Seroprevalence and Risk Factors of Toxoplasmosis in Togo

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Abstract

Objectives: Toxoplasmosis is caused by *Toxoplasma gondii*. 50 to 70% of African are contaminated and 60% to 80% in Togo. This parasitic infection involves disorders in immunocompromised persons and pregnant women. The purpose of this study is to evaluate the seroprevalence of toxoplasmosis and the risk factors of contamination in the Togolese population. **Methods:** Prospective studies from January 2012 to December 2015 concerned 7076 patients. A survey on the risk factors for *Toxoplasma gondii* contamination has done. The seroprevalence was monitored by examining serological antibodies IgG and IgM using ELISA method. Sampling of 200 excreta of domestic cats were analyzed by coproscopy examinations **Results:** The average age was 32.61±6.80 years old. The seroprevalence of IgM was 2.85%; and for IgG 59.7%. IgG were high in men (67.0% *vs*. 58.6%, $p<0.0001$); IgM were high in women (2.81% vs. 1.85%, $p<0.01$). In this study, we observed 57.49% immunized subjects. Regarding potential risk factors for contamination, the survey revealed that 80.5% of subjects consume raw garden produce, 65.4% non-potable and 8.3%

undercooked meat. In cats, 20.5% of them excreted oocysts of *Toxoplasma gondii*. **Conclusion:** The prevalence of the toxoplasmosis was high in the Togolese population because the risks factors were variable and the level of contamination were high too. The country's health authorities, especially for the pregnant women, should install sensitization and prevention programs on the risks of the contamination of toxoplasmosis. The high proportion of the young subjects affected constitutes a socio-economical danger for the country**.**

Keywords: Toxoplasmosis, seroprevalence, risk factors, Togo

Introduction

Toxoplasmosis is one of the most common parasitic infections in humans, caused by an Apicomplexa protozoan: *Toxoplasma gondii,* a ubiquitous parasite of warm-blooded animals. The definitive hosts are felids (Dubey, 2008; Angel *et al.,* 2014). It has a very wide variety of intermediates hosts and can parasitize all types of cells. Horizontal infection generally occurs through the ingestion of raw or undercooked meat that contains cysts (encysted bradyzoites), through the ingestion of contaminated food or in drinking water with oocysts. The transplacental transmission from mother infected occurs during pregnancy (Montoya and Liesenfeld, 2004; Dubey, 2008 ; Dubey *et al.,* 2012; Hammond-Aryee *et al*., 2014).

In the immunocompetent subjects, toxoplasmosis is asymptomatic in 80% of cases. However, ganglionic affection with fever, lymphadenopathy and asthenia can be observed. In immunocompromised patients, toxoplasmosis caused life-threatening infections such as encephalitis, lethargy, ataxia, coma, pneumonia, chorioretinitis. Encephalitis toxoplasmosis is the leading cause of death in patients (Webster, 2001; Labalette *et al*., 2002; Montoya and Liesenfeld, 2004; Dubey and Jones, 2008). The severity of infections is related to the decrease of T-cells and IFN-γ. This involves rapidly proliferating of tachyzoites leading to tissue necrosis (Labalette *et al*., 2002; Dubey and Jones, 2008; Montoya and Remington, 2008).

Symptoms of infection in pregnant women are rare. However, acute infection can lead the lesions of the placenta and induce serious type of foetopathies. The consequences of congenital infection are spontaneous abortions, stillbirths, premature births and multi-visceral lesions (Pinard et al., 2003; Dubey and Jones, 2008; Montoya and Remington, 2008). The common abortions in patients with toxoplasmosis can be partially attribute to the presence of anti-Cardio-Lipin (A'aiz et al., 2014). The gestational age and the rate of trans placental infection are in correlation and an inverse relationship has been found between the severity of the lesions or clinical

signs and the gestational age at which maternal seroconversion occurs (Montoya and Remington, 2008; Bamba *et al*., 2012).

In neonates and infants, *Toxoplasma gondii* infection causes congenital malformations (hydrocephalus or microcephaly), chorioretinitis, convulsions, intracranial calcification, mental retardation, visual disturbances, learning difficulties (Dubey and Jones, 2008; Uttah *et al*., 2013). Toxoplasmosis in intermediate hosts has been associated with the development of neurological disorders (Fond *et al*., 2012; Angel *et al*., 2014).

Toxoplasmosis diagnosis is mainly based on the presence of anti-*Toxoplasma gondii* antibodies (IgG and IgM) in the serum. Several tests can be used as dye test, immuno fluorescent study test, latex agglutination enzyme linked immunosorbent assay (ELISA) (Hamad and Kadir, 2013). It is estimated that one-third of the humanity has this parasitic infection (Montoya and Liesenfeld, 2004; Hammond-Aryee *et al.,* 2014). In Africa, 50% to 70% of the populations are infected. The seroprevalence varied widely in different regions of the world according to sanitation level of the population, dietary habits, contacts with cats or contaminated soil and climatic zones. It has been reported that the prevalence is higher (> 60%) in humid and forest areas, and less than 50% in dry or desert areas (Millogo *et a*l, 2000 ; Studeničová *et al*. 2006 ; Dubey *et al.*, 2012; Linguissi 2012; Pangui *et al.*, 2013).

In Togo, the seroprevalence is estimated between 60% and 80% (Agbo *et al.*, 1991; Grunitzky *et al.,* 1995, Balogou *et al*. 2007). Despite the high rate of the toxoplasmosis in Togo, recent data are not available. The aim of this study was to determine the prevalence of *Toxoplasma gondii* infection in the population for the effective management of the groups that were exposed to high risk of contamination.

Material and methods

Studies populations and laboratory analysis on prevalence

A prospective study from January 2012 to December 2015 concerned 7076 patients of all ages (6158 female and 918 male) were oriented for serological tests for detection of *Toxoplasma gondii* infection.

They were attended to National Institute of Hygiène of Togo, which approved the study.

Collection of specimens

Blood sample were withdrawn, from patients in the serological laboratory of National Institute of Hygiène (Reference center of medical analyzes of Togo), centrifuged (1000-2000 rpm) and sera were collected kept at 4°C.

Serological tests

Serological tests were performed by immunoenzymatic method (ELISA):

-for qualitative detection of anti-*Toxoplasma gondii* IgM antibodies using Platelia ™ Toxo IgM : ref 72841 (BIO-RAD, Marnes la Coquette, France);
-for quantitative determination of anti-*Toxoplasma gondii* IgG antibodies using Platelia ™ Toxo IgG: ref 72840 (BIO-RAD, Marnes la Coquette, France).

Information about patients:
For this prospective study, a paper has been established for each patient from the registers of the laboratory, concerning the : age, sex, profession, level of education, living area, requesting service of the analysis, and tests results.

A survey on the risk factors

Epidemiological investigation on the risk factors to determine the sources of contamination was performed in the residential areas of the patients. Houses were randomly selected and a total of 300 were visited. The inhabitants were subjected to a structured survey and the questions were about the presence of cats or other animals (dog, mouse ..) in the house, culinary habits, sources of drinking water, food, knowledge and pathologies related to toxoplasmosis.

Animals and fecal sample

After the interrogation of the subjects, excreta of the cats were collected in the houses. A total of 450 cats *(Felis catus*) were identified during the study and 200 excreta samples were collected in plastic bags and stored at 4°C. The excreta samples were analyzed by coproscopy method after enrichment with Willis liquid (saturated solution of NaCl: density 1.2), by waterline technique. The identification of *Toxoplasma gondii* oocysts was performed after optical microscope (x10, x40) observation, using reference manual of the laboratory of the National Direction of Livestock of Togo.

Statistical methods

Data analysis was performed using GraphPad 6.1 software. Quantitative results were expressed by mean±standard deviation and in percentage. The different groups were compared by Student test. The correlation coefficients were determined using Pearson test. The frequencies were calculated according to the formulas:

All subjects = 100 X (Number of Women + Men considered) / Total population (Women + Men)
Women = 100 X (Number of Women Considered) / Total Women Population
Men = 100 X (Number of men considered) / Total population of men
The results were considered significant for $p < 0.05$).

The prevalence (P) has been calculated according to the formula:
*P = n/N * 100; (n= number of positives samples and N = total number of tested samples)*

Results

The study population characteristics

The sex ratio was 0.15 in favor of women (87.30%). The mean age was 32.61 ± 6.8 years old (males 34.07±10.55 years old; females 32.39±6.0 years old). The age groups of 31-45 years old and 16-30 years old were the most represented respectively 62.4% and 34.6% of the studied subjects (figure 1).

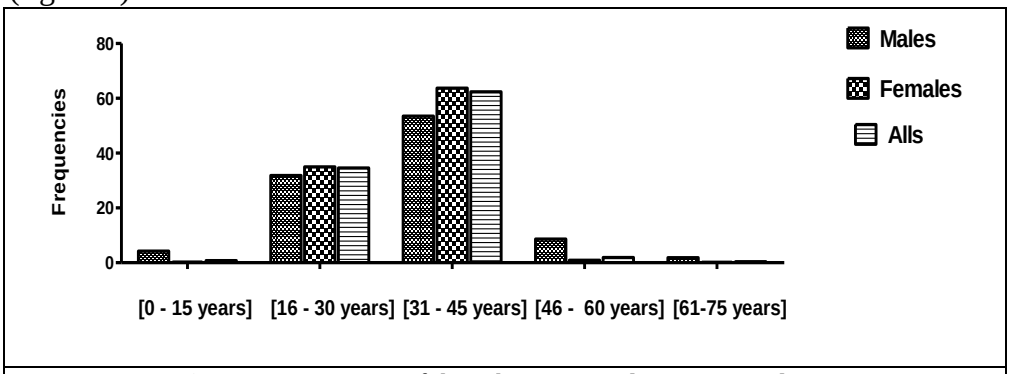

Figure1: Repartition of the subjects according to age and sex.

Seroprevalence of IgG and IgM antibodies according to the age

Considering the age group and its relation with the distribution of seropositive *Toxoplasma gondii* antibodies (figures 2a and 2b), we found that the prevalence of IgG seropositivity was high (> 45%) in all age groups. The age group of 46-60 years old had the highest percentage (67.8%) of positive results, followed by the age group of 16-30 years old (57.8%) and 31-45 years old (55.4%).

For IgM, the highest seropositivity rate of *Toxoplasma gondii* antibodies was 5.74%, in the age group of 46-60 years old, followed by 2.85% and 2.23% in the age groups 31-45 years old and 16-30 years old respectively. However, it is a lack of IgM in the age group of 0-15 years old and 61-75 years old. A positive correlation was observed between the age and the prevalence of IgG (R = 0.68) and IgM (R = 0.97) in male. In female, the correlation was positive for IgM (R = 0.99).

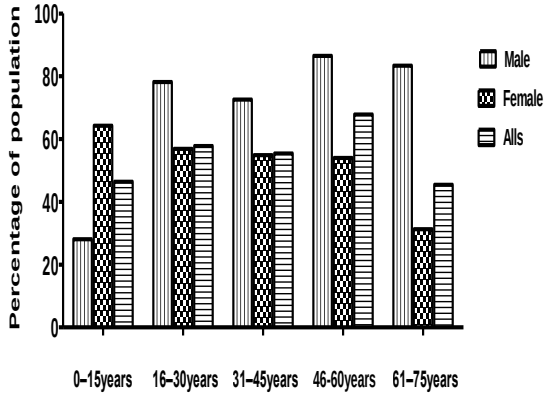

Figure 2a: Seroprevalence of IgG antibodies according to the age and sex

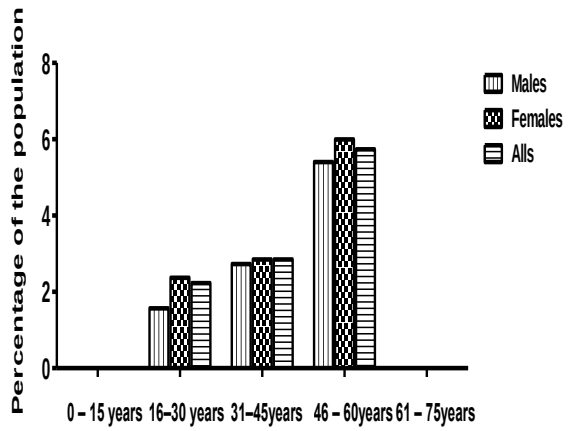

Figure 2b: Seroprevalence of IgM antibodies according to the age and sex

Toxoplasmosis serology in studied population

Table 1 reveals seroprevalence of toxoplasmosis in 7076 subjects included in the study, 4223 (59.7%) were seropositive for *Toxoplasma gondii* antibodies IgG and 190 (2.69%) were positive for *Toxoplasma gondii* antibodies IgM. The frequency of anti-toxoplasmosis IgG was significantly higher in the males (67.0% *vs*. 58.6%, p <0.0001). The rate of anti-toxoplasmosis IgM was significantly higher in the female (2.81% *vs*. 1.85%, p <0.01). In this study, we observed 4067 (57.49%) immunized subjects; females 56.35% (3469/6158) and males 65.14% (598/918).

Table 1. Toxoplasmosis serology according to sex

Antibodies profil	**Female (n=6158)**	**Male (n=918)**	**Alls (7076)**
	Number (%)	Number (%)	Number (%)
IgM positive	**173 (2,81)**	**17 (1,85)**	**190 (2,69)**
IgG positive	**3608 (58,61)**	**615 (66,99)**	**4223 (59,7)**
IgM positive/IgG negative or IgM positive/IgG positive (Recent infection or seroconversion)	46 (0,75)	0 (0)	46 (0,65)
IgM negative/IgG positive (immunized)	3469 (56,35)	598 (65,14)	4067 (57,49)
IgM negative /IgG negative(no immunized)	2516 (40,87	303 (33,01	2819 (39,85)

Results of the survey on the risk factors

The Analysis of the data collected during the survey revealed that: according to educational status, 88.2% of the subjects has school education (32.30% for primary level, 45.4% for secondary level and 10.5% for high

school level). In the study population, 12.2% had a knowledge of toxoplasmosis.

According to their occupation, 96.5% of the subjects were often employees, traders or housewives and 3.5% were women farmers.

Concerning the feed habits, regarding dietary habits, the survey data revealed that 80.5% of subjects consume raw garden products, 65.4% non-potable water and 8.3% undercooked meat.

Result of fecal samples analysis

The microscopic observation of the fecal samples of the cats revealed that 20.5% of them excreted *Toxoplasma gondii* oocysts.

Discussion

The aim of this prospective study was to determine the seroprevalence of toxoplasmosis in the Togolese population. The high rates of subjects in the age group of 16 to 45 years old, representing 97%, confirm the demographic characteristics of developing countries, as Togo, where the majority of the populations is under 40 years old (POPIN).

We found that the seroprevalence for IgG antibodies was 59.7% and the one for IgM was 2.7%. The significant high prevalence in men for IgG, ($p < 0.0001$) has already been reported in other studies (Coelho *et al.,* 2003). Anti-toxoplasmosis IgG and IgM antibodies, are serological markers of *Toxoplasma gondii* infection and are synthesized during primary infection (Pinard et al., 2003; Dubey, 2008; Dubey and Jones, 2008; Hamad and Kadir, 2013; Hammond-Aryee *et al.,* 2014). Indeed, by the action of T lymphocytes and cytokines, especially IFN-γ, parasites encyst in tissues with a weak immune response (brain, muscle, eyes). The antibodies IgM, IgE, IgA were produced during the acute phase of the infection of *Toxoplasma gondii*. The anti-toxoplasmosis IgG production during the chronic phase providing lifelong protection (Kaneko *et al.,* 2004; Couper *et al.,* 2005; Montoya and Remington, 2008; Dubey *et al.,* 2012). IgM is involved in immune responses by stopping the entry of the parasites into the cells and its intracellular replication (Kaneko *et al.,* 2004; Couper *et al.,* 2005). Therefore, in the immunized women the risk of reactivation of the cysts is reduced. However, in non-immunized women, the risk of infection increases with the age of pregnancy, and the fetus malformations associated with congenital toxoplasmosis increase when the infection occurs earlier in pregnancy. Toxoplasmosis contracted during the first trimester of pregnancy is responsible for spontaneous abortions, stillbirths, premature births and multi-

visceral lesions (Pinard et al., 2003; Dubey and Jones, 2008; Montoya and Remington, 2008). For this reason, the 40.87% of non-immunized women in this study have a high risk of contamination **(**Avelino *et al.,* 2004; Hammond-Aryee *et al*., 2014). This explains why the screening of toxoplasmosis is a part of health tests advised, but not mandatory in the assessment of pregnancy in many African countries as Togo. In developed countries pregnant women benefit from a special monitoring where *Toxoplasma gondii* serology is one of the examinations required in early pregnancy (Pinard *et al*., 2003; Adou-Bryn *et al*., 2004; Lopes-Mori *et al*, 2011; Hamad and Kadir, 2013; Koffi *et al.,* 2015).

In this study, the significant positive correlation, between age and prevalence of IgG (R = 0.68) in men and IgM (R = 0.99) in men and women, is explained by the increasing of the risks of infection according to the age and the persistence of infection during the life of patients. This observation is confirmed by the increasing of the seroprevalence from 45% for age group of 1-15 years old to 67.8% for age group of 46-60 years old (Garcia *et al.,* 1999; Cantos *et al.,* 2000). 37.5%

Former studies have shown that seroprevalence varies according to climatic, geographical and socio-economic conditions. The prevalences found in this study are lower than the one found in Central Europe: Slovakia and Czech Republic with 24.2% and 31.1% respectively (Svobodova *et al.,* 1998; Studeničová *et al*. 2006), in Irak with 37.5% (Hamad and Kadir, 2013). In France the prevalence was 43.8% (Berger *et al*., 2009) and in Scotland it was 27% (William *et al*., 1981).

In West Africa, a decrease of the prevalence of toxoplasmosis was observed from Senegal with 40.2% (Faye *et al.,* 1998), Mali, 27% (Dinkorma *et al.,* 2013) to Mauritania, 14.3% (Monjour *et al.,* 1983). The seroprevalence of 59.7% of this study is similar to those observed in Benin, 53.6% (Rodier *et al.,* 1995), in Burkina, 57.8% (Millogo *et al*., 2000; Linguissi *et al.,* 2012), in Ivory Coast, 58.7% (Koffi *et al*., 2015). It is lower than the one observed in Nigeria, 75.4% (Onadeko *et al.,* 1996) and Ghana, 92.5% (Ayi *et al*., 2009). In Central and Eastern Africa the prevalence is above 50% (Bisvigou *et al.,* 2009; Pangui *et al*., 2013; Yobi et al., 2014).

The seroprevalence of anti-toxoplasma antibodies found in this study is in correlation with the results of previous works in Togo. Comparing these results with the studies done 40 years ago in Togo, we observed no variation in seroprevalences (53% to 80%) in both sexes of all healthy and pregnant women (Agbo *et al.,* 1991; Balogou *et al.,* 2007; Apetse *et al.,* 2015). These results could be explained by the fact that for decades, hygiene and dietary

habits, food preservation quality have not changed to prevent the contamination by *Toxoplasma gondii* which infection remains a public health problem in Togo. This confirms our results of the survey on the risks factors of the contamination by *Toxopalma gondii* that revealed the rate of 80.5% of subjects that consume raw fruits and vegetables, 65.4% use non-potable water, 8.3% eat undercooked meat. In addition, 65% of the subjects have the contact with cats that 20.5% of them excreted *Toxoplasma gondii* oocysts.

The lack of public health schemes to manage the spread of this pathogen places African populations at risk of ongoing and possibly increasing incidence and prevalence, as well as a corresponding increase in mortality and morbidity due to toxoplasmosis (Koffi *et al.,* 2015, Soumana *et al.,* 2016).

Conclusion

The results of this study revealed that the seroprevalence of toxoplasmosis remains high in the Togolese population. The data observed in this study should allow the country's health authorities to establish sensitization and prevention programs for all age group of the population to explain how to avoid contamination by the food, water and soil. In the houses, the presence of cats (definitive hosts of *Toxoplasma gondii*) untreated by veterinarians is a permanent danger of reinfection by the inhabitants. For the mainly female population, the program should focus on the risks of fetal diseases and embryopathies related to *Toxoplasma gondii* infection during pregnancy. An effort should be made to make serological tests available for early detection of infection. Women of childbearing age who are not immunized against *Toxoplasma gondii* represent a group of high risk of contamination. The high proportion of subjects aged 15-45 years old, the most active and most affected by toxoplasmosis, constitutes a serious danger for the socio-economic development of the country.

In addition, 65% of the subjects have the contact with cats that 20.5% of them excreted *Toxoplasma gondii* oocysts.

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Conflict of interests

The authors have no conflicts of interest.

Ethical considerations

Ethical consideration has been completely observed by the authors.

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