
EUROTOXO

European TOXO PREVENTION Project

Prevention of Congenital Toxoplasmosis:
A European initiative on the state-of-science

Systematic review of risk factors for *Toxoplasma gondii* infection in pregnant women

Panel 3: prevention and screening issues

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TABLE OF CONTENTS

ABSTRACT	4
INTRODUCTION	5
1. BACKGROUND	5
2. PHYSIOPATHOLOGY	5
3. OBJECTIVES	6
METHODS	6
RESULTS	8
OVERALL	8
EX-YUGOSLAVIA, SERBIA-MONTENEGRO	8
NORWAY	8
ITALY	9
FRANCE	9
EUROPEAN RESEARCH NETWORK ON CONGENITAL TOXOPLASMOSIS	9
DISCUSSION	11
PUBLIC HEALTH IMPLICATIONS	12
RESEARCH IMPLICATIONS	13
ACKNOWLEDGEMENTS	13
REFERENCES	13

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Abstract

Objective: To review the specific routes and risk factors of Toxoplasmosis infection in pregnant women in order to improve the control measures for primary prevention of congenital toxoplasmosis.

Methods: We performed a review using a MEDLINE request focus on the followings keywords: pregnancy or prenatal care, toxoplasmosis, and risk factor. We pre-selected the comparative study designs, including cohort or case-control studies conducted in incident (or recently infected) pregnant women, women of reproductive age or in newborn infected and studying risk factors of toxoplasma infection.

Results: Out of the 131 papers eligible in pubmed, 28 fulfilled the criteria for pre-selection and finally only five the criteria of risk factor design in eligible population. Comparing the findings in seven European centres in Belgium, Denmark, France, Italy, Norway, Yugoslavia, and Switzerland, it appears that risk factors for Toxoplasma infection vary according to local food customs, food hygiene and lifestyles. Inadequately cooked or raw meat is the main risk factor for infection consistently identified in all centres. Several types of meats are susceptible to be involved according to centres: beef, lamb or game meats. Pork is inconsistently reported. The type of cooking may also play a role but the risk remains unclear when eating cured meat or frozen meat. Weak associations are reported for tasting, or handling raw meat during food preparation. Contact with soil contributed to a substantial minority of infections: this risk could include consumption of raw or unwashed vegetables or gardening. Owning a pet cat was not reported as a risk factor for infection in all but one study. The study of the population attributable fraction showed that 30% to 63% of seroconversions were due to consumption of undercooked or cured meat products and 6% up to 17% were a result of soil contact.

Conclusion: Overall, only few studies contribute to the evidence-based knowledge of route of Toxoplasma infection in pregnant women. All the findings support the fact that the oral route is the source of infection involving mainly food preparation and consumption, with raw meat instead of pet owning as it is generally believed by most of the physicians and general population. This information is helpful in guiding appropriate health education message for pregnant women and general population.

Introduction

1. Background

Congenital toxoplasmosis is caused by a protozoan parasite, which is transmitted to the foetus when the woman acquires toxoplasma infection for the first time during pregnancy. While toxoplasmosis infection in women is often benign, congenital toxoplasmosis could lead to severe sequelae for the foetus and the newborn with visual or neurological impairment or death (Ambroise-Thomas and Petersen 2000; Joynson and Wreghitt 2001; Montoya and Liesenfeld 2004).

In Europe, the epidemiology of congenital toxoplasmosis varies across the European countries as also its trends over the past few decades. The public health programs to prevent congenital toxoplasmosis differ also between European countries without any consensus on their indication (Joynson and Wreghitt 2001): some countries perform a compulsory antenatal screening (Austria, France); other countries perform a neonatal screening (Denmark) while some others do not perform any screening but recommend hygienic control measures (United-Kingdom, Norway, Finland).

The EUROTOXO project was a European consensus initiative launched in 2002 and aimed at defining the implications of current scientific knowledge for a research agenda and policy decisions on how best to prevent congenital toxoplasmosis and its consequences.

Because of the asymptomatic nature of most of *Toxoplasma* infection, the specific routes are not well known. However, primary prevention in pregnant women may take place in the prevention of congenital toxoplasmosis, as it is a preventable disease. Pregnant women and their general practitioners and obstetricians need to be informed about the specific risk factors for toxoplasmosis to lower the risk of congenital infection.

2. Physiopathology

Toxoplasma gondii has a complex life cycle (Figure 1) consisting of three different forms (Ambroise-Thomas and Petersen 2000; Montoya and Liesenfeld 2004; Kravetz and Federman 2005):

1. Tachyzoïte: during the acute stage of infection, this form of the parasite invades and replicates within macrophages;
2. Bradyzoïte: during latent infections, this form of the parasite is present in tissue cysts; and
3. Sporozoïte: this form of the parasite is found in oocysts, which are environmentally resistant. Cats (Felidae) are the definitive hosts of *Toxoplasma*. Cats become infected with *T. gondii* by carnivorousism. During acute infections, cats excrete unsporulated (i.e., uninfected) oocysts in their feces; after several days to several weeks, depending on environmental conditions, the oocysts sporulate and become infectious. Under favourable conditions (i.e., in warm, moist soil), oocysts can remain infectious for approximately one year. Oocysts are remarkably resistant to disinfectants, freezing, and drying, but are killed by heating to 67°C for 10 minutes, by cooling to -13°C (Hill and Dubey 2002) or by gamma irradiation (0.5 kGy) (Dubey 1996).

Toxoplasmosis can be transmitted to humans by several routes (Figure 1):

1. Ingestion of raw or inadequately cooked infected meat or eat uncooked foods that have come in contact with contaminated meat.
2. Ingestion of oocysts that cats have passed in their faeces from fecally contaminated hands, either in a cat litter box or outdoors in soil (e.g., soil from gardening or unwashed fruits or vegetables or water).
3. Transmission to the foetus through placenta.

4. Organ transplantation or blood transmission from a seropositive donor to a seronegative recipient.
5. Accidental inoculation of tachyzoites in laboratory personnel with contaminated needles or infected animals. The parasites form tissue cysts, most commonly in skeletal muscle, myocardium, and brain; these cysts may remain throughout the life of the host.

It has been reported that pregnant women have a significant 2.2 times higher risk of converting for toxoplasmosis than non-pregnant women, and 7.7 times higher if they are adolescents (Avelino, Campos et al. 2003). This greater vulnerability of pregnant women to this parasite is probably due to alterations in the immune mechanisms inherent to gestation, resulting from suppression of immune response because of the necessity of tolerance to the graft (foetus) and/or as a consequence of hormone imbalances characteristic of the gestational condition (Daunter 1992). Thus, it is plausible that pregnant women and young women at risk of pregnancy potentiating susceptibility to toxoplasmosis.

Effective primary prevention of congenital toxoplasmosis requires identifying specific risk factors for infection in pregnant women.

3. Objectives

This report is aimed at reviewing the knowledge on the routes and risk factors of Toxoplasma infection specifically in pregnant women to formulate appropriate health education counselling for primary prevention.

Methods

We performed a systematic search of published papers reported in PUBMED bibliographic database (from 1966 to 2005) using the following research keywords:

(Toxoplasmosis) AND (Pregnancy or prenatal care) AND Risk factor*.

There was no language restriction.

Reference lists in recent book chapters and review articles written by the authors were also used.

Preselection of individual manuscripts was based on the following criteria based on the abstract content: comparative study design including cohort or case-control study in incident (or recently infected) pregnant women or reproductive age or in newborn infected studying for risk factors of toxoplasma infection. Studies studying prevalent cases were excluded. Articles selected were data extracted.

Because of the heterogeneity between detailed risk factors assessed between studies, no summary odds ratio was computed.

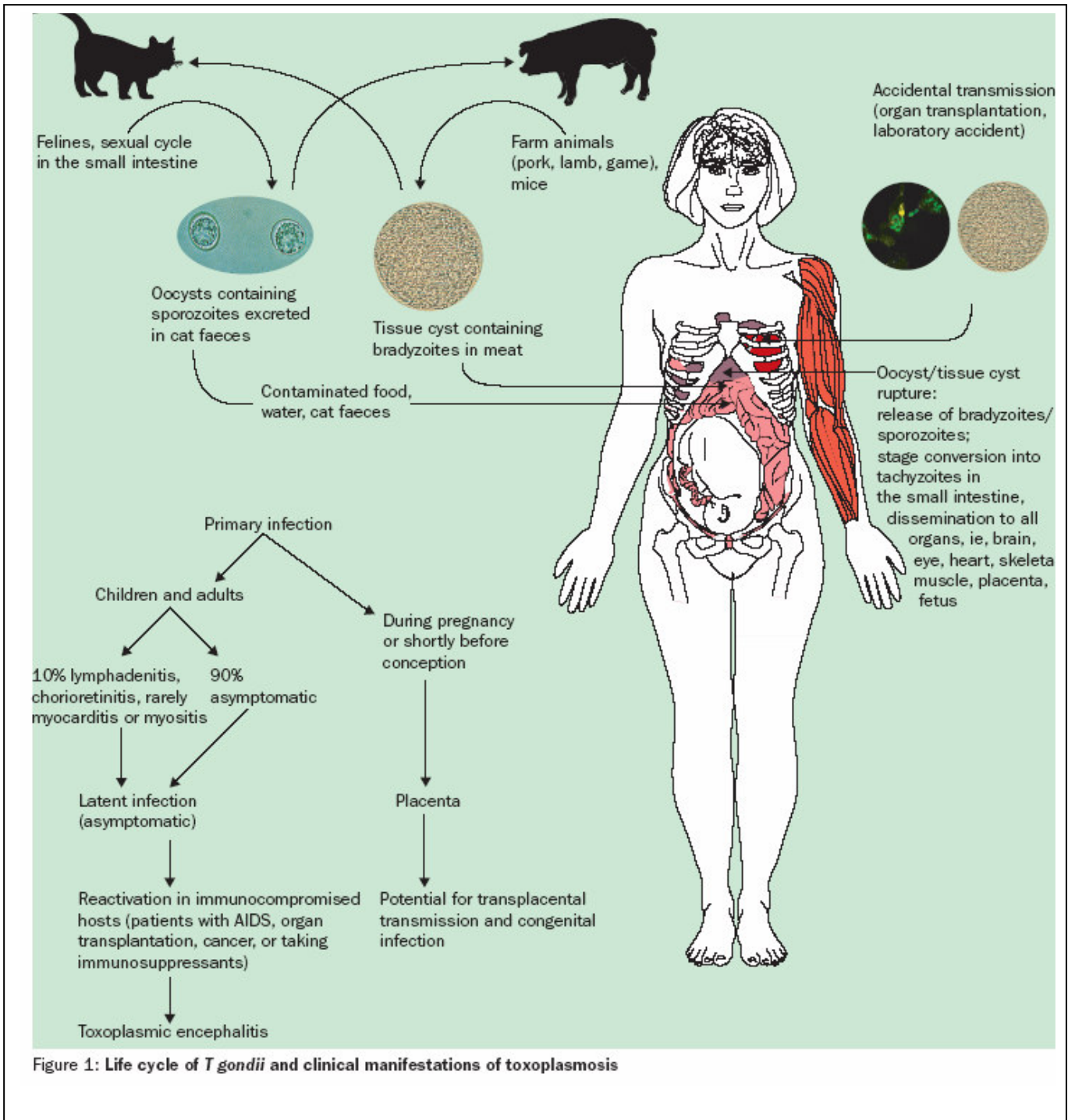


Figure 1: Life cycle of *T gondii* and clinical manifestations of toxoplasmosis

Reprinted from "Figure 1 from THE LANCET, V363: 1965-1976, Montoya JG and Liesenfeld O: "Toxoplasmosis," © 2004, with permission from Elsevier"

Results

Overall

Through June 2005, 131 articles were selected in Pubmed using the keys words cited of whom 93 were eligible original papers for this review. Among those, 28 were pre-selected on the abstract based on comparative study design of whom only four were risk factor studies conducted in incident pregnant women or newborns for toxoplasmosis. One study was conducted among women of reproductive age. These studies are summarised in the Table 1. Most of the other risk factor studies were conducted in pregnant women seroprevalent for toxoplasmosis.

Ex-Yugoslavia, Serbia-Montenegro

A systematic sample of 1157 women of reproductive age, aged 15 to 49 years in Belgrade, ex-Yugoslavia, was interviewed using a questionnaire during a 4-year period (1988-1991) (Bobic, Jevremovic et al. 1998). This transversal study design provided a robust level of evidence when researching for exposure at risk of toxoplasma infection while epidemiological data were collected simultaneously with serological data. The overall mean rate of *Toxoplasma gondii* infection was high, estimated to be 77% increasing from age from 57% to 93%, and decreased significantly over the study period ($p < 0.01$). Of the potential environmental risk factors examined, only consumption of undercooked meat was found to be associated with *Toxoplasma gondii* infection (Table 1). In a subgroup analysis of women below age 20 years, exposure to soil (farming, gardening) was also found to be associated with *Toxoplasma gondii* infection (OR = 10.3, CI = 2.7-38.6). Cat ownership itself was not associated with Toxoplasma infection ($p=0.326$).

Norway

From 1992 to 1994, a prospective case-control study designed to identify preventable risk factors for *Toxoplasma gondii* infection in pregnancy was conducted in Norway (Kapperud, Jennum et al. 1996). Case-patients were identified through a serologic screening program encompassing 37,000 pregnant women and through sporadic antenatal testing for Toxoplasma infection. A total of 63 pregnant women with serologic evidence of recent primary T. gondii infection and 128 seronegative control women matched by age, stage of pregnancy, expected date of delivery, and geographic area were enrolled. The following factors were found to be independently associated with an increased risk of *Toxoplasma gondii* seroconversion during pregnancy: eating raw or undercooked lamb; washing kitchen knives infrequently after preparation of raw meat prior to handling another food item; cleaning the cat litter box; eating raw or undercooked minced meat products; eating raw or undercooked pork; and eating unwashed raw vegetables or fruits (Table 1). In univariate analysis, travelling to countries outside of Scandinavia was identified as a significant risk factor, but this variable was not independently associated with infection after data were controlled for factors more directly related to the modes of infection. Four of these six risk factors involve contact with undercooked meat, which allows direct ingestion of tissue cysts. Cleaning the litter box allows contact with oocysts if the cat was infected in the past two weeks. Following exposure to contaminated cat litter, subjects must then touch their mouths for faecal-oral transmission to occur. Eating unwashed vegetables also allows faecal-oral transmission of oocysts, as outdoor cats are known to deposit their faeces in gardens, a risk factor that is likely to be independent of cat ownership since many outdoor cats roam to neighbouring sites to deposit their faeces.

Italy

In Naples, between November 1991 and June 1994, risk factors for toxoplasma infection were compared in 42 recently infected women (as assessed by detection of specific IgM+IgG in serum) and 2096 susceptible, IgG negative women (Buffolano, Gilbert et al. 1996). Recent infection was strongly associated with frequency of consumption of cured pork and raw meat. Overall, 45% of susceptible women reported eating cured pork or raw meat at least once a month. Eating cured pork or raw meat at least once a month increased the risk of toxoplasma infection threefold (OR: 3.1 95% CI: 1.6-6.0). Based on these results and assuming a causal relationship, avoidance of monthly consumption of cured pork and raw meat by pregnant women in Naples could reduce the risk of toxoplasma infection by approximately 48% (95% confidence interval: 32% 84%).

France

In France, where prenatal screening is mandatory in susceptible seronegative women, a case-control study was undertaken in 1995 to identify risk factors for Toxoplasma infection during pregnancy (Baril, Ancelle et al. 1999). A total of 80 pregnant women who seroconverted to Toxoplasma were matched with 80 pregnant women who had repeatedly negative tests. The women were interviewed by telephone, using a standardized questionnaire, to determine socio-demographic characteristics, exposure to possible risk factors and the type of information on prevention received during pregnancy. The risk factors for Toxoplasma infection included in a multivariate analysis were in a decreasing order as follows: poor hand hygiene (OR = 9.9; 95%CI: 0.8-125), consumption of undercooked beef (OR = 5.5; 95%CI: 1.1-27), having a pet cat (OR =4.5; 95%CI: 1.0-19.9), frequent consumption of raw vegetables outside the home (OR = 3.1; 95%CI: 1.2-7.7) and consumption of undercooked lamb (OR = 3.1; 95%CI: 0.85-14). Pork was not reported as a risk factor for infection. In addition, receipt of printed documentary advice on prevention (leaflets, books or magazines) was associated with a lower risk of infection (OR = 2.2; 95%CI: 1.1-4.4). In fact, in this study, only two risk factors were significantly with infection: exposure to undercooked beef and frequent consumption of raw vegetables outside the home whereas the other were not statistically significant due to probable lack of statistical power.

European Research Network on Congenital Toxoplasmosis

The last published study was a large multicenter European case-control study in six cities conducted in in Belgium, Denmark, Italy, Norway, and Switzerland, and involving 252 cases and 708 controls (Cook, Gilbert et al. 2000). Multivariate analyses were adjusted for age, location, period between diagnosis of infection, period between diagnosis and interview. This study reported that contact with raw or undercooked beef, lamb, or other meat (venison, horse, rabbit, whale, game birds), as well as with soil, were independent risk factors for *Toxoplasma gondii* seroconversion during pregnancy. In addition, travel outside of Europe, the United States, and Canada was a risk factor for seroconversion. Tasting meat during cooking was of borderline importance. Consumption of pork was not identified as a risk for infection. Multiple different cat exposures were assessed, but none were found to be significant risk factors for toxoplasmosis infection. Specifically, having a cat or kitten at home, cleaning the litter box, and owning a cat that hunts were not risk factors for *Toxoplasma gondii* seroconversion.

The study of the population attributable fraction showed that 30% to 63% of seroconversions were due to consumption of undercooked or cured meat products according to the different centres and 6% up to 17% were a result of soil contact.

Table 1: Routes and risk factors for *Toxoplasma gondii* infection in incident pregnant women or reproductive age (study design and chronological order). Literature synthesis.

Place and period of study Study reference	Study design	Population	Routes and risk factors: OR or aOR for adjusted analysis adjusted analysis
Ex-Yugoslavia, Serbia-Montenegro 1988-1991 (Bobic, Jevremovic et al. 1998)	Transversal study	Incident women of reproductive age aged 15-49 years in Belgrade (N=1157)	Undercooked meat consumption: OR: 1.6, 95% CI: 1.2-2.1 In women < age 20, exposure to soil: OR: 10.3, 95% CI: 2.7-38.6 Pet cats: no influence
Norway, 1992-1994 (Kapperud, Jennum et al. 1996)	Case-control study	63 incident pregnant women for <i>T. gondii</i> infection (cases) versus 128 seronegative controls matched by age, gestational age, geographic area	Eating raw or undercooked mutton: aOR =11.4, p=0.005 Washing the kitchen knives after preparation of raw meat: aOR =7.3, p=0.04 Cleaning the cat litter box: aOR =5.5, p=0.02 Eating raw or undercooked minced meat products: aOR =4.1, p=0.007 Eating raw or undercooked pork: aOR =3.4, p=0.03 Eating unwashed raw vegetables or fruits: aOR =2.4, p=0.03
Italy, 1991-1994 (Buffolano, Gilbert et al. 1996)	Case-control study	42 pregnant women recently infected (IgM+IgG) versus 2096 seronegative controls	Eat cured pork: OR: 2.9, 95% CI: 1.6-5.5 Eat raw meat: OR: 2.6, 95% CI: 1.4-4.7 Gardening: OR: 2.0, 95% CI: 1.1-3.7 Ever owned a cat: OR: 0.7, 95% CI: 0.2-2.4
France, 1995 (Baril, Ancelle et al. 1999)	Case-control study	80 incident pregnant women for <i>T. gondii</i> infection (cases) versus 80 seronegative controls matched by gestational age, geographic area, and physicians	Undercooked beef: aOR: 5.5, 95% CI: 1.1-27 Poor hand hygiene: aOR: 9.9, 95% CI: 0.8-125 Undercooked lamb: aOR: 3.4, 95% CI: 0.85-14 Raw vegetables eaten outside home: aOR: 3.1, 95% CI: 1.0-19.9 Pet cat aOR: 4.5, 95% CI: 1.0-19.9
Six large European cities (Naples, Lausanne, Copenhagen, Oslo, Brussels, Milan) 1994-1995 (Cook, Gilbert et al. 2000)	Case-control study	252 incident pregnant women (cases) versus 858 seronegative controls (of whom 150 were excluded). Analyses adjusted for age, location, period between diagnosis of infection, period between diagnosis and interview	Raw or undercooked lamb: aOR: 3.13, 95% CI: 1.4-7.2 Raw or undercooked beef: aOR: 1.73, 95% CI: 1.1-7.2 Raw or undercooked pork: aOR: 1.40, 95% CI: 0.7-2.8 Other meat: aOR: 4.12, 95% CI: 1.1-7.2 Contact with soil: aOR: 1.81, 95% CI: 1.2-2.7 Taste meat cooking: aOR: 1.52, 95% CI: 1.0-2.4 Cat: aOR: 1.26, 95% CI: 0.7-2.4 Eat raw sausage: aOR: 0.91, 95% CI: 0.5-1.6 Eat dry to cured meat: aOR: 0.82, 95% CI: 0.7-1.4 Eat salami: aOR: 1.231, 95% CI: 0.9-2.0 Eat frozen meat: aOR: 0.77, 95% CI: 0.5-1.2 Unpasteurised milk: aOR: 1.47, 95% CI: 0.9-2.5 Use of microwave cooker: aOR: 1.30, 95% CI: 0.8-2.3 Working with animals: aOR: 1.50 95% CI: 0.8-2.7 Travel outside Europe, US or Canada: aOR: 2.33, 95% CI: 1.3-4.1 Living on farm: aOR: 1.15, 95% CI: 0.6-2.2

aOR : adjusted odds ratio; RR: relative risk; 95% CI: 95% confidence interval

Discussion

There is no biological test to distinguish infections from oocysts ingestion transmitted by cats, or soil from tissue cysts ingestion from infected meats (Dubey 2000; Hill and Dubey 2002). Therefore, epidemiological surveys remain the most useful way of assessing the relative importance of different sources of *Toxoplasma gondii* infection in human beings. This epidemiological approach is not effective when subjects are chronically infected. So, we selected papers reporting risk factors for acute toxoplasmosis in women who recently acquired infection. Consequently, these studies conducted among incident pregnant women allow determining specific sources of *Toxoplasma gondii* infection in this population. However, case-control studies, four of the five reported studies, are not the best evidence level design to investigate a risk factor: the risk exposure was investigated using personal retrospective interviews, which may introduce an information bias. Indeed, this recall bias is frequent in case-control study design when comparing infected versus healthy women. Only, one study was a transversal design with simultaneous information on both risk and disease infection but was conducted in childbearing women. This latter conducted in a large sample showed consistent results with the case-control study findings.

Overall, only few studies contribute to the evidence-based knowledge of route of infection in pregnant women and adjusting for confounding factors. Only one multicenter study allowing comparative adjusted risk factor analysis is available (Cook, Gilbert et al. 2000). Comparing the findings in seven European studies obtained in Belgium, Denmark, France, Italy, Norway, Yugoslavia, and Switzerland, it appears that risk factors for *Toxoplasma* infection vary according to local food customs, food hygiene and lifestyles. All the findings support the fact that the oral route is probably the major source of infection involving food preparation and consumption, with raw meat, responsible of 30% to 63% of all infections (Cook, Gilbert et al. 2000).

Inadequately cooked or raw meat is therefore the main risk factor for infection with toxoplasma consistently identified in all centres (Hill and Dubey 2002). Several types of meats are susceptible to be involved in the transmission of tissue cysts and are identified as risk factor according to centres: beef, lamb or game meats. Pork well identified previously in Norway and Italy (Buffolano, Gilbert et al. 1996; Kapperud, Jenum et al. 1996) was surprisingly not reported as a route for infection in the Cook's study (Cook, Gilbert et al. 2000; Dubey 2000). In fact, veterinary control measures exist already for a long time regarding pigs also involved in the transmission of other infection disease such as Trichinellosis (Dubey 1996). It could be hypothesised that the presence of tissue cyst in pork has decreased at the time the Cook's study and that pregnant women most aware of this specific risk tended to thoroughly cook longer pork than other meats. There are other risk factors reported to be associated with infected meat: age of animal, farm conditions, animal life conditions, and type of meat non-skeletal muscle has higher density of oocyst than skeletal meat. In fact, a full answer to this question would require determining the prevalence of *Toxoplasma* cysts in meat before its release on the consumer market. The type of cooking may also play a role but the risk remains unclear when eating cured meat or frozen meat. Weak associations are reported for tasting, or handling raw meat during food preparation.

Contact with soil or vegetables were identified in four of the five studies. Contact with soil contributed to a substantial minority of infections (6% to 17%) (Cook, Gilbert et al. 2000): this risk could include consumption of raw or unwashed vegetables or gardening. Soil contact through gardening allows contact with infective oocysts deposited by any recently infected cat. While oocysts take one to five days to become infective, they can remain infective in soil for up to one year.

Owning a pet cat was not reported as a risk factor for infection in all but one study. This fact, in apparent contradiction with the life cycle of *Toxoplasma* could be explained by the fact that this risk

factor is one of the most acknowledged risk factor in pregnant women compared to the others. Indeed, a study conducted in the US reported that the highest level of knowledge among pregnant women was about cats and *T. gondii*; 61% responded that the organism is shed in the faeces of infected cats and 60% responded that people could acquire toxoplasmosis by changing cat litter. There was a low level of knowledge about other risk factors; only 30% of the women were aware that *T. gondii* might be found in raw or undercooked meat (Jones, Ogunmodede et al. 2003). Given such knowledge, pregnant women at risk (owning a pet cat) may have better hand hygiene than those who are not exposed to cats considering they are not at risk and who would have a poorer hand hygiene leading to a higher risk of infection from greater routes such as meat.

Although knowledge on precise risk factors for toxoplasma infection is incomplete, this review identifying some specific routes of infection in pregnant women is informative and had several public health and research implications in the current perspective of preventing congenital toxoplasmosis.

Public health implications

Information about how to avoid toxoplasmosis in pregnancy could be a cost effective approach to preventing congenital toxoplasmosis (Conyn-van Spaendonck and van Knapen 1992; Lopez, Dietz et al. 2000; National Collaborating Centre for Women's and Children's Health 2003). Based on the knowledge of these identified risk factors for primary toxoplasmosis, pregnant women should be appropriately advised by their obstetricians and primary care providers on how to lower the risk of congenital toxoplasmosis by avoiding risk factor exposure. Public health prevention campaigns should consequently focus on the appropriate risk factors mainly on foods and eating habits, and hand hygiene in order to give health advice. The single most important health message for pregnant women in all centres in the study is to avoid eating any meat (raw or cured) that has not been thoroughly cooked. The importance of other risk factors varied between geographical centres. Consequently, advice to ensure that all fruit and vegetables are thoroughly peeled and washed and to avoid soil contact, working with animals, or drinking unpasteurised milk may be justified. Contact with cats, although not identified as a risk factor in the recent studies play a role in the life cycle of *Toxoplasma gondii* and should remained in prevention messages to be consistent with the common knowledge. However, these latter information messages should not reduce the emphasis on meat risk.

Finally, recommendations to prevent congenital toxoplasma infection in pregnant women could be detailed as follows:

- Washing hands before handling food;
- Cooking meat to a safe temperature (i.e., one sufficient to kill *Toxoplasma*);
- Cleaning cooking surfaces and utensils after they have contacted raw or cured meat, poultry, seafood, or unwashed fruits or vegetables;
- Peeling or thoroughly washing fruits and vegetables before eating;
- Avoiding cat faeces in soil or changing cat litter or, if no one else is available to change the cat litter, using gloves, then washing hands thoroughly;
- Wearing gloves and thoroughly washing hands after gardening or handling soil.

Where it is offered, health information delivered to pregnant women as a primary prevention intervention is frequently inappropriate and incomplete. In the USA, toxoplasmosis-related knowledge and practices among pregnant women is low and inappropriate, except for the risk of transmission from cats (Jones, Ogunmodede et al. 2003). Indeed, only 30% of women are aware that *Toxoplasma gondii* may be found in raw or undercooked meat. The content and mean of information of health

education advises for pregnant women need to be thoroughly considered. Therefore, health education and control measures must be improved.

Research implications

Further research is required to determine the cysts viability in meat products and their condition to develop potential primary prevention strategies susceptible to control these risk factors in general population and in pregnant women.

Primary prevention of toxoplasma infection in the general population may also have a great impact on morbidity and mortality from toxoplasmosis than strategies confined to pregnant women. The feasibility and effects of veterinary public health strategies, such as meat labelling and improved farm hygiene should be explored. These preventive interventions should aim to reduce prevalence of infection in meat, improve labelling of meat according to farming and processing methods.

Studies are also required to determine the ways and the effect of health messages focussed on the appropriate risk factors to improve the quality and consistency of health information given to pregnant women. These studies would study in pregnant and their physicians, knowledge, attitudes, behaviour and practices regarding the prevention of congenital toxoplasmosis and the incidence of Toxoplasma infection in pregnant women. This specific question on the effectiveness of primary prevention using health education in pregnant women is being explored in another EUROTOXO report (Gollub, Leroy et al. 2005).

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