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

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17

18 **ABSTRACT:**

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

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

24

25 **Search Strategy.**

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

28

29 **Selection criteria.**

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

34

35 **Data collection and analysis**

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

43

44 **Conclusion.**

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

47 **Fundings: No**

48

49 **KEYWORDS:** Toxoplasmosis; pregnancy; primary prevention; hygiene measures

50

51

52 **What's already known about this topic?**

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

56

57 **What does this study add?**

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

60

61 **INTRODUCTION:**

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

72 At the time of screening in early pregnancy, it is recommended to give written and oral
73 information to pregnant women about primary prevention. Primary prevention measures have
74 since been reaffirmed and updated on several occasions, notably in 1983 and 1996 in a
75 weekly publication of the epidemiological bulletin by the Haut Conseil de la santé publique
76 (HCSP), historically the Conseil Supérieur d'Hygiène Publique de France (CSHPPF) until 2006
77 (5–7). These measures aim to reduce the incidence of toxoplasma seroconversion during
78 pregnancy in seronegative women. However, toxoplasmosis prevention management during
79 pregnancy differs between countries.

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

82

83 **MATERIALS AND METHODS**

84 We followed the standard MOOSE and PRISMA criteria when conducting this systematic
85 review and reporting the results (8,9). A systematic literature search was conducted for
86 articles focused on congenital toxoplasmosis prevention, toxoplasmosis prevention during
87 pregnancy, toxoplasmosis prevention and hygiene measures which were published between
88 1970 and August 2020, using the databases of PubMed, Scope Med, EMBASE, and the
89 Cochrane library. Searches were performed using Medical Subject Heading terms and the free
90 keywords: (“congenital toxoplasmosis” OR “toxoplasmosis during pregnancy”) AND
91 (“prevention” OR “Hygiene measures”) AND (“Cohort” OR “Case-control” OR “Reviews”).
92 Furthermore, the reference lists of retrieved articles were manually scrutinized to identify
93 potential relevant studies. Two reviewers (KW and OP) independently screened the titles and
94 abstracts of the studies to identify all potential eligible studies using a predefined data
95 extraction form. Then, they independently evaluated studies for inclusion, and studies were
96 included in the systematic review if they met the following criteria: 1) cohort or case control
97 studies; 2) the risk factors of toxoplasmosis seroconversion during pregnancy; the utility of
98 hygiene measures to prevent seroconversion during pregnancy; knowledge assessment of
99 hygiene measure by health care providers and pregnant women; and assessment of hygiene
100 measures effectiveness and how they are applied and followed by pregnant women. The
101 following data were extracted: the first author’s last name, year of publication, study location,
102 study design, risk factors, exposure assessment, outcome assessment, odds ratios, with their
103 confidence intervals.

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

106 **RESULTS**

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

110 **Risk factors of toxoplasmosis infection during pregnancy.**

111 In 2021, Thebault *et al* published a meta-analysis of the main identified and known risk
112 factors for toxoplasmosis (10). In this meta-analysis, the quality assessment stage was passed
113 by 213 primary studies investigating risk factors for sporadic infection with *Toxoplasma*
114 *gondii*, which were conducted between 1983 and 2016 (80.5% after 2000). Excluding
115 susceptible populations other than pregnant women, and some risk factors, 187 publications
116 were retained for meta-analysis. The meta-analysis of toxoplasma sporadic infections
117 revealed the significance of transmission by environmental factors such as contact with soil
118 and contact with animals, in particular cats. The consumption of raw or undercooked meat
119 and unwashed vegetables significantly increased the odds of acquiring the disease. Shellfish
120 and raw milk were identified as significant sources of toxoplasmosis. Almost all meat
121 categories were identified as risk factors: pork, poultry, beef, processed meat, lamb, and game
122 meat. Contaminated drinking water may play a role in the acquisition of infection. Moreover,
123 the lack of hygiene in preparing food was identified as a risk factor. A significant risk factor
124 for pregnant women is traveling abroad (10).

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

132 In the meta-analysis of the ANSES (*Agence nationale de sécurité sanitaire de l'alimentation,*
133 *de l'environnement et du travail*), the authors point out that the information collected like
134 seroprevalence or food preparation are not directly comparable between them(11). The
135 serological tests to identify cases are not the same depending on the countries from which the
136 studies were conducted. Eating habits are not the same, with differences in the preparation of
137 foodstuffs whose contamination levels can vary greatly from one country to another. Among
138 the studies taken into account for the meta-analysis, only 15% of those were conducted in
139 Europe, in environments comparable to the French situation. In 2005, the Eurotox group
140 published a literature review of the on dietary and behavioral risk factors associated with *T.*
141 *gondii* contamination in pregnant women (12). This review included five studies carried out
142 between 1996 and 2000 in Europe, among them three case-control studies with pregnant
143 women who had a toxoplasmic seroconversion during pregnancy (13–15). The other two
144 studies were cross-sectional (16,17). The objective of these studies was to assess the
145 environmental risk factors associated with acute toxoplasmosis during pregnancy. There were
146 some discrepancies between the studies in terms of data collection, population size, inclusion
147 criteria, which may explain differences in the results found in these publications. The risk
148 factors investigated were generally the same, however Bobic *et al* (18) and Cook *et al* (14)
149 did not investigate the consumption of poorly washed fruits and vegetables as a risk factor.
150 The study populations and inclusion criteria differed depending on the authors. Only the study
151 by Baril *et al* considered the certainty of seroconversion during pregnancy as a selection
152 criterion (14). The date of seroconversion in the other studies was uncertain. Moreover in
153 2005, the French Food Safety Agency (*Agence Française de Sécurité Sanitaire des Aliments –*
154 *AFSSA –*) provided a synthesis of the literature on behavioural and dietary risk factors
155 associated with *T. gondii* contamination (7). The AFSSA considered the five studies taken into
156 account by Eurotox as well as a sixth, older, cross-sectional study of Swiss pregnant women

157 carried out in 1987 and concluded the same as Eurotox (7). This cross-sectional study
158 included 280 women with a *T. gondii* IgG positive cord sample at delivery and 279 women
159 with an IgG negative cord sample. Overall, consumption of undercooked meat was the only
160 risk factor identified by all the studies with an Odd Ratio (OR) varying between 1.6 [95% CI:
161 1.2-2.1] and 11.4, p=0.00513.

162 Some risk factors are not established: Unpasteurized dairy products, shellfish, wild boar meat,
163 pork, poultry, game, are not identified as risk factors in France (18). With regards to food
164 consumption, undercooked meat, unwashed vegetables, raw milk, and shellfish are risk
165 factors only for positive *Toxoplasma* serology. Those results are in accordance with the
166 analysis of published outbreaks, showing that raw or undercooked meat was the origin of
167 44.7% of the outbreaks and raw vegetables of 5.3% (19)). These factors were not all sought in
168 the questionnaire of the Baril et al. study (8, 12). Moreover, it is possible that these
169 differences can be explained by the cooking practices of the meats (more or less cooked) or
170 by a lesser contamination of these foods in France (farming methods). Moreover,
171 environmental exposure (gardening, farm life) is not identified in the French studies. A recent
172 study confirmed the roles of drinking water, plants, raw milk and shellfish as risk factors for
173 *T.gondii* contamination in pregnant women (10).

174 **Risk factors of congenital toxoplasmosis.**

175 Since then, in 2014, Carellos *et al* studied the risk factors associated with congenital
176 toxoplasmosis in Brazil (20). This was a case-control study in Minas Gerais, including 175
177 women who had a child with congenital toxoplasmosis and 278 control women who delivered
178 without congenital toxoplasmosis. Factors associated with a lower risk of congenital
179 toxoplasmosis were a higher maternal age (OR = 0.89; CI 95% = 0.85-0.93), a higher level of
180 education (OR = 0.85; CI95% = 0.78-0.92), access to drinking water (OR = 0.21; CI95% =
181 0.08-0.51), and housing with flush toilets (OR = 0.18; CI95% = 0.04-0.78). Factors associated

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

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

207 70% of pregnant women are not immune to *T. gondii*, and 0.2-0.25% become infected during
208 pregnancy. In case of congenital toxoplasmosis, prompt initiation of treatment reduces the
209 occurrence of cerebral signs and symptoms, as well as retinal lesions(24). Binquet and al.(23)
210 showed that prenatal screening is cost-effective as compared to neonatal screening in
211 moderate prevalence areas. In addition, prenatal screening, by providing closer follow-up of
212 women at risk increases the number of occasions for education avoiding toxoplasmosis.
213 Though most international societies do not recommend systematic screening for mainly
214 financial reasons, if congenital toxoplasmosis appears benign in France today, it is probably
215 thanks to screening and the possibility of early treatment of fetuses and/or newborns. In
216 Germany, systematic screening is not recommended, but Lange and al. (25) encourage its
217 implementation. In the United States, systematic screening is not recommended. This country
218 represents a combination of parasite and host diversity, with substantial resources for
219 management of this disease but inadequate allocation of these resources. The absence of
220 mandatory gestational screening and a fragmented healthcare system with insufficient
221 insurance coverage and access results in a poor understanding of the true scope of congenital
222 toxoplasmosis there, and financial concerns limit access to screening(26). In undeveloped
223 countries, like Marocco or Columbia, the absence of systematic screening for toxoplasmosis
224 due to a lack of means has already shown its impact(26). Screening and management of
225 congenital toxoplasmosis depends above all on public health policy and the wealth of
226 countries(26).

227 **Established and recognized preventive measures of toxoplasmosis infection during**
228 **pregnancy.**

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

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

235 **Dietary recommendations.** Undercooking meat is the most widely documented risk factor in
236 the literature. The main epidemiological studies in pregnant women all conclude that there is
237 an over-risk of eating undercooked meat (13–17,27). Meat likely to contain cysts must be
238 cooked at a temperature above 67 degrees Celsius. In practice, this temperature corresponds
239 to a meat that does not allow red juice to run out when cutting. It was determined by Dubey in
240 1990 who studied the effect of temperature on the infectivity of cysts in infected pork (28).
241 Dubey was thus able to establish a thermal destruction curve estimating that a temperature of
242 67 degrees Celsius must be reached in the heart of the meat to achieve total inactivation of the
243 cysts. There is no advantage to the microwave over other cooking methods (29). Microwaves
244 were studied under experimental conditions with partial effectiveness on the infectivity of
245 *T.gondii* cysts at 65 degrees (41). All types of meat are susceptible to infection by *T.gondii*
246 and should therefore be cooked at more than 67 degrees, including venison, although beef and
247 mutton appear to be preferentially implicated in the French study by Baril et al (14). The
248 AFSSA reminds that the prevalence of toxoplasmosis is variable in cattle. It is higher in sheep
249 and leads to a high frequency of abortions in this species. In France, the contamination of
250 cattle from which meat intended for consumption comes was shown for sheep and lamb meat
251 by Halos et al in 2010 who estimated the overall seroprevalence of *T.gondii* was 17.7% (11.6-
252 31.5%) for lambs and 89% (73.5-100%) for adult sheep (P<0.0001) (30). No significant
253 difference was observed between imported and French meats. *T.gondii* contamination of meat
254 was also shown for beef by Blaga et al in 2019 ((31)) who were able to estimate the level of
255 toxoplasma infection of sheep, cattle and pig meat in France, (31,32,32,33). The
256 seroprevalence of toxoplasmosis ranged from 3% to 69.5% depending on the species and

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

271 According to AFSSA (7) in 2004, the frequency of contamination of domestic poultry could
272 represent a potential risk for humans, but parasitological data from experimental infections in
273 chicken, pigeon, duck and prevalence studies in chicken show that the parasites are mainly
274 localized in the brain, heart, to a lesser degree in other viscera and more rarely in muscles
275 (35–38). The risk of contamination is theoretically not zero but has not been assessed in
276 France. The prevalence of *T.gondii* in chickens was studied in a literature review by Dubey in
277 2010, which recalls that chickens are considered one of the most important hosts in the
278 epidemiology of *T.gondii* infection because they are an efficient source of infection for cats
279 and because humans can be infected by this parasite after eating infected chicken meat that
280 has not been properly cooked (39). The global prevalence of *T.gondii* infection in chickens is
281 very high. It has been estimated in chickens kept in backyard flocks to be close to 100%. In

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

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

304 **Water consumption.** Water consumption as a source of contamination has been recently
305 demonstrated in a review (47). Application of PCR for detection of *T. gondii* in water has
306 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.

332 **Pet care measures.** The last indispensable measures are precautions for cats. The handling of
333 cat litter is particularly inadvisable. Cleaning should be done with boiling water and the
334 wearing of gloves is strongly recommended. It is the cats, as the definitive hosts, who ensure
335 the spread of oocysts by contaminating the environment. Overall, the prevalence in feral cats
336 appears to be higher than in domestic cats (58). In a 2018 study by Simon et al, the dynamics
337 of seroconversion of *T.gondii*, was studied in five populations of cats living in Ardennes
338 farms in France (59). Seroprevalence varied between farms, from 15% to 73%, suggesting
339 differential exposure of cats to *T.gondii*. On highly exposed farms, cats could be infected
340 before the age of six months. Seroconversion rates ranged from 0.42 to 0.96 seroconversions
341 per cat per year and were higher in fall and winter than in spring and summer. These results
342 suggested variations in *T.gondii* exposures by season and farm. Seroprevalence of *T.gondii*
343 was estimated at 52.7% in a 2010 study by Afonso et al. in domestic cats living in rural areas
344 in France (60): seroconversion rates varied from 0.26 to 0.39 seroconversions per cat per year.
345 In 2006, Afonso et al estimated the prevalence of *T.gondii* in an urban stray cat population at
346 18.6% between 1993 and 2004. The prevalence of T.gondii is heterogeneous depending on
347 location, environment and season. Within the same location, cats excrete oocysts only very
348 episodically. It is therefore impossible to predict the real risk associated with a cat at a given
349 time. However, epidemiological studies have identified contact with cats as a risk factor for
350 *T.gondii*.

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

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

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

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

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

366

367

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

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

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

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

389

390 **Unestablished and hypothetical preventive measures of toxoplasmosis infection during**
391 **pregnancy.**

392 Freezing the meat at a temperature of -12 degrees Celsius or lower for at least 3 days to
393 destroy the cysts. This measure is the result of Dubey's experiments on pork in 1988 (63). The
394 required freezing time depends on the thickness of the piece of meat to be inactivated. The
395 larger the piece, the longer the freezing time required to reach a potentially deep cyst. This
396 explains why industrially frozen meat can be consumed without risk, whereas domestic
397 freezing may not be sufficient to destroy the cysts. Sporulated oocysts found on plants remain
398 viable and potentially infectious after constant freezing for 28 days at -21 degrees Celsius
399 (64,65). The *AFSSA* also proposes measures whose effectiveness is to be further evaluated
400 (7). Among them, the consumption of marinated, salted or smoked meat could also be
401 avoided. In this regard, the consumption of dried or salted pork has been identified as a risk
402 factor by Buffolano et al OR = 2.9 [IC95%:1.6-5.5] (16). Experiments on pieces of mutton
403 meat in 1992 by Lundén et al suggest that smoking or salting procedures may be effective in
404 controlling the infective power of cysts (66). Smoking was studied by injecting a solution of
405 sodium chloride into the meat before it was smoked at a temperature not exceeding 50°C for
406 24 to 48 hours. Salting was evaluated on pieces of meat from 200 to 360 g put in plastic bags

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

414 Among the preventive measures are cited the consumption of seafood although no *T.gondii*
415 infection linked to seafood consumption has yet been found in France, *ANSES* states in 2018
416 that shellfish (oysters and mussels) have been identified as a risk factor for contamination in
417 two meta-analysis (10,68). The presence and survival of oocysts in shellfish and other
418 foodstuffs from the sea are suspected by indirect arguments such as the existence of cases of
419 marine mammals infected with *T.gondii* (69–71). More recently, in 2017, a Chinese study
420 revealed the presence of *T. gondii* oocysts in oysters sold in a market in China (72). A total of
421 26 of the 998 oysters tested tested positive by PCR amplification (2.61%). This study suggests
422 that oysters have the ability to filter and retain oocysts in their tissues. Another 2014 Chinese
423 study of 3432 shellfish showed low (N=5/3432) but not zero contamination of *T.gondii* in
424 these species (73). In a New Zealand study in 2018, *T.gondii* contamination in the form of
425 sporulated oocysts was detected in 16.4% (N=13) of a sample of 104 commercial mussels
426 (74). In the 2018 meta-analysis, *ANSES* concludes that seafood consumption is associated
427 with an increased risk of *T.gondii* in the general population (10,11). Seafood should be
428 thoroughly cooked to inactivate oocysts.

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

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

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

443 **Knowledge of primary prevention measures by health professionals.**

444 Several studies around the world have assessed practitioners' knowledge, attitudes, and practices
445 regarding prevention of infections in pregnancy. In 2009 in United States(76), among 305
446 gynecologists interviewed, about 84% reported counseling pregnant women about preventing
447 infection from *T.gondii*. The majority reported time constraints were a barrier to counseling,
448 although most reported educational materials would be helpful. In 2012, Sellier et al studied
449 the knowledge and practices of midwives on the primary prevention of maternal toxoplasmic
450 infections during pregnancy in France(77). This was a survey of 139 midwives working in
451 the public, private or liberal sectors in the Rhône-Alpes region, by means of a questionnaire.
452 The midwives had satisfactory theoretical knowledge of toxoplasmosis with between 76.5%
453 and 100% correct answers depending on the items for 102 participants. However, 49% forgot
454 to recommend good hand hygiene, 38% did not adapt the advice given to the profile of their
455 patients and 62% did not repeat the advice at the end of pregnancy. There is little other work
456 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

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

491 **Knowledge of primary prevention measures by pregnant women.**

492 In France there are four dating from the 1990s cited in the AFSSA and HAS reports which
493 evaluated the levels of knowledge by pregnant women of measures to prevent toxoplasmosis
494 during pregnancy(83,84). Pregnant women's knowledge levels were considered satisfactory
495 in three of these studies because between 71% and 96% of the women included could cite two
496 means of preventing toxoplasmosis(14,85,86). In the fourth study, less than half of the women
497 included could cite two ways to prevent toxoplasmosis(87). In two studies, seronegative
498 women were better informed than immunized women(14,86). In a third study, no difference
499 in knowledge was found between seronegative women and immunized women(87). The
500 fourth study did not specify the knowledge in seropositive patients for *T.gondii*(85). The
501 heterogeneity of the results can be partly explained by methodological differences: for
502 example, in the study by Baril et al(14), the pregnant women interviewed were all
503 seronegative in early pregnancy, which was not the case in the other three studies, which
504 therefore included women who were seropositive in early pregnancy and therefore may not
505 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

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

537 **Health education programs are what works.**

538 In 2008 a review of the literature by the Eurotox group attempted to answer the question of
539 the effectiveness of measures or programs for the primary prevention of *T.gondii* infections in
540 pregnant women(89). A total of four studies and two unpublished works met the inclusion
541 criteria. All studies had methodological shortcomings. The first, a Belgian study, supported a
542 significant decrease in the incidence of seroconversion to *T.gondii* after the introduction of
543 intensive information on toxoplasmosis among selected pregnant women(90). The second
544 study was Polish and concluded in favour of a significant increase in knowledge after the
545 implementation of a multi-faceted public health education program(91). The third study was
546 Canadian and supported increased knowledge and behaviour change in the group that
547 received specific information compared to the control group(92). The last study was French
548 and did not show any significant change in risk behaviours as a result of information provided
549 by a doctor(93). This review highlighted the weakness of the literature in this area and the
550 lack of studies measuring actual seroconversion. There was only suggestive evidence that
551 health education approaches could help reduce the risk of congenital toxoplasmosis, but this
552 problem already required further study using a more rigorous methodology and research
553 design. Among the unpublished works, the Risk Assessment, Information, Awareness,
554 randomized controlled trial evaluated the effect of a prenatal toxoplasmosis education
555 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;

631 4/Contact with the soil (especially through gardening).

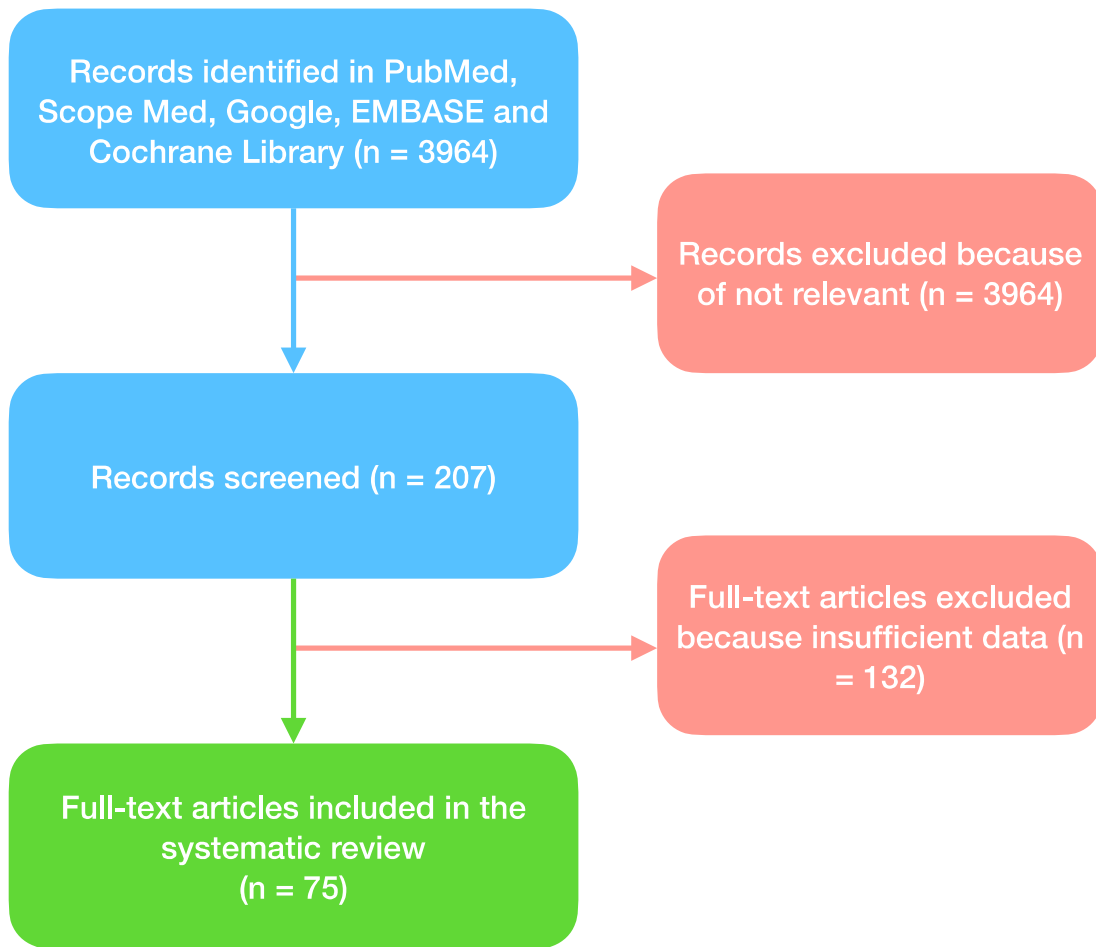
632 In addition to the already known risk factors for transmission of congenital toxoplasmosis,
633 this systematic review described evidence for the transmission way of toxoplasmosis by
634 shellfish, and drinking water. Raw milk (goat/cows) and game meat are also highly suspected
635 to have this role.

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

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

647

648 **Figure 1.** Flow diagram of study selection process.
649



650

651

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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