

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

Karl Wehbe, Lucille Pencole, Martin Lhuaire, Jeanne Sibiude, Laurent Mandelbrot, Isabelle Villena, Olivier Picone

# ▶ To cite this version:

Karl Wehbe, Lucille Pencole, Martin Lhuaire, Jeanne Sibiude, Laurent Mandelbrot, et al.. Hygiene measures as primary prevention of toxoplasmosis during pregnancy: A systematic review. Journal of Gynecology Obstetrics and Human Reproduction, 2022, 51 (3), pp.102300. 10.1016/j.jogoh.2021.102300. hal-03520417

# HAL Id: hal-03520417 https://hal.univ-reims.fr/hal-03520417v1

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

Karl Wehbe<sup>1</sup>, Lucille Pencole<sup>5</sup>, Martin Lhuaire<sup>2,3</sup>, Jeanne Sibiude<sup>5</sup>, Laurent Mandelbrot<sup>5</sup>, Isabelle Villena<sup>4</sup>, Olivier Picone<sup>5</sup>

#### **Authors' affiliations:**

- 1 Département de Gynécologie-Obstétrique Institut Mère Enfant Alix de Champagne Centre Hospitalier Universitaire (CHU), 51092 REIMS
- 2 Department of Plastic, Reconstructive and Aesthetic Surgery, Hôpital Européen Georges Pompidou, Assistance Publique des Hôpitaux de Paris, Université de Paris, Paris, France
- 3 Department of Organogenesis and Anatomy, URDIA, EA4465, UFR Biomedical des Saints-Pères, Université de Paris, Paris, France
- 4 Université Reims Champagne Ardenne, EA7510 et Centre National de Référence de la Toxoplasmose, Centre de Ressources Biologiques Toxoplasma, Service de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Reims, Reims, France.
- 5 Assistance Publique-Hôpitaux de Paris, Service de Gynécologie-Obstétrique, Hôpital Louis Mourier, Colombes, France; Université de Paris; Inserm IAME-U1137, Paris, France; FHU PREMA; Groupe de Recherche sur les Infections pendant la grossesse (GRIG)

Correspondance to:
Pr Olivier Picone
Service de Gynécologie Obstétrique
Hôpital Louis Mourier, APAP, 178 rue des renouillets, 92700 Colombes, France
Olivier.picone@php.fr
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

1	Hygiene measures as primary prevention of toxoplasmosis during pregnancy: a
2	systematic review
3	
4	Short running head: Hygiene measures as primary prevention of toxoplasmosis during
5	pregnancy
6	
7	Conflict of interest statements: None.
8	
9	Role of funding source: None.
10	
11	Words count: 6931 words (without abstract and references)
12	
13	Number of figures: 1
14	
15	Number of tables: 0
16	
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
- 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.

- 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
- 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	<b>KEYWORDS:</b> Toxoplasmosis; pregnancy; primary prevention; hygiene measures
50 51	What's almosty lyngyyn ab aut this taria?
52 53 54 55 56	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.
57 58 59	What does this study add?  Hygiene measures are effective and applicable primary prevention to reduce toxoplasmosis and avoid congenital toxoplasmosis and its consequences.

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

## **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.

82

83

81

# 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

84

85

86

87

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

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.

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131

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

132

133

134

135

136

137

138

139

140

141

142

143

144

145

146

147

148

149

150

151

152

153

154

155

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,

# Risk factors of congenital toxoplasmosis.

T.gondii contamination in pregnant women (10).

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% = 0.04-0.78). Factors associated

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

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

182

183

184

185

186

187

188

189

190

191

192

193

194

195

196

197

198

199

200

201

202

203

204

205

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

228 pregnancy.

207

208

209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

226

227

229

230

231

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

232

233

234

235

236

237

238

239

240

241

242

243

244

245

246

247

248

249

250

251

252

253

254

255

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

257

258

259

260

261

262

263

264

265

266

267

268

269

270

271

272

273

274

275

276

277

278

279

280

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

282

283

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

299

300

301

302

303

304

305

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

307

308

309

310

311

312

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

**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 <u>T.gondii</u> 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

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347

348

349

350

351

352

353

354

355

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

407

408

409

410

411

412

413

414

415

416

417

418

419

420

421

422

423

424

425

426

427

428

429

430

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

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

457

458

459

460

461

462

463

464

465

466

467

468

469

470

471

472

473

474

475

476

477

478

479

480

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

in the same way depending on the study (by telephone or face-to-face). The questions asked were different or differently worded, which could lead to different responses from patients. Elsewhere in the world, a U.S. study was conducted in 2002 with pregnant women who were interviewed to determine their knowledge about toxoplasmosis and their infection control practices. Volunteer obstetricians from the American College of Obstetricians and Gynecologists recruited the 403 participants who completed the questionnaire (88). Among these women, 48% had indicated that they had received information about toxoplasmosis; however, only 7% knew that they had been tested for this disease. Forty percent of the women surveyed knew that toxoplasmosis is caused by an infection, but 21% thought a poison was the cause. The highest level of knowledge was about the role of cats in toxoplasmosis; 61 percent of participants responded that the parasite is excreted in the feces of infected cats and 60 percent responded that people could become infected by changing the cat litter. The level of knowledge about other risk factors was low; only 30% of the women knew that T.gondii can be found in raw or undercooked meat. The level of knowledge about modes of contamination was associated with education, age and ethnicity. Nevertheless, a high percentage of women reported that they did not eat undercooked meat during pregnancy and that they used good hygienic measures, such as washing their hands after handling raw meat, gardening or changing cat litter. The authors concluded that, with the exception of the risk of transmission by cats, the knowledge of pregnant women about toxoplasmosis was low. However, toxoplasmosis prevention practices appeared to be generally good.

506

507

508

509

510

511

512

513

514

515

516

517

518

519

520

521

522

523

524

525

526

527

528

529

530

# Implementation of primary prevention measures by pregnant women.

Preventive measures can only be effective if healthcare professionals are aware of the risk factors and the advice to be given to pregnant women. Pregnant women must then be well informed and aware of these preventive measures, which mean that this knowledge must have 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.

531

532

533

534

535

536

537

538

539

540

541

542

543

544

545

546

547

548

549

550

551

552

553

554

555

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,

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

556

557

558

559

560

561

562

563

564

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

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

581

582

583

584

585

586

587

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

604

changes were observed. Change in pet hygiene behaviors: informed women performed significantly better than uninformed women (p<0.05); Change in food hygiene behaviors: informed women performed significantly better than uninformed women with respect to cooking roast beef (p < 0.05) and hamburgers (p < 0.01); other items were already good in the pre-test. In terms of personal hygiene, informed women behaved significantly better than others only in the sub-group of women who were not unemployed (p<0.05). Only 5% of women in the intervention group recalled that they had received specific information on toxoplasmosis prevention in prenatal classes. The authors concluded that prenatal education can help to change the behavior of pregnant women, including personal, food and pet-related hygiene. There were no results on the incidence of toxoplasmosis by group. The metaanalysis of Di Mario et al. also includes the results of the Risk Assessment, Information, Awareness, randomized controlled trial using data presented by Wallon et al. in 2006(96). Overall, current data do not demonstrate the effectiveness of primary prevention measures for congenital toxoplasmosis on the incidence of the disease. The results are only in favor of an effect on the behavior of pregnant women with regard to preventive measures. Further wellconducted studies are needed to substantiate the question, but this work is costly and technically difficult to implement (number of subjects to be included, strong decreasing incidence of the disease...).

624

625

623

606

607

608

609

610

611

612

613

614

615

616

617

618

619

620

621

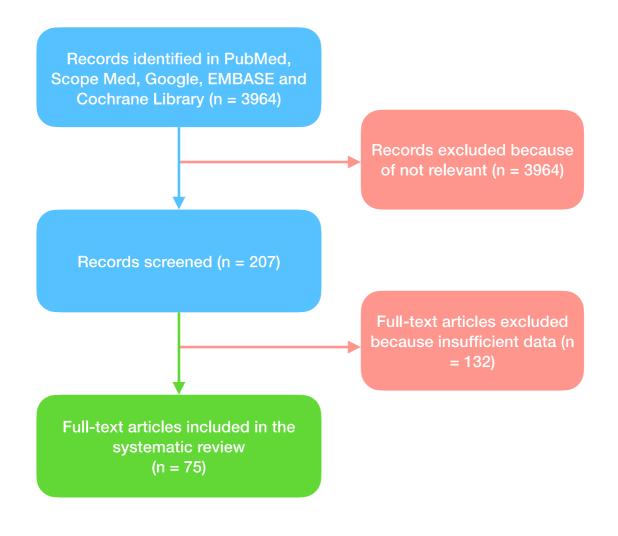
622

## Conclusion.

- With current epidemiological data, it has been possible to identify a proven number of risk
- 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.

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



# **Authors contribution:**

- All persons listed as authors have contributed substantially to the design, performance,
- 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.

#### References

- 1. Thulliez P, Daffos F, Forestier F. Diagnosis of Toxoplasma infection in the pregnant
- woman and the unborn child: current problems. Scand J Infect Dis Suppl. 1992;84:18–22.
- Practice bulletin no. 151: Cytomegalovirus, parvovirus B19, varicella zoster, and
- toxoplasmosis in pregnancy PubMed [Internet]. [cited 2021 May 24]. Available from:
- https://pubmed.ncbi.nlm.nih.gov/26000539/
- 664 3. Paquet C, Yudin MH. No. 285-Toxoplasmosis in Pregnancy: Prevention, Screening,
- and Treatment. J Obstet Gynaecol Can. 2018 Aug;40(8):e687–93.
- 666 4. Guidance | Antenatal care for uncomplicated pregnancies | Guidance | NICE [Internet].
- NICE; [cited 2021 May 24]. Available from:
- 668 https://www.nice.org.uk/guidance/cg62/chapter/1-Guidance#screening-for-infections
- 5. InVS | BEH n°16 (16 avril 1996). Facteurs de risque d'acquisition de la toxoplasmose
- 670 chez les femmes enceintes en 1995 (France). Recommandations. Note de la rédaction
- [Internet]. [cited 2019 Jun 16]. Available from:
- http://invs.santepubliquefrance.fr/beh/1996/9616/
- 673 6. Toxoplasmose | ameli.fr | Assuré [Internet]. [cited 2021 Aug 29]. Available from:
- 674 https://www.ameli.fr/assure/sante/themes/toxoplasmose
- 7. Derouin F, Bultel C, Roze S, et al. Toxoplasmose: état des connaissances et
- 676 évaluation du risque lié à l'alimentation : rapport du groupe de travail "Toxoplasma gondii"
- de l'Afssa NLM Catalog NCBI [Internet]. [cited 2020 Jun 20]. Available from:
- 678 https://www.ncbi.nlm.nih.gov/nlmcatalog/101310165
- 8. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in
- epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in
- 681 Epidemiology (MOOSE) group. JAMA. 2000 Apr 19;283(15):2008–12.
- 682 9. Moher D, Liberati A, Tetzlaff J, et al. Preferred Reporting Items for Systematic
- Reviews and Meta-Analyses: The PRISMA Statement. PLOS Medicine. 2009
- 684 juil;6(7):e1000097.
- 685 10. Thebault A, Kooh P, Cadavez V, et al. Risk factors for sporadic toxoplasmosis: A
- 686 systematic review and meta-analysis. Microbial Risk Analysis. 2020 Aug 6;100133.
- AVIS et RAPPORT de l'Anses relatif à l'attribution des sources des maladies
- 688 infectieuses d'origine alimentaire | Anses Agence nationale de sécurité sanitaire de
- l'alimentation, de l'environnement et du travail [Internet]. [cited 2019 Jul 21]. Available
- 690 from: https://www.anses.fr/fr/content/avis-et-rapport-de-lanses-relatif-%C3%A0-lattribution-
- des-sources-des-maladies-infectieuses-0
- 692 12. Leroy WV, Hadjichristodoulou C, Leroy DV, et al. European TOXO PREVENTION
- 693 Project, Systematic review of risk factors for Toxoplasma gondii infection in pregnant
- women. Panel 3: prevention and screening issues 2005.
- 695 13. Cook AJ, Gilbert RE, Buffolano W, et al. Sources of toxoplasma infection in pregnant
- 696 women: European multicentre case-control study. European Research Network on Congenital
- 697 Toxoplasmosis. BMJ. 2000 Jul 15;321(7254):142–7.
- 698 14. Baril L, Ancelle T, Goulet V,et al. Risk factors for Toxoplasma infection in
- 699 pregnancy: a case-control study in France. Scand J Infect Dis. 1999;31(3):305–9.
- 700 15. Kapperud G, Jenum PA, Stray-Pedersen B, et al. Risk factors for Toxoplasma gondii
- infection in pregnancy. Results of a prospective case-control study in Norway. Am J
- 702 Epidemiol. 1996 Aug 15;144(4):405–12.
- 703 16. Buffolano W, Gilbert RE, Holland FJ, et al. Risk factors for recent toxoplasma
- infection in pregnant women in Naples. Epidemiol Infect. 1996 Jun;116(3):347–51.

- 705 17. Bobić B, Jevremović I, Marinković J, et al. Risk factors for Toxoplasma infection in a
- reproductive age female population in the area of Belgrade, Yugoslavia. Eur J Epidemiol.
- 707 1998 Sep;14(6):605–10.
- 708 18. Augustin J-C, Kooh P, Bayeux T, et al. Contribution of Foods and Poor Food-
- Handling Practices to the Burden of Foodborne Infectious Diseases in France. Foods. 2020
- 710 Nov 11;9(11).
- 711 19. Meireles LR, Ekman CCJ, Andrade HF de, et al HUMAN TOXOPLASMOSIS
- 712 OUTBREAKS AND THE AGENT INFECTING FORM. FINDINGS FROM A
- 713 SYSTEMATIC REVIEW. Rev Inst Med Trop Sao Paulo. 2015 Oct;57(5):369–76.
- 714 20. Carellos EVM, de Andrade GMQ, Vasconcelos-Santos DV, et al. Adverse
- socioeconomic conditions and oocyst-related factors are associated with congenital
- toxoplasmosis in a population-based study in Minas Gerais, Brazil. PLoS ONE.
- 717 2014;9(2):e88588.
- 718 21. Dubey JP, Ferreira LR, Alsaad M, et al. Experimental Toxoplasmosis in Rats Induced
- 719 Orally with Eleven Strains of Toxoplasma gondii of Seven Genotypes: Tissue Tropism,
- 720 Tissue Cyst Size, Neural Lesions, Tissue Cyst Rupture without Reactivation, and Ocular
- Lesions. PLoS One [Internet]. 2016 May 26 [cited 2021 May 24];11(5). Available from:
- 722 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4882154/
- 723 22. Carme B, Demar M, Ajzenberg D, Severe acquired toxoplasmosis caused by wild
- 724 cycle of Toxoplasma gondii, French Guiana. Emerging infectious diseases. 2009
- 725 Apr;15(4):656–8.
- 726 23. Binquet C, Lejeune C, Seror V, al. The cost-effectiveness of neonatal versus prenatal
- screening for congenital toxoplasmosis. PLoS One. 2019 Sep 18;14(9):e0221709.
- 728 24. Picone O, Fuchs F, Benoist G, et al. Toxoplasmosis screening during pregnancy in
- 729 France: Opinion of an expert panel for the CNGOF. J Gynecol Obstet Hum Reprod. 2020
- 730 Sep;49(7):101814.
- 731 25. Lange AE, Thyrian JR, Wetzka S, et al. The impact of socioeconomic factors on the
- efficiency of voluntary toxoplasmosis screening during pregnancy: a population-based study.
- 733 BMC Pregnancy Childbirth. 2016 Jul 29;16:197.
- 734 26. El Bissati K, Levigne P, Lykins J, et al. Global initiative for congenital toxoplasmosis:
- an observational and international comparative clinical analysis. Emerg Microbes Infect. 2018
- 736 Sep 27;7:165.
- 737 27. Sturchler D, Berger R, Just M. Die konnatale Toxoplasmose in der Schweiz. Schwei
- 738 Med Wochenschr. 1987;117:161–7.
- 739 28. Dubey JP, Kotula AW, Sharar A, et al. Effect of high temperature on infectivity of
- Toxoplasma gondii tissue cysts in pork. J Parasitol. 1990 Apr;76(2):201–4.
- 741 29. Dubey JP. The scientific basis for prevention of Toxoplasma gondii infection: studies
- on tissue cyst survival, risk factors and hygiene measures. In: Ambroise-Thomas P., Petersen
- P.E. (eds) Congenital toxoplasmosis; 271-5. Springer, Paris; 2000.
- Halos L, Thébault A, Aubert D, et al. An innovative survey underlining the significant
- level of contamination by Toxoplasma gondii of ovine meat consumed in France. Int J
- 746 Parasitol. 2010 Feb;40(2):193–200.
- 747 31. Blaga R, Aubert D, Thébault A, et al. Toxoplasma gondii in beef consumed in France:
- regional variation in seroprevalence and parasite isolation. Parasite. 2019;26:77.
- 749 32. Villena I, Blaga R. Coopération médico-vétérinaire et réduction de l'incidence de la
- toxoplasmose en France. Bulletin de l'Académie Vétérinaire de France. 2018 Jan 1;171:87.
- 751 33. Djokic V, Blaga R, Aubert D, et al. Toxoplasma gondii infection in pork produced in
- 752 France. Parasitology. 2016 Apr;143(5):557–67.
- 753 34. Roqueplo C, Blaga R, Jean-Lou M,. Seroprevalence of Toxoplasma gondii in hunted
- vild boars (Sus scrofa) from southeastern France. Folia Parasitol. 2017 25;64.

- 755 35. Dubey JP, Ruff MD, Camargo ME, et al. Serologic and parasitologic responses of
- domestic chickens after oral inoculation with Toxoplasma gondii oocysts. Am J Vet Res.
- 757 1993 Oct;54(10):1668–72.
- 758 36. Biancifiori F, Rondini C, Grelloni V, et al. Avian toxoplasmosis: experimental
- 759 infection of chicken and pigeon. Comp Immunol Microbiol Infect Dis. 1986;9(4):337–46.
- 760 37. Kaneto CN, Costa AJ, Paulillo AC, et al. Experimental toxoplasmosis in broiler
- 761 chicks. Vet Parasitol. 1997 May;69(3–4):203–10.
- 762 38. Bártová E, Dvoráková H, Bárta J, et al. Susceptibility of the domestic duck (Anas
- platyrhynchos) to experimental infection with Toxoplasma gondii oocysts. Avian Pathol.
- 764 2004 Apr;33(2):153–7.
- 765 39. Dubey JP. Toxoplasma gondii infections in chickens (Gallus domesticus): prevalence,
- 766 clinical disease, diagnosis and public health significance. Zoonoses Public Health. 2010
- 767 Feb;57(1):60–73.
- 768 40. Wallace GD. Experimental transmission of Toxoplasma gondii by filth-flies. Am J
- 769 Trop Med Hyg. 1971 May;20(3):411–3.
- 770 41. Wallace GD. Experimental transmission of Toxoplasma gondii by cockroaches. J
- 771 Infect Dis. 1972 Nov;126(5):545–7.
- Wallace GD. Intermediate and transport hosts in the natural history of Toxoplasma
- 773 gondii. Am J Trop Med Hyg. 1973 Jul;22(4):456–64.
- 774 43. Saitoh Y, Itagaki H. Dung beetles, Onthophagus spp., as potential transport hosts of
- feline coccidia. Nippon Juigaku Zasshi. 1990 Apr;52(2):293–7.
- 776 44. Kniel KE, Lindsay DS, Sumner SS, et al. Examination of attachment and survival of
- Toxoplasma gondii oocysts on raspberries and blueberries. J Parasitol. 2002 Aug;88(4):790–778 3.
- 779 45. Hall SM, Pandit A, Golwilkar A, et al. How do Jains get toxoplasma infection?
- 780 Lancet. 1999 Aug 7;354(9177):486–7.
- 781 46. Roghmann MC, Faulkner CT, Lefkowitz A, et al. Decreased seroprevalence for
- 782 Toxoplasma gondii in Seventh Day Adventists in Maryland. Am J Trop Med Hyg. 1999
- 783 May;60(5):790–2.
- 784 47. Shapiro K, Bahia-Oliveira L, Dixon B, et al. Environmental transmission of
- 785 Toxoplasma gondii: Oocysts in water, soil and food. Food and Waterborne Parasitology. 2019
- 786 Jun 1;15:e00049.
- 787 48. Bahia-Oliveira G-M. Toxoplasma gondii [Internet]. Global Water Pathogen Project.
- 788 Michigan State University, UNESCO; 2015 [cited 2021 Jun 20]. Available from:
- 789 https://www.waterpathogens.org/book/toxoplasma-gondii
- 790 49. Triviño-Valencia J, Lora F, Zuluaga JD, et al. Detection by PCR of pathogenic
- 791 protozoa in raw and drinkable water samples in Colombia. Parasitol Res. 2016
- 792 May;115(5):1789–97.
- 793 50. Sotiriadou I, Karanis P. Evaluation of loop-mediated isothermal amplification for
- detection of Toxoplasma gondii in water samples and comparative findings by polymerase
- chain reaction and immunofluorescence test (IFT). Diagn Microbiol Infect Dis. 2008
- 796 Dec;62(4):357–65.
- 797 51. Sroka J, Wójcik-Fatla A, Dutkiewicz J. Occurrence of Toxoplasma gondii in water
- from wells located on farms. Annals of agricultural and environmental medicine: AAEM.
- 799 2006:
- 800 52. Wells B, Shaw H, Innocent G, et al. Molecular detection of Toxoplasma gondii in
- water samples from Scotland and a comparison between the 529bp real-time PCR and ITS1
- 802 nested PCR. Water Res. 2015 Dec 15;87:175–81.

- 803 53. Aubert D, Villena I. Detection of Toxoplasma gondii oocysts in water: proposition of
- a strategy and evaluation in Champagne-Ardenne Region, France. Mem Inst Oswaldo Cruz.
- 805 2009 Mar;104(2):290–5.
- 806 54. Dubey JP. Toxoplasmosis a waterborne zoonosis. Veterinary parasitology. 2004 Dec
- 807 9;126(1–2):57–72.
- 808 55. Shapiro K, Conrad PA, Mazet JAK, et al. Effect of estuarine wetland degradation on
- transport of Toxoplasma gondii surrogates from land to sea. Appl Environ Microbiol. 2010
- 810 Oct;76(20):6821-8.
- 811 56. Newman AP, Aitken D, Antizar-Ladislao B. Stormwater quality performance of a
- macro-pervious pavement car park installation equipped with channel drain based oil and silt
- 813 retention devices. Water Res. 2013 Dec 15;47(20):7327–36.
- 814 57. Virahsawmy HK, Stewardson MJ, Vietz G, et al. Factors that affect the hydraulic
- performance of raingardens: implications for design and maintenance. Water Sci Technol.
- 816 2014;69(5):982–8.
- 817 58. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans.
- 818 Int J Parasitol. 2000 Nov;30(12–13):1217–58.
- 819 59. Simon JA, Pradel R, Aubert D, et al. A multi-event capture-recapture analysis of
- 820 Toxoplasma gondii seroconversion dynamics in farm cats. Parasit Vectors. 2018 Jun
- 821 8;11(1):339.
- 822 60. Afonso E, Thulliez P, Gilot-Fromont E. Local meteorological conditions, dynamics of
- seroconversion to Toxoplasma gondii in cats (Felis catus) and oocyst burden in a rural
- 824 environment. Epidemiol Infect. 2010 Aug;138(8):1105–13.
- 825 61. Maldonado YA, Read JS, Diseases C on I. Diagnosis, Treatment, and Prevention of
- 826 Congenital Toxoplasmosis in the United States. Pediatrics [Internet]. 2017 Feb 1 [cited 2021]
- 827 May 24]; Available from:
- https://pediatrics.aappublications.org/content/early/2017/01/26/peds.2016-3860
- 829 62. CDC Toxoplasmosis Prevention & Control [Internet]. [cited 2021 May 24].
- 830 Available from: https://www.cdc.gov/parasites/toxoplasmosis/prevent.html
- 831 63. Dubey JP. Long-term persistence of Toxoplasma gondii in tissues of pigs inoculated
- with T gondii oocysts and effect of freezing on viability of tissue cysts in pork. Am J Vet Res.
- 833 1988 Jun;49(6):910–3.
- 834 64. Frenkel JK, Dubey JP. Effects of freezing on the viability of toxoplasma oocysts. J
- 835 Parasitol. 1973 Jun;59(3):587–8.
- 836 65. Smith JL. Foodborne Toxoplasmosis. Journal of Food Safety. 1991;12(1):17–57.
- 837 66. Lundén A, Uggla A. Infectivity of Toxoplasma gondii in mutton following curing,
- smoking, freezing or microwave cooking. Int J Food Microbiol. 1992 Apr;15(3–4):357–63.
- 839 67. Fredericks J, Hawkins-Cooper DS, Hill DE, et al. Low salt exposure results in
- inactivation of Toxoplasma gondii bradyzoites during formulation of dry cured ready-to-eat
- pork sausage. Food Waterborne Parasitol. 2019 Jun;15:e00047.
- 842 68. Nayeri T, Sarvi S, Daryani A. Toxoplasma gondii in mollusks and cold-blooded
- animals: a systematic review. Parasitology. 2021 Mar 11;1–9.
- 844 69. Dubey JP, Beattie CP. Toxoplasmosis of Animals and Man. Boca Raton, FL: CRC
- 845 Press, 1988. Parasitology. 1990 Jun;100(3):500–1.
- 846 70. Dubey JP, Zarnke R, Thomas NJ, et al. Toxoplasma gondii, Neospora caninum,
- 847 Sarcocystis neurona, and Sarcocystis canis-like infections in marine mammals. Vet Parasitol.
- 848 2003 Oct 30;116(4):275–96.
- Miller MA, Gardner IA, Kreuder C, et al. Coastal freshwater runoff is a risk factor for
- Toxoplasma gondii infection of southern sea otters (Enhydra lutris nereis). Int J Parasitol.
- 851 2002 Jul;32(8):997–1006.

- 852 72. Cong W, Zhang N-Z, Hou J-L, et al. First detection and genetic characterization of
- 853 Toxoplasma gondii in market-sold oysters in China. Infect Genet Evol. 2017;54:276–8.
- 854 73. Zhang M, Yang Z, Wang S, et al. Detection of Toxoplasma gondii in shellfish and fish
- 855 in parts of China. Vet Parasitol. 2014 Feb 24;200(1–2):85–9.
- 856 74. Coupe A, Howe L, Burrows E, et al. First report of Toxoplasma gondii sporulated
- oocysts and Giardia duodenalis in commercial green-lipped mussels (Perna canaliculus) in
- 858 New Zealand. Parasitol Res. 2018 May;117(5):1453–63.
- 859 75. Boughattas S. Toxoplasma infection and milk consumption: Meta-analysis of
- assumptions and evidences. Crit Rev Food Sci Nutr. 2017 Sep 2;57(13):2924–33.
- 861 76. Ross DS, Rasmussen SA, Cannon MJ, et al. Obstetrician/Gynecologists' Knowledge,
- Attitudes, and Practices regarding Prevention of Infections in Pregnancy. Journal of Women's
- 863 Health. 2009 Aug 1;18(8):1187–93.
- 864 77. Sellier Y, Dupont C, Peyron F, et al. Prévention des infections toxoplasmiques
- maternelles en cours de grossesse: connaissances et pratiques de sages-femmes de la région
- 866 Rhône-Alpes (France). Rev med perinat. 2012 Mar 1;4(1):9–16.
- 867 78. Binquet C. Evaluation des stratégies de dépistage et de prise en charge de la
- toxoplasmose congénitale [thèse]. Dijon: Université de Bourgogne; 2003.
- 869 79. Kravetz JD, Federman DG. Prevention of toxoplasmosis in pregnancy: knowledge of
- 870 risk factors. Infect Dis Obstet Gynecol. 2005 Sep;13(3):161–5.
- 871 80. Jones JL, Dietz VJ, Power M, et al. Survey of obstetrician-gynecologists in the United
- States about toxoplasmosis. Infect Dis Obstet Gynecol. 2001;9(1):23–31.
- 873 81. Davis SM, Anderson BL, Schulkin J, et al. Survey of obstetrician-gynecologists in the
- United States about toxoplasmosis: 2012 update. Arch Gynecol Obstet. 2015
- 875 Mar;291(3):545–55.
- 876 82. da Silva LB, de Oliveira R de VC, da Silva MP, et al. Knowledge of Toxoplasmosis
- among Doctors and Nurses Who Provide Prenatal Care in an Endemic Region. Infect Dis
- Obstet Gynecol [Internet]. 2011 [cited 2019 Jul 18];2011. Available from:
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3124125/
- 880 83. Toxoplasmose | Anses Agence nationale de sécurité sanitaire de l'alimentation, de
- l'environnement et du travail [Internet]. [cited 2019 Jun 16]. Available from:
- https://www.anses.fr/fr/content/toxoplasmose
- 883 84. Surveillance sérologique et prévention de la toxoplasmose et de la rubéole au cours de
- la grossesse et dépistage prénatal de l'hépatite B Pertinence des modalités de réalisation
- [Internet]. Haute Autorité de Santé. Available from: https://www.has-
- sante.fr/jcms/c\_893585/fr/surveillance-serologique-et-prevention-de-la-toxoplasmose-et-de-
- 887 la-rubeole-au-cours-de-la-grossesse-et-depistage-prenatal-de-l-hepatite-b-pertinence-des-
- 888 modalites-de-realisation
- 889 85. Goulet V, Le Magny F, Iborra M. Enquête sur la connaissance des mesures
- préventives contre la toxoplasmose auprès de femmes venant d'accoucher. BEH. 1990;4:14-5.
- 891 86. Carme B, Lenne E, Tirard V, et al. Etude épidémiologique de la toxoplasmose chez les
- 892 femmes enceintes à Amiens (Picardie). Nécessité d'une enquête nationale. Médecine et
- 893 Maladies Infectieuses. 1994 Dec 1;24(12):1271–3.
- 894 87. Wallon M, Malleret MR, Mojon M, et al. Toxoplasmose congénitale, évaluation de la
- politique de prévention. Presse Med. 1994;23:1467-70.
- 896 88. Jones JL, Ogunmodede F, Scheftel J, et al. Toxoplasmosis-related knowledge and
- practices among pregnant women in the United States. Infect Dis Obstet Gynecol.
- 898 2003;11(3):139–45.
- 899 89. Gollub EL, Leroy V, Gilbert R, et al, European Toxoprevention Study Group
- 900 (EUROTOXO). Effectiveness of health education on Toxoplasma-related knowledge,

- behaviour, and risk of seroconversion in pregnancy. Eur J Obstet Gynecol Reprod Biol. 2008
- 902 Feb;136(2):137–45.
- 903 90. Breugelmans M, Naessens A, Foulon W. Prevention of toxoplasmosis during
- pregnancy--an epidemiologic survey over 22 consecutive years. J Perinat Med.
- 905 2004;32(3):211–4.
- 906 91. Pawlowski ZS, Gromadecka-Sutkiewicz M, Skommer J, Pet al. Impact of health
- 907 education on knowledge and prevention behavior for congenital toxoplasmosis: the
- 908 experience in Poznań, Poland. Health Educ Res. 2001 Aug;16(4):493–502.
- 909 92. Carter AO, Gelmon SB, Wells GA, et al. The effectiveness of a prenatal education
- 910 programme for the prevention of congenital toxoplasmosis. Epidemiol Infect. 1989
- 911 Dec;103(3):539-45.
- 912 93. Foulon W, Naessens A, Ho-Yen D. Prevention of congenital toxoplasmosis. J Perinat
- 913 Med. 2000;28(5):337–45.
- 914 94. Nguyen Hoang Hanh DT. Master Recherche "Epidémiologie et Biostatistique".
- 915 Evolution des connaissances et des comportements au cours d'un programme d'éducation
- 916 prénatale pour la prévention primaire de la toxoplasmose congénitale chez les femmes
- 917 enceintes séronégatives pour la toxoplasmose, région de Lyon, 1994-1995. Bordeaux:
- 918 Université Victor Segalen; 2004.
- 919 95. Di Mario S, Basevi V, Gagliotti C, et al. Prenatal education for congenital
- 920 toxoplasmosis. Cochrane Database Syst Rev. 2015 Oct 23;(10):CD006171.
- 921 96. Wallon M, Nguyen Hoang Hanh DT, Peyron F, et al. Impact of health education for
- 922 the primary prevention of Toxoplasma infection in pregnancy: lessons from the ERIS
- 923 study.16th European congress of clinical microbiology and infectious diseases (ECCMID),
- 924 Nice (France), April 1–4, 2006.

925