Hygiene measures as primary prevention of toxoplasmosis during pregnancy: A systematic review

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Version of Record: https://www.sciencedirect.com/science/article/pii/S2468784721002361
Manuscript_3dac5ddf3e02cf6e9c6e1607b6e965bd

Hygiene measures as primary prevention of toxoplasmosis during pregnancy: a systematic review

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Short running head: Hygiene measures as primary prevention of toxoplasmosis during pregnancy

Conflict of interest statements: None.

Role of funding source: None.

Words count: 6931 words (without abstract and references)

Number of figures: 1

Number of tables: 1

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ABSTRACT:

Background. Hygiene measures are recommended to prevent toxoplasmosis during pregnancy, although screening for seroconversion in pregnant women currently are debated and practices vary among countries.

Objectives: The purpose of this systematic literature review was to assess the effectiveness of hygiene measures during pregnancy to prevent toxoplasmosis infection.

Search Strategy.

We followed the standard MOOSE and PRISMA criteria when conducting this systematic review and reporting the results.

Selection criteria.

A systematic literature search was conducted for studies focused on congenital toxoplasmosis prevention, toxoplasmosis prevention during pregnancy, toxoplasmosis prevention and hygiene measures, which were published between 1970 and August 2020, using the databases of PubMed, Scope Med, EMBASE, and the Cochrane library.

Data collection and analysis

Our literature search identified 3964 articles, 3757 were excluded after review of title or abstract and 67 studies were considered relevant to the subject. We reviewed risk factors for toxoplasmosis infection during pregnancy and for congenital toxoplasmosis, preventive measures for toxoplasmosis during pregnancy, including: dietary recommendations, pet care measures, environmental measures, knowledge of risk factors and ways to control toxoplasmosis infection, knowledge of risk factors for infection by health professionals, knowledge of primary prevention measures by pregnant women.

Conclusion.

Hygiene measures are effective and applicable primary prevention to reduce toxoplasmosis and avoid congenital toxoplasmosis and its consequences.

Fundings: No

KEYWORDS: Toxoplasmosis; pregnancy; primary prevention; hygiene measures

What's already known about this topic?

Hygiene measures are recommended to prevent toxoplasmosis during pregnancy, although screening for seroconversion in pregnant women currently are debated and practices vary among countries.

What does this study add?

Hygiene measures are effective and applicable primary prevention to reduce toxoplasmosis and avoid congenital toxoplasmosis and its consequences.

INTRODUCTION:

Systematic biological screening of toxoplasmosis in seronegative pregnant women dates back to 1978 in France and is also performed in several other countries as Austria and Brazil, but it is not recommended in most countries (1–4). At the end of the 1970's, toxoplasmosis seroprevalence was high in women of childbearing age, but incidence of maternal infection during pregnancy was so high justifying the recommendation of primary prevention. In most countries including France, the prevalence and incidence of toxoplasmosis of seroconversion decreased sharply over the last 30 years. Congenital toxoplasmosis may lead to fetopathy, hydrocephalus, and death. Most often, the disease is asymptomatic at birth, but may lead to chorioretinitis that can be diagnosed only later in life. The risk of brain damage in higher in case of infection in early pregnancy.

At the time of screening in early pregnancy, it is recommended to give written and oral information to pregnant women about primary prevention. Primary prevention measures have since been reaffirmed and updated on several occasions, notably in 1983 and 1996 in a weekly publication of the epidemiological bulletin by the Haut Conseil de la santé publique (HCSP), historically the Conseil Supérieur d'Hygiène Publique de France (CSHPF) until 2006 (5–7). These measures aim to reduce the incidence of toxoplasma seroconversion during pregnancy in seronegative women. However, toxoplasmosis prevention management during pregnancy differs between countries.

The purpose of this systematic review was to assess the effectiveness of hygiene measures during pregnancy to prevent toxoplasmosis infection.

MATERIALS AND METHODS

We followed the standard MOOSE and PRISMA criteria when conducting this systematic review and reporting the results (8,9). A systematic literature search was conducted for articles focused on congenital toxoplasmosis prevention, toxoplasmosis prevention during pregnancy, toxoplasmosis prevention and hygiene measures which were published between 1970 and August 2020, using the databases of PubMed, Scope Med, EMBASE, and the Cochrane library. Searches were performed using Medical Subject Heading terms and the free keywords: ("congenital toxoplasmosis" OR "toxoplasmosis during pregnancy") AND ("prevention" OR "Hygiene measures") AND ("Cohort" OR "Case-control" OR "Reviews"). Furthermore, the reference lists of retrieved articles were manually scrutinized to identify potential relevant studies. Two reviewers (KW and OP) independently screened the titles and abstracts of the studies to identify all potential eligible studies using a predefined data extraction form. Then, they independently evaluated studies for inclusion, and studies were included in the systematic review if they met the following criteria: 1) cohort or case control studies; 2) the risk factors of toxoplasmosis seroconversion during pregnancy; the utility of hygiene measures to prevent seroconversion during pregnancy; knowledge assessment of hygiene measure by health care providers and pregnant women; and assessment of hygiene measures effectiveness and how they are applied and followed by pregnant women. The following data were extracted: the first author's last name, year of publication, study location, study design, risk factors, exposure assessment, outcome assessment, odds ratios, with their confidence intervals.

Data synthesis and statistical analysis: In this review no specific statistical analysis were performed.

RESULTS

Our literature search identified 3964 articles and 3757 were excluded after review of title or abstract (Figure 1). Two hundred and seven articles were further reviewed. We excluded 140 studies because they were considered as reporting insufficient data.

Risk factors of toxoplasmosis infection during pregnancy.

In 2021, Thebault *et al* published a meta-analysis of the main identified and known risk factors for toxoplasmosis (10). In this meta-analysis, the quality assessment stage was passed by 213 primary studies investigating risk factors for sporadic infection with *Toxoplasma gondii*, which were conducted between 1983 and 2016 (80.5% after 2000). Excluding susceptible populations other than pregnant women, and some risk factors, 187 publications were retained for meta-analysis. 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 (10).

Several limitations exist in the search for risk factors for toxoplasmosis contamination in pregnant women. Risk factor studies classify patients primarily on the basis of seroprevalence, which is an indicator of *T. gondii* infection but does not allow precise dating of the time of infection. As a result, there may be a significant time lag in studies between the collection of information on exposures of infected cases to the factors sought and the date of infection which implies a bias. This would imply that the risk factor under investigation is present consistently over time, which is not in fact the case.

In the meta-analysis of the *ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail)*, the authors point out that the information collected like seroprevalence or food preparation are not directly comparable between them(11). The serological tests to identify cases are not the same depending on the countries from which the studies were conducted. Eating habits are not the same, with differences in the preparation of foodstuffs whose contamination levels can vary greatly from one country to another. Among the studies taken into account for the meta-analysis, only 15% of those were conducted in Europe, in environments comparable to the French situation. In 2005, the Eurotoxo group published a literature review of the on dietary and behavioral risk factors associated with *T. gondii* contamination in pregnant women (12). This review included five studies carried out between 1996 and 2000 in Europe, among them three case-control studies with pregnant women who had a toxoplasmic seroconversion during pregnancy (13–15). The other two studies were cross-sectional (16,17). The objective of these studies was to assess the environmental risk factors associated with acute toxoplasmosis during pregnancy. There were some discrepancies between the studies in terms of data collection, population size, inclusion criteria, which may explain differences in the results found in these publications. The risk factors investigated were generally the same, however Bobic *et al* (18) and Cook *et al* (14) did not investigate the consumption of poorly washed fruits and vegetables as a risk factor. The study populations and inclusion criteria differed depending on the authors. Only the study by Baril *et al* considered the certainty of seroconversion during pregnancy as a selection criterion (14). The date of seroconversion in the other studies was uncertain. Moreover in 2005, the French Food Safety Agency (*Agence Française de Sécurité Sanitaire des Aliments – AFSSA* –) provided a synthesis of the literature on behavioural and dietary risk factors associated with *T. gondii* contamination (7). The *AFSSA* considered the five studies taken into account by Eurotoxo as well as a sixth, older, cross-sectional study of Swiss pregnant women

carried out in 1987 and concluded the same as Eurotoxo (7). This cross-sectional study included 280 women with a *T. gondii* IgG positive cord sample at delivery and 279 women with an IgG negative cord sample. Overall, consumption of undercooked meat was the only risk factor identified by all the studies with an Odd Ratio (OR) varying between 1.6 [95% CI: 1.2-2.1] and 11.4, p=0.00513.

Some risk factors are not established: Unpasteurized dairy products, shellfish, wild boar meat, pork, poultry, game, are not identified as risk factors in France (18). With regards to food consumption, undercooked meat, unwashed vegetables, raw milk, and shellfish are risk factors only 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% (19)). These factors were not all sought in the questionnaire of the Baril et al. study (8, 12). Moreover, it is possible that these differences can be explained by the cooking practices of the meats (more or less cooked) or by a lesser contamination of these foods in France (farming methods). Moreover, environmental exposure (gardening, farm life) is not identified in the French studies. A recent study confirmed the roles of drinking water, plants, raw milk and shellfish as risk factors for *T.gondii* contamination in pregnant women (10).

Risk factors of congenital toxoplasmosis.

Since then, in 2014, Carellos *et al* studied the risk factors associated with congenital toxoplasmosis in Brazil (20). This was a case-control study in Minas Gerais, including 175 women who had a child with congenital toxoplasmosis and 278 control women who delivered without congenital toxoplasmosis. Factors associated with a lower risk of congenital toxoplasmosis were a higher maternal age (OR = 0.89; CI 95% = 0.85-0.93), a higher level of education (OR = 0.85; CI95% = 0.78-0.92), access to drinking water (OR = 0.21; CI95% = 0.08-0.51), and housing with flush toilets (OR = 0.18; CI95% = 0.04-078). Factors associated

with an increased risk of congenital toxoplasmosis were the presence of cats in the vicinity (OR = 2.27; CI 95% = 1.27-4.06), cat ownership (OR = 1.90; CI95% = 1.09-3.31), handling of soil (OR = 2.29; CI95% = 1.32-3.96) and consumption of fresh meat that has not been frozen (OR = 3.97; CI95% = 2.17-7.25). Sub-group analysis showed that water-related factors (access to drinking water, flushing toilets) were significant for the rural population only. The authors concluded that the risk of congenital toxoplasmosis is associated with a low socioeconomic level and that maternal exposure to sources of *T.gondii* varies with socioeconomic level. In populations with low socioeconomic levels, the main source of infection could be related to oocysts with water as the main vector. These data suggest that the prevention of congenital toxoplasmosis should be tailored to the reality of the target population. The prevention message focuses on the known risk factors. Finally, although the results of the studies included in these systematic reviews must be interpreted with caution because of the methodological differences between them and their variable quality, three types of dietary and behavioral factors seem to be associated with the risk of acquiring toxoplasmosis in pregnant women or women of childbearing age: consumption of undercooked meat, consumption of inadequately cleaned raw vegetables and poor hand hygiene. These results must be carefully analyzed, because in South America, *Toxoplasma* strains are more virulent than in Europe or in North America, so the circulating genotypes are not the same (10,21,22). The clinical presentation for this strains is more severe in adults (22) and we can suppose that during pregnancy, this could increase rate of contamination and seroconversion in some countries.

The toxoplasmosis screening program in pregnant women differs from country to country and is a source of disagreement among scientists. France, Austria, and Slovenia have prenatal screening program(23). In France, a recent analyze of the practices have been conducted(24), because of the decreasing incidence of this infection and the cost of testing. In France, about

70% of pregnant women are not immune to *T. gondii*, and 0.2-0.25% become infected during pregnancy. In case of congenital toxoplasmosis, prompt initiation of treatment reduces the occurrence of cerebral signs and symptoms, as well as retinal lesions(24). Binquet and al.(23) showed that prenatal screening is cost-effective as compared to neonatal screening in moderate prevalence areas. In addition, prenatal screening, by providing closer follow-up of women at risk increases the number of occasions for education avoiding toxoplasmosis. Though most international societies do not recommend systematic screening for mainly financial reasons, if congenital toxoplasmosis appears benign in France today, it is probably thanks to screening and the possibility of early treatment of fetuses and/or newborns. In Germany, systematic screening is not recommended, but Lange and al. (25) encourage its implementation. In the United States, systematic screening is not recommended. This country represents a combination of parasite and host diversity, with substantial resources for management of this disease but inadequate allocation of these resources. The absence of mandatory gestational screening and a fragmented healthcare system with insufficient insurance coverage and access results in a poor understanding of the true scope of congenital toxoplasmosis there, and financial concerns limit access to screening(26). In undeveloped countries, like Marocco or Columbia, the absence of systematic screening for toxoplasmosis due to a lack of means has already shown its impact(26). Screening and management of congenital toxoplasmosis depends above all on public health policy and the wealth of countries(26).

Established and recognized preventive measures of toxoplasmosis infection during pregnancy.

In France, where toxoplasmosis screening is mandatory, preventives measures include a number of hygiene and dietary precautions published in the weekly epidemiological bulletin by the CSHPF in 1996 (5). The *AFSSA* classifies the measures published by the *CSHPF* in

two categories: " essential measures " supported by studies with a high level of evidence and " other measures " for which there is no scientific justification with a sufficient level of evidence (7). They are formulated such as follows:

Dietary recommendations. Undercooking meat is the most widely documented risk factor in the literature. The main epidemiological studies in pregnant women all conclude that there is an over-risk of eating undercooked meat (13–17,27). Meat likely to contain cysts must be cooked at a temperature above 67 degrees Celsius. In practice, this temperature corresponds to a meat that does not allow red juice to run out when cutting. It was determined by Dubey in 1990 who studied the effect of temperature on the infectivity of cysts in infected pork (28). Dubey was thus able to establish a thermal destruction curve estimating that a temperature of 67 degrees Celsius must be reached in the heart of the meat to achieve total inactivation of the cysts. There is no advantage to the microwave over other cooking methods (29). Microwaves were studied under experimental conditions with partial effectiveness on the infectivity of *T.gondii* cysts at 65 degrees (41). All types of meat are susceptible to infection by *T.gondii* and should therefore be cooked at more than 67 degrees, including venison, although beef and mutton appear to be preferentially implicated in the French study by Baril et al (14). The *AFSSA* reminds that the prevalence of toxoplasmosis is variable in cattle. It is higher in sheep and leads to a high frequency of abortions in this species. In France, the contamination of cattle from which meat intended for consumption comes was shown for sheep and lamb meat by Halos et al in 2010 who estimated the overall seroprevalence of *T.gondii* was 17.7% (11.6-31.5%) for lambs and 89% (73.5-100%) for adult sheep (P<0.0001) (30). No significant difference was observed between imported and French meats. *T.gondii* contamination of meat was also shown for beef by Blaga et al in 2019 ((31)) who were able to estimate the level of toxoplasma infection of sheep, cattle and pig meat in France, (31,32,32,33). The seroprevalence of toxoplasmosis ranged from 3% to 69.5% depending on the species and

origin of the meat. The seroprevalence increased with the age of the animals, and this parameter had a significant effect on the level of seroprevalence for each species. A significant difference in *T. gondii* infection (3% vs. 6.3%, P=0.004) was observed between above-ground and free-range swine production. *T.gondii* contamination of French meats was also demonstrated in pigs by Djokic et al in 2016 with an overall seroprevalence in pigs estimated between 2 and 9%, and in wild boar by Roqueplo et al in 2017 (33,34). In the latter study, the seroprevalence of *T.gondii* was 16.8% among the 841 boars examined. These observations highlight the importance of remembering that for any consumer, and particularly for sensitive populations (pregnant women, immunodeficient people), the best means of prevention is cooking meat thoroughly . Avoid eating marinated, smoked or grilled meat (as may be the case with venison). An altenative is to freeze meat to -12°C during minimum three days, which is also efficient to destroy the cysts. In France, there is no health control on *T.gondii*, which is not routinely detected in slaughterhouses because of the complexity of measuring prevalence in livestock.

According to *AFSSA* (7) in 2004, the frequency of contamination of domestic poultry could represent a potential risk for humans, but parasitological data from experimental infections in chicken, pigeon, duck and prevalence studies in chicken show that the parasites are mainly localized in the brain, heart, to a lesser degree in other viscera and more rarely in muscles (35–38). The risk of contamination is theoretically not zero but has not been assessed in France. The prevalence of *T.gondii* in chickens was studied in a literature review by Dubey in 2010, which recalls that chickens are considered one of the most important hosts in the epidemiology of *T.gondii* infection because they are an efficient source of infection for cats and because humans can be infected by this parasite after eating infected chicken meat that has not been properly cooked (39). The global prevalence of *T.gondii* infection in chickens is very high. It has been estimated in chickens kept in backyard flocks to be close to 100%. In

free-range chickens, it was estimated to be 30-50%. *ANSES* also proposes to control insects that can be considered as a passive vector of oocysts (40–43).

The washing of raw vegetables is one of the indispensable measures according to the AFSSA, whose consumption is a risk factor for *T.gondii* contamination, all the more so as they are soiled with soil. The importance of washing raw vegetables consumed raw is recalled. An additional precaution could be taken in the case of consumption of raw vegetables in restaurants outside the home (7). Regarding the detection of *T.gondii* in foodstuffs of plant origin, there are few direct arguments for the presence of oocysts on these surfaces. Oocysts have never been found in fruits and vegetables intended for human consumption. However, it is known from experimental studies with mouse bioassays that oocysts can adhere to and survive on fruits and vegetables for human consumption (44). The oocysts were able to survive 8 weeks at 4 degrees on raspberry and blueberry berries and infect mice fed with these berries. Indirectly, the transmission of *T.gondii* to humans by oocysts is demonstrated by the high rate of seropositivity (between 24 and 47%) in certain vegetarian populations (45,46). Finally, the consumption of raw vegetables prepared outside the home has been identified as a risk factor by Baril et al (14). In the study by Kapperud et al, consumption of raw vegetables or unwashed fruits was associated with an increased risk of *T.gondii* infection. As for meats, there is no surveillance system for *T.gondii* in foodstuffs from vegetable origin in France. Action of washing is to detaching the oocysts of the vegetables. When preparing meals: wash vegetables and herbs carefully, especially if they are earthy and eaten raw. Wash kitchen utensils and worktops thoroughly. Wash hands after contact with raw vegetables, fruit or meat and before eating. Good hands and utensil hygiene are of major importance.

Water consumption. Water consumption as a source of contamination has been recently demonstrated in a review (47). Application of PCR for detection of *T. gondii* in water has been applied in numerous studies worldwide, and recently reviewed by Bahia-Oliveira et

307 al.(48). In Colombia, the prevalence of *T. gondii* DNA in 46 samples of drinking water was
308 58.6% (49). Similar prevalences were reported in raw and treated water in Bulgaria at 48%
309 (50) and in Poland at 37.5% (51). In comparison, lower prevalences of Toxoplasma in water
310 have been reported via real-time PCR in Scotland at 8.7% (N = 1411) (52), and in France's
311 Champagne-Ardenne region at 7.7% (N = 482), where some of the positive samples were
312 obtained from public drinking water(53). Humans and susceptible animal hosts can be
313 exposed to *T.gondii* oocysts in the environment through drinking water contaminated with
314 felid feces, but oocysts can survive various inactivation procedures especially those using
315 chemical reagents (54). For example, oocysts remain viable in water even after exposure to
316 aqueous 2% sulfuric acid for at least 18 months at 4 °C; they also resist detergents or
317 disinfectant solutions such as sodium hypochlorite. Drinking-water treatment plants using
318 chlorination as the sole method of disinfection could therefore supply water containing
319 infective oocysts (47).
320 As recommendation to pregnant women, filtered or bottled water should be consumed if
321 living or travelling in an endemic region (40). They should avoid recreating in fresh or marine
322 waters in endemic regions, or in non-endemic regions if in close proximity to overland runoff
323 from heavily populated zones. Produce should be washed with drinking water (or with filtered
324 or bottled water if living or travelling in an endemic region). Municipal and ecosystem-level
325 management strategies should be implemented to reduce the overall flux of oocysts mobilized
326 to nearshore waters through runoff. Specific recommendations include wetland preservation
327 and restoration(55), replacement of impermeable surfaces such as asphalt with alternative
328 permeable paving options(56), and storm-water treatment processes including bioswales and
329 raingardens(57). Finally, common household products such as detergents, antimicrobial
330 soaps, and bleach are not effective at killing oocysts, and their use for this purpose is not
331 recommended.

Pet care measures. The last indispensable measures are precautions for cats. The handling of cat litter is particularly inadvisable. Cleaning should be done with boiling water and the wearing of gloves is strongly recommended. It is the cats, as the definitive hosts, who ensure the spread of oocysts by contaminating the environment. Overall, the prevalence in feral cats appears to be higher than in domestic cats (58). In a 2018 study by Simon et al, the dynamics of seroconversion of *T.gondii*, was studied in five populations of cats living in Ardennes farms in France (59). Seroprevalence varied between farms, from 15% to 73%, suggesting differential exposure of cats to *T.gondii*. On highly exposed farms, cats could be infected before the age of six months. Seroconversion rates ranged from 0.42 to 0.96 seroconversions per cat per year and were higher in fall and winter than in spring and summer. These results suggested variations in *T.gondii* exposures by season and farm. Seroprevalence of *T.gondii* was estimated at 52.7% in a 2010 study by Afonso et al. in domestic cats living in rural areas in France (60): seroconversion rates varied from 0.26 to 0.39 seroconversions per cat per year. In 2006, Afonso et al estimated the prevalence of *T.gondii* in an urban stray cat population at 18.6% between 1993 and 2004. The prevalence of T.gondii is heterogeneous depending on location, environment and season. Within the same location, cats excrete oocysts only very episodically. It is therefore impossible to predict the real risk associated with a cat at a given time. However, epidemiological studies have identified contact with cats as a risk factor for *T.gondii*.

To summarize, avoid direct contact with objects that could be contaminated by cat excrement (such as litter boxes, dirt) and wear gloves whenever handling these objects. Disinfect cat litter boxes with bleach is not efficient on oocysts.

Environmental measures. Hand washing is retained as an essential hygiene measure. Poor hand hygiene is associated with an increased risk of toxoplasmic contamination in the study by Baril et al (14). Contact with soil is found in the study by Cook et al. as also being

associated with an increased risk of contamination and is equated with hand hygiene as a risk factor (13). The Afssa specifies that these measures must be extended to the hygiene of the kitchen utensils incriminated in the work of Kapperud et al (7,15). According to the *AFSSA* meta-analysis in 2018, poor hand hygiene is associated with an increased risk of *T.gondii* contamination in pregnant women OR 1.5 (11).

To summarize, avoid direct contact with soil and wear gardening gloves. Wash hands after gardening activities even if gloves are worn.

These measures are based on the identification of risk factors, knowledge of the mechanisms of contamination and techniques for reducing the infectious potential of cysts and oocysts.

Knowledge of risk factors and ways to control the infectivity of cysts or oocysts.

At present, it is not possible to specify the respective proportion of the different modes of infection through ingestion of *T.gondii* due to the persistence of uncertainties about the sources of contamination. The identification of risk factors for contamination has made it possible to propose preventive measures and information to pregnant women or immunodeficents patients who are seronegative for *T.gondii.* These measures would theoretically have to be adapted to each pregnant woman, but each real risk cannot be precisely quantified. Since it is not possible to target the risk factors for each patient and to provide tailor-made information, the information has been generalized in the same way for all pregnant women, regardless of their actual exposure. The relevance of these measures for pregnant women was analyzed by *AFSSA* in 2005 in the light of the data available in the literature at that time (7). The *AFSSA* insists on the fact that these recommendations are much more concise than those sometimes found on the Internet or in various documents. It is also recalled that short recommendations have a positive effect on the motivation to follow them,

while too many recommendations discourage and dissuade pregnant women from making the effort to apply them.

Recommendations in UK and Australia are the same than in France. On the other hand, American recommendations (61,62) are more strict. For example, contact with mucous membranes should be avoided when handling raw meat, gloves should be worn when handling raw meat, cats should be kept indoors, stray cats should not be handled or adopted while the woman is pregnant and cat litter box should be changed daily.

Unestablished and hypothetical preventive measures of toxoplasmosis infection during pregnancy.

Freezing the meat at a temperature of -12 degrees Celsius or lower for at least 3 days to destroy the cysts. This measure is the result of Dubey's experiments on pork in 1988 (63). The required freezing time depends on the thickness of the piece of meat to be inactivated. The larger the piece, the longer the freezing time required to reach a potentially deep cyst. This explains why industrially frozen meat can be consumed without risk, whereas domestic freezing may not be sufficient to destroy the cysts. Sporulated oocysts found on plants remain viable and potentially infectious after constant freezing for 28 days at -21 degrees Celsius (64,65). The *AFSSA* also proposes measures whose effectiveness is to be further evaluated (7). Among them, the consumption of marinated, salted or smoked meat could also be avoided. In this regard, the consumption of dried or salted pork has been identified as a risk factor by Buffolano et al OR = 2.9 [IC95%:1.6-5.5] (16). Experiments on pieces of mutton meat in 1992 by Lundén et al suggest that smoking or salting procedures may be effective in controlling the infective power of cysts (66). Smoking was studied by injecting a solution of sodium chloride into the meat before it was smoked at a temperature not exceeding 50°C for 24 to 48 hours. Salting was evaluated on pieces of meat from 200 to 360 g put in plastic bags

with 30 to 50 g of sodium chloride and 25 to 40 g of sucrose for 64 hours at 4°C. In both cases, the procedures removed the infective power of *T.gondii* cysts. However, the authors pointed out that the exact mechanism of efficacy on the infectivity of the cysts is poorly known and could be related to the changes in osmotic pressure associated with the addition of salt and sugar in meat. Moreover, recent study (67) confirm the safety of ready to eat products containing pork with respect to T. gondii prepared using typical NaCl concentrations at or above 1.3%, and industry standard fermentation and drying procedures.

Among the preventive measures are cited the consumption of seafood although no *T.gondii* infection linked to seafood consumption has yet been found in France, *ANSES* states in 2018 that shellfish (oysters and mussels) have been identified as a risk factor for contamination in two meta-analysis (10,68). The presence and survival of oocysts in shellfish and other foodstuffs from the sea are suspected by indirect arguments such as the existence of cases of marine mammals infected with *T.gondii* (69–71). More recently, in 2017, a Chinese study revealed the presence of *T. gondi* oocysts in oysters sold in a market in China (72). A total of 26 of the 998 oysters tested tested positive by PCR amplification (2.61%). This study suggests that oysters have the ability to filter and retain oocysts in their tissues. Another 2014 Chinese study of 3432 shellfish showed low (N=5/3432) but not zero contamination of *T.gondii* in these species (73). In a New Zealand study in 2018, *T.gondii* contamination in the form of sporulated oocysts was detected in 16.4% (N=13) of a sample of 104 commercial mussels (74). In the 2018 meta-analysis, *ANSES* concludes that seafood consumption is associated with an increased risk of *T.gondii* in the general population (10,11). Seafood should be thoroughly cooked to inactivate oocysts.

The consumption of raw goat's milk was also one of the measures whose effectiveness needed to be further evaluated. The *ANSES* recalled that it had been the cause of some cases of toxoplasmosis and reasonably advocated avoiding it. In the meta-analysis of 2018, the

identification of raw milk as a risk factor was based on 16 publications for the general population and 27 in pregnant women. In 2017, a literature review by Boughattas on milk consumption and toxoplasma infection reported, despite heterogeneous data, that the main source of infection was goat's milk (75).

Although developed from a scientific substrate, these measures to prevent toxoplasmic infection can only be effective if they are properly followed. In order to do so, they must be well disseminated to health professionals caring for women of childbearing age outside and during pregnancy. It is also necessary to evaluate the knowledge of pregnant women informed by these professionals and the impact of these measures on women's behaviour. Moreover, the psychological and dietary impact of the implementation of primary prevention measures in pregnant women is not evaluated in the literature.

Knowledge of primary prevention measures by health professionnals.

Several studies around the world have assessed praticians' knowledge, attitudes, and practices regarding prevention of infections in pregnancy. In 2009 in United States(76), among 305 gynecologists interviewed, about 84% reported counseling pregnant women about preventing infection from *T.gondii.* The majority reported time constraints were a barrier to counseling, although most reported educational materials would be helpful. In 2012, Sellier et al studied the knowledge and practices of midwives on the primary prevention of maternal toxoplasmic infections during pregnancy in France(77). This was a survey of 139 midwives working in the public, private or liberal sectors in the Rhône-Alpes region, by means of a questionnaire. The midwives had satisfactory theoretical knowledge of toxoplasmosis with between 76.5% and 100% correct answers depending on the items for 102 participants. However, 49% forgot to recommend good hand hygiene, 38% did not adapt the advice given to the profile of their patients and 62% did not repeat the advice at the end of pregnancy. There is little other work on the subject. We have data from general practitioners in Burgundy in a work by Binquet

457 which highlighted a poor level of knowledge of the modes of contamination by these
458 doctors(78). Nevertheless, it was an anonymous survey with a low response rate of only 25%
459 with no comparison between participants and non-participants making it difficult to interpret
460 the results. Elsewhere in the world, there are cross-sectional studies conducted in the United
461 States in 1999 and 2005, with an update in 2012, which highlight the limits of health
462 professionals' knowledge in this field(79,80). The study by Kravetz and Federman included a
463 random sample of 49 obstetricians, 40 internists and 13 family doctors, and highlighted in
464 particular the poor prioritization of risk factors for contamination by practitioners and their
465 overestimation of the weight of contact with cats(79). The authors concluded that there was a
466 need for more information to be provided to health professionals, especially family doctors
467 and internists. The Jones et al. study was updated in 2015(81). It consisted of a questionnaire
468 sent to 1056 members of the American College of Obstetricians and Gynecologists (ACOG).
469 The results showed a minimum of correct answers between 19.7% and 40.3% depending on
470 whether or not the members included in the study were members of the Collaborative
471 Ambulatory Research Network (CARN). Of the participants, 80.2% had not diagnosed any
472 acute maternal *T.gondii* infection in the past 5 years. Among them, 12.7% had correctly
473 identified the screening role of IgG avidity testing, 42.6% had performed serological
474 screening for *T. gondii* in some asymptomatic pregnant women, and 62.1% had used
475 appropriate approaches. Health care professionals in the northeastern United States were 2.02
476 times more likely to perform routine screening than those in the West ($p = 0.025$). Female
477 physicians were 1.48 times more likely than male physicians ($p = 0.047$) to offer routine
478 screening. Participants felt that updating the ACOG recommendations on the screening and
479 management of acute *T.gondii* infection in pregnancy was useful. In 2011, a Brazilian study
480 by da Silva et al looked at health professionals' knowledge of risk factors(82). In Brazil, an
481 endemic region, the prevention of congenital toxoplasmosis most often relies on serological

screening of pregnant women. According to the authors, many cases could be prevented by simple precautions during pregnancy. The objective of this study was to assess knowledge about toxoplasmosis among professionals working in prenatal care in this high-prevalence region, a questionnaire was administered to 118 nurses and physicians. It included questions on diagnosis, clinic and prevention. Regarding prevention, 97.4% of professionals agreed that cats are the animal that eliminates the parasite in the stool, but 51.7% said that dogs also eliminate oocysts. The greatest number of errors was highlighted in relation to the education of non-immune pregnant women in relation to raw vegetables with only 5.2% of correct answers.

Knowledge of primary prevention measures by pregnant women.

In France there are four dating from the 1990s cited in the *AFSSA* and HAS reports which evaluated the levels of knowledge by pregnant women of measures to prevent toxoplasmosis during pregnancy(83,84). Pregnant women's knowledge levels were considered satisfactory in three of these studies because between 71% and 96% of the women included could cite two means of preventing toxoplasmosis(14,85,86). In the fourth study, less than half of the women included could cite two ways to prevent toxoplasmosis(87). In two studies, seronegative women were better informed than immunized women(14,86). In a third study, no difference in knowledge was found between seronegative women and immunized women(87). The fourth study did not specify the knowledge in seropositive patients for *T.gondii*(85). The heterogeneity of the results can be partly explained by methodological differences: for example, in the study by Baril et al(14), the pregnant women interviewed were all seronegative in early pregnancy, which was not the case in the other three studies, which therefore included women who were seropositive in early pregnancy and therefore may not have had any recall of information on toxoplasmosis. The questionnaires were not completed

506 in the same way depending on the study (by telephone or face-to-face). The questions asked
507 were different or differently worded, which could lead to different responses from patients.
508 Elsewhere in the world, a U.S. study was conducted in 2002 with pregnant women who were
509 interviewed to determine their knowledge about toxoplasmosis and their infection control
510 practices. Volunteer obstetricians from the American College of Obstetricians and
511 Gynecologists recruited the 403 participants who completed the questionnaire(88). Among
512 these women, 48% had indicated that they had received information about toxoplasmosis;
513 however, only 7% knew that they had been tested for this disease. Forty percent of the women
514 surveyed knew that toxoplasmosis is caused by an infection, but 21% thought a poison was
515 the cause. The highest level of knowledge was about the role of cats in toxoplasmosis; 61
516 percent of participants responded that the parasite is excreted in the feces of infected cats and
517 60 percent responded that people could become infected by changing the cat litter. The level
518 of knowledge about other risk factors was low; only 30% of the women knew that *T.gondii*
519 can be found in raw or undercooked meat. The level of knowledge about modes of
520 contamination was associated with education, age and ethnicity. Nevertheless, a high
521 percentage of women reported that they did not eat undercooked meat during pregnancy and
522 that they used good hygienic measures, such as washing their hands after handling raw meat,
523 gardening or changing cat litter. The authors concluded that, with the exception of the risk of
524 transmission by cats, the knowledge of pregnant women about toxoplasmosis was low.
525 However, toxoplasmosis prevention practices appeared to be generally good.
526 **Implementation of primary prevention measures by pregnant women.**
527 Preventive measures can only be effective if healthcare professionals are aware of the risk
528 factors and the advice to be given to pregnant women. Pregnant women must then be well
529 informed and aware of these preventive measures, which mean that this knowledge must have
530 an impact on the behaviour of pregnant women. In France, only one study (1994) cited in the

AFSSA report evaluated the degree of application of measures to prevent toxoplasmosis by seronegative pregnant women at the beginning of pregnancy(83). The results were poor, with only 17 per cent of pregnant women having satisfactorily implemented preventive measures. No significant association was found between the degree of implementation of preventive measures and age, parity or socio-professional category. On the other hand, preventive behaviors were associated with women's level of knowledge.

Health education programs are what works.

In 2008 a review of the literature by the Eurotoxo group attempted to answer the question of the effectiveness of measures or programs for the primary prevention of *T.gondii* infections in pregnant women(89). A total of four studies and two unpublished works met the inclusion criteria. All studies had methodological shortcomings. The first, a Belgian study, supported a significant decrease in the incidence of seroconversion to *T.gondii* after the introduction of intensive information on toxoplasmosis among selected pregnant women(90). The second study was Polish and concluded in favour of a significant increase in knowledge after the implementation of a multi-faceted public health education program(91). The third study was Canadian and supported increased knowledge and behaviour change in the group that received specific information compared to the control group(92). The last study was French and did not show any significant change in risk behaviours as a result of information provided by a doctor(93). This review highlighted the weakness of the literature in this area and the lack of studies measuring actual seroconversion. There was only suggestive evidence that health education approaches could help reduce the risk of congenital toxoplasmosis, but this problem already required further study using a more rigorous methodology and research design. Among the unpublished works, the Risk Assessment, Information, Awareness, randomized controlled trial evaluated the effect of a prenatal toxoplasmosis education program on the incidence of seroconversions during pregnancy, the level of knowledge,

556 preventive attitudes and behaviors of pregnant women, and the impact of a prenatal
557 toxoplasmosis education program on the incidence of seroconversions during pregnancy(94).
558 It was a multi-centre project carried out between 1994 and 1995 in seven departments of the
559 Rhône-Alpes region. A total of 5,023 seronegative pregnant women had been recruited in the
560 first trimester of their pregnancy by general practitioners and obstetric gynaecologists. These
561 women were randomized into groups. The first group consisted of 3,268 pregnant women
562 who had received information through an educational audiovisual support providing specific
563 information on toxoplasmosis in addition to the information usually provided. The other
564 group consisted of 1755 pregnant women who had received the usual information. The low
565 incidence of seroconversions during pregnancy in both groups (13/2,591 in the intervention
566 group and 4/1,358 in the control group; p=0.35) did not reveal any significant difference
567 according to the level of information the pregnant women had received about the risk factors
568 for *T.gondii* contamination. The seroconversion rates for toxoplasmosis detected during the
569 study did not differ between groups (RR = 1.70; 95% CI = 0.56 to 5.21; N = 3949).
570 Concerning the study of behavioral changes in the two groups of women, the statistical
571 analysis only took into account the women who had completed the study questionnaires,
572 which were 1953 (60%) women in the first group and 837 women (48%) in the second group.
573 At inclusion, 92% of the pregnant women knew the risk of infection associated with eating
574 undercooked beef, 90% knew the risk associated with eating poorly washed salad and 82%
575 knew the risk associated with handling cat litter. On the other hand, only 55% of women were
576 aware of the prevention of hand washing after handling raw meat. In addition, 88% of women
577 reported washing vegetables and fruit intended to be eaten raw. However, of the 97% who
578 had eaten meat at least once, only 55% had always eaten it well cooked. Overall, there was a
579 significant but small improvement in women's level of knowledge about toxoplasmosis and
580 its prevention among those patients who had been informed and who had a good knowledge

581 of the disease at inclusion in the study as well as a level of education above the baccalaureate
582 level. However, no association was found between preventive measure behaviors and group
583 assignment (information vs. no information). There were no significant differences in
584 behaviors related to cooking meat (OR = 1.21; 95% CI = 0.98-1.50) or hand washing (OR =
585 1.01; 95% CI = 0.83-1.22). Differences in behaviors were associated with the level of
586 knowledge and attitudes toward prevention at inclusion. Since 2009, Di Mario et al have been
587 trying to answer the question of the effectiveness of prenatal education to prevent congenital
588 toxoplasmosis through a review of the literature conducted by the Cochrane Database(95).
589 Only randomized or quasi-randomized controlled trials evaluating any type of prenatal
590 educational intervention for *T.gondii* infection during pregnancy and how to avoid it could be
591 included and considered in this meta-analysis. The authors note that when the protocol for this
592 systematic review was first published in 2006, no other systematic review on the effectiveness
593 of prenatal education for the congenital prevention of toxoplasmosis was available. They
594 specify that of the six studies considered in the literature review by Gollub et al in 2008, only
595 two were randomized controlled trials (Carter 1989; Wallon 2006), the other trials being
596 observational studies (Breugelmans 2004; Foulon 2000; Nguyen 2004; Pawlowski 2001). The
597 authors did not provide a meta-analysis of the data due to the lack of standardization in the
598 reporting of results. The first study was already included in the 2008 review and compared
599 two randomly assigned groups of women, which is why it was also included in Di Mario's
600 meta-analysis(92). The study was conducted in Ontario, Canada, and involved 432 pregnant
601 women who had attended early prenatal classes in six centres. It was a cluster randomized
602 trial. In this study, 26 groups were randomized to attend a 10-minute presentation on
603 toxoplasmosis prevention during the first prenatal education class. The remaining 26 groups
604 were randomized not to receive this information during the prenatal education class. Pre- and
605 post-training questionnaires were conducted. Among the informed patients, the following

606 changes were observed. Change in pet hygiene behaviors: informed women performed
607 significantly better than uninformed women (p<0,05) ; Change in food hygiene behaviors:
608 informed women performed significantly better than uninformed women with respect to
609 cooking roast beef (p<0.05) and hamburgers (p<0.01); other items were already good in the
610 pre-test. In terms of personal hygiene, informed women behaved significantly better than
611 others only in the sub-group of women who were not unemployed (p<0,05). Only 5% of
612 women in the intervention group recalled that they had received specific information on
613 toxoplasmosis prevention in prenatal classes. The authors concluded that prenatal education
614 can help to change the behavior of pregnant women, including personal, food and pet-related
615 hygiene. There were no results on the incidence of toxoplasmosis by group. The meta-
616 analysis of Di Mario et al. also includes the results of the Risk Assessment, Information,
617 Awareness, randomized controlled trial using data presented by Wallon et al. in 2006(96).
618 Overall, current data do not demonstrate the effectiveness of primary prevention measures for
619 congenital toxoplasmosis on the incidence of the disease. The results are only in favor of an
620 effect on the behavior of pregnant women with regard to preventive measures. Further well-
621 conducted studies are needed to substantiate the question, but this work is costly and
622 technically difficult to implement (number of subjects to be included, strong decreasing
623 incidence of the disease...).
624
625 **Conclusion.**
626 With current epidemiological data, it has been possible to identify a proven number of risk
627 factors for food and behavioral contamination relevant to each country worldwide situation
628 1/Consumption of meat, especially if it is undercooked;
629 2/Consumption of vegetables;
630 3/Contact with cats;

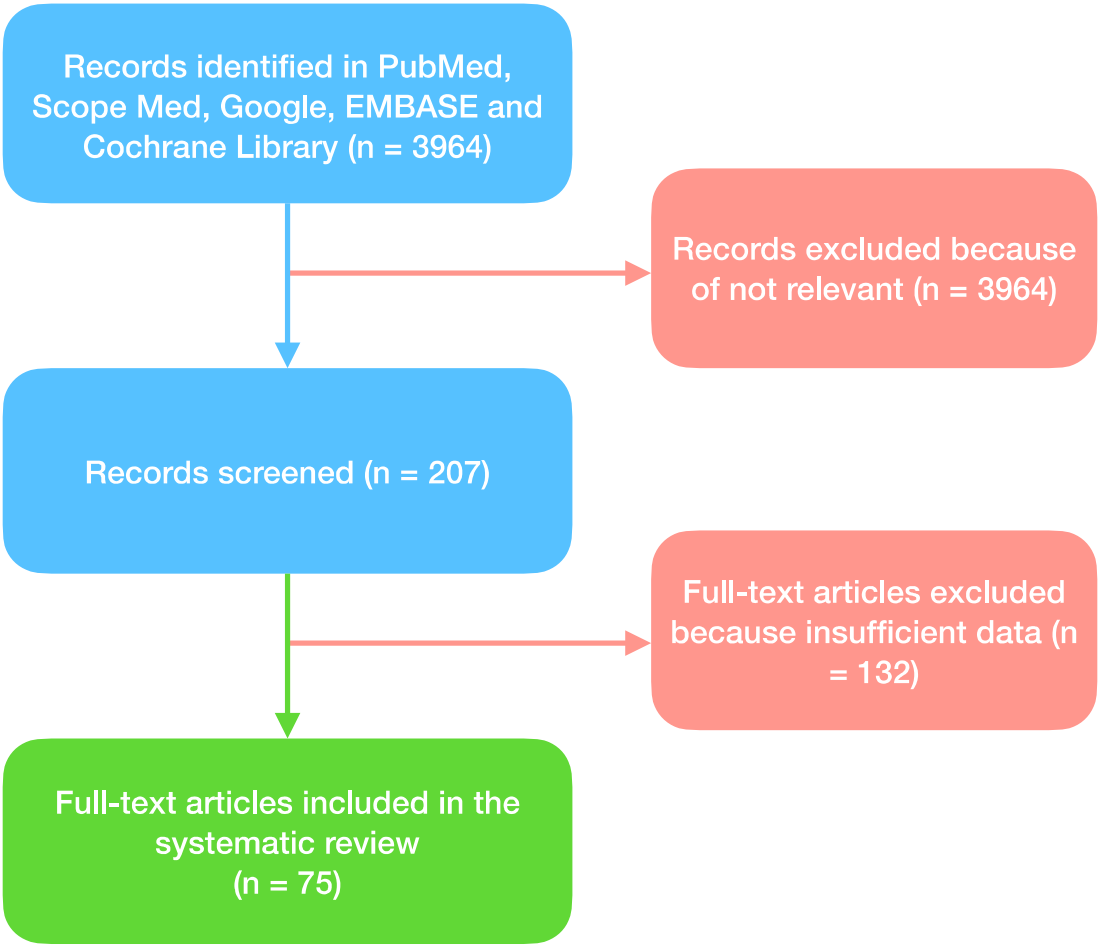
4/Contact with the soil (especially through gardening).

In addition to the already known risk factors for transmission of congenital toxoplasmosis, this systematic review described evidence for the transmission way of toxoplasmosis by shellfish, and drinking water. Raw milk (goat/cows) and game meat are also highly suspected to have this role.

There is not a high level of evidence that demonstrate that the implementation of prevention measures reduces the incidence of seroconversions in pregnant women. However, it seems reasonable to avoid known risk factors.

Compliance with these preventive measures requires that health professionals must give the information to pregnant women in a clear manner. This implies that health professionals must be fully trained on toxoplasmosis, and the known risk factors for contamination. The information provision must be well-organized during prenatal care visits. A recent opinion of expert panel of French experts on toxoplasmosis including gynecologists, pediatricians and parasitologists still recommend pursuing the screening program for prevention of congenital toxoplasmosis. Hygiene measures and pregnancy screening program represent the two main primary prevention tools to avoid congenital toxoplasmosis and its consequences.

Figure 1. Flow diagram of study selection process.



652 **Authors contribution:**

653 All persons listed as authors have contributed substantially to the design, performance,
654 analysis, and reporting of this work.

655 **KW, ML, IV, LM, OP**: collected data, analyzed data, wrote paper.

656 **KW, LP, JS, OP:** Designed study, analyzed data, wrote paper.

657

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