = 84.05; p-val = 0.001 s^2=0.489 I^2=43.607

*N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2). **** including non-compliant toilets or lack of toilets in the main dwelling; (at) the analysis type is significant: results are given for multivariate estimates

Table 2. Meta-analysis results on disaggregated risk factors for positive *Toxoplasma* serology

Main risk factor	Population	Geographical area	Specific class or specific population	Pooled OR [95% CI]	N/n*	Risk factor p-val	Publi-cation bias p-val	Points removed **	Heterogeneity analysis***
Animals									
Cat	All	All	Mixed	1.711 [1.463 - 2.001]	63/112	<.0001	0.465	0	τ^2=0.607 QE(df=311) = 1191;p-val < .0001 I^2=52.37 s^2=0.552
			Pregnant	1.631 [1.445 -1.841]	70/150	<.0001			
			Children	1.653 [1.328 - 2.057]	13/52	<.0001			
Food									
Meat	Mixed	Oceania excluded (1 OR excluded)	Other red meats	1.897 [1.480 - 2.431]	23/48	<.0001	0.037	8	τ^2= 1.499 QE(df=274)= 668.8;p-val < .0001 s^2=0.717 I^2=67.63
			Pork	2.114 [1.411 - 3.169]	15/23	0.001			
			Others	1.734 [1.544 - 1.947]	54/145	<.0001			
			Poultry	1.623 [1.147 - 2.297]	11/15	0.006			
			Processed meat	1.365 [1.075 - 1.733]	13/26	0.011			
			Beef	1.635 [1.170 - 2.285]	15/23	0.004			
Meat	Pregnant	All	Other red meats	1.822 [1.279 - 2.595]	11/33	0.001	0.002	0	τ^2=1.608 QE(df=235)= 996.1;p-val < .0001 s^2=0.579 I^2=73.50
			Others	1.592 [1.354 - 1.871]	60/106	<.0001			
			Poultry	1.514 [1.130 - 2.028]	11/19	0.005			
			Processed meat	1.532 [1.201 - 1.953]	9/27	0.001			
			Beef	2.052 [1.576 - 2.672]	11/29	<.0001			
Other red meats	Pregnant	All	Lamb meat	1.832 [1.148 - 2.922]	14/4	0.011	0.260	0	τ^2=0.762 QE(df=19) = 41.75;p-val = 0.002 s^2=0.754 I^2=50.27
Other red meats	Mixed	All	Lamb meat	2.404 [1.189 - 4.859]	5/6	0.015	0.096	2	τ^2=0.859 QE(df=42) = 66.08;p-val = 0.010 s^2=0.633 I^2=57.615
			Boar meat	2.487 [1.814 - 3.409]	5/8	<.0001			
			Goat meat	1.667 [1.212 - 2.294]	7/8	0.002			
			Venison/ram/horse meat	1.566 [1.221 - 2.008]	9/12	0.001			
Produce	Mixed	All	Vegetables	1.866 [1.491 - 2.335]	33/52	<.0001	0.879	2	τ^2=0.297 QE(df=56) = 198.6;p-val < .0001 s^2=0.522 I^2=36.29
Produce	Pregnant	All	Vegetables	1.372 [1.198 - 1.571]	34/57	<.0001	0.784	0	τ^2=0.145 QE(df=62) = 124.3; p-val < .0001 ; s^2=0.234 I^2=38.32
Seafood	All	All	Mollusks	1.917 [1.395 - 2.636]	4/9	<.0001	0.087	0	τ^2=0.019

									QE(df=11) = 14.95; p-val = 0.185 s^2=0.258 I^2=6.867

*N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2)

Table 3. Effect of poor handling on the pooled association between positive *Toxoplasma* serology and consumption of meat, vegetables and dairy

Risk Factor	Risk factor precise	Pooled OR [95% CI]	N/n*	p-val risk factor	Ratio Poor handling to Base [95% CI]	Points removed**	Publication bias p-val	Heterogeneity analysis***
Meat	Raw	1.876 [1.289 - 2.729]	41/71	0.075	1.232 [0.979 - 1.551]	2	0.0001	τ^2= 0.229 QE(df=490) =1534;p-val < .0001 ; s^2=0.229 I^2=50
	Undercooked	1.685 [1.209 - 2.347]	80/139	0.287	1.107 [0.918 - 1.334]			
	Base	1.523 [1.317 - 1.759]	56/283	<.0001	_			
Fruit & vegetables	Unwashed	1.789 [1.204 - 2.658]	39/59	0.001	1.489 [1.206 - 1.839]	0	0.647	τ^2=0.502 QE(df=27) = 355.0; p-val < .0001 s^2=0.394 I^2=56.015
	Base	1.202 [0.999 - 1.446]	14/28	0.052	_			
Cheese and milk	Raw	1.459 [0.939 - 2.267]	39/52	0.015	1.430 [1.073 - 1.905]	0	0.108	τ^2=0.337 QE(df=69) = 195.5; p-val < .0001 s^2=0.349 I^2=49.128
	Base	1.019 [0.874 - 1.189]	10/19	0.802	_			

*N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2).