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Parasitology

Seroprevalence of Toxoplasmosis in Pregnant Women in the Fes-Meknes Region of Morocco: Apropos of 285 Cases

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Abstract **Original Research Article**

Introduction: Toxoplasmosis is a common infection with a worldwide distribution. It affects homeotherms and has a significant impact on human health, particularly in cases of pregnancy or immunodepression. The aim of this study was to assess the seroprevalence of this parasitosis and determine the main risk factors associated with positive serology in pregnant women in the Fès-Meknès region. ***Material and Method*:** This is a cross-sectional study carried out between April 2019 and March 2020, involving 285 pregnant women from the Fès-Meknès region, followed up in the gynecology department of the Moulay Ismail Military Hospital in Meknès and in the two gynecology-obstetrics departments of the Hassan II University Hospital in Fès. Serological detection of immunoglobulin G was performed to estimate the seroprevalence of toxoplasmosis. ***Results and Discussion*:** The seroprevalence of toxoplasmosis was 49.1%, suggesting that a significant proportion of women are non-immune and therefore at risk of congenital toxoplasmosis. Seroprevalence increases with age and illiteracy, and is higher in urban than in rural areas. ***Conclusion*:** Our study highlights the usefulness of preconception screening for toxoplasmosis, the value of serological surveillance of non-immune women during pregnancy, and the importance of implementing educational programs for women of childbearing age to reduce the burden of abortion, fetal death and infant sequelae attributable to this parasitosis.

Keywords: Toxoplasma gondii - Seroprevalence - Pregnant women - Fez – Meknes.

INTRODUCTION

Toxoplasmosis is a parasitic infection caused by an intracellular protozoan of the class Coccidia: Toxoplasma gondii. It is a ubiquitous, cosmopolitan anthropozoonosis [1]. Transmission to humans occurs mainly through consumption of meat contaminated with cysts, ingestion of food or water contaminated with oocysts released into the environment by felids, or congenital vertical transmission. More rarely, tachyzoites may be transmitted through tissue transplants, transfusion of blood products or consumption of unpasteurized milk [2].

This is one of the most widespread infections worldwide. It is estimated that between 25% and 30% of the human population is infected. Numerous factors can modify seroprevalence, which explains the variations between continents, countries, regions and communities within the same region. Hot, humid climates are responsible for high prevalences of toxoplasmosis in tropical countries, while dry or cold climates are associated with low prevalences. Prevalence increases with age. It varies according to eating habits, cultural and ethnic practices, and the presence and number of cats. A high socio-economic level, with better hygiene conditions, developed agricultural systems, and better access to drinking water and sanitation, considerably reduces this prevalence. Conversely, low socio-economic status increases the seroprevalence of toxoplasmosis [3].

Infection is most often latent, but certain severe forms are particularly dreaded in immunocompromised subjects and newborns infected in utero [1]. Indeed, primary infection during pregnancy in a non-immunized woman can lead to congenital toxoplasmosis, with

clinical manifestations ranging from spontaneous abortion to neurological and ocular damage of varying severity, depending on the age of the pregnancy [4]. The frequency and potential severity of T. gondii infection call for effective prevention, diagnosis and management, particularly in non-immune pregnant women. Screening for toxoplasmosis is based on serology. Consequently, prevention of congenital toxoplasmosis requires serological surveillance of pregnant women to establish their immunological status, to identify non-immune pregnant women, to limit the risk of contamination by applying prevention advice, and to diagnose maternal seroconversion as early as possible, in order to offer appropriate treatment.

MATERIALS AND METHODS

Type, duration and setting of study: This was a cross-sectional epidemiological study carried out in the gynecology department of the Moulay Ismail military hospital in Meknes and the gynecology-obstetrics departments 1 and 2 of the Hassan II University Hospital in Fez, in collaboration with the immunoserology laboratory of the Moulay Ismail military hospital in Meknes. The study was spread over a 12-month period, from April 01, 2019 to March 31, 2020.

Study population: the study population consisted of 285 women residing in the Fès-Meknès region seen in consultation, with a confirmed pregnancy, regardless of gestational age, and whose medical records and toxoplasmosis serology results were accessible. All patients were informed of the interest of this study and agreed to participate. For non-immune patients, measures to prevent toxoplasmosis were explained and encouraged**.**

Data collection and serological analysis: Data were collected on pre-established forms, by interviewing pregnant women and using their medical records. An anonymous questionnaire was drawn up to ensure complete and targeted collection of data relevant to our study. The questionnaire was organized into 5 parts, and collected data relating to demographic characteristics: age, occupation, place of residence, classification of current pregnancy, gestational age; socio-economic characteristics: level of education and economic and social status; dietary habits and hygiene: Consumption of raw or poorly cooked vegetables, consumption of untreated or poorly treated water, consumption of undercooked meat, contact with cats, notion of gardening and contact with the soil, washing of vegetables and fruit; Knowledge of toxoplasmosis: Has the patient ever heard of toxoplasmosis, nature of information, sources of information **;** Immune status: Toxoplasmosis serology of previous pregnancies, pre-conception serology, previous serology of current pregnancy, current serology. The tests used to perform the serologies varied according to the laboratory chosen by the parturient. The ARCHITECT Toxo IgM IgG (ABBOTT) and LIAISON Toxo IgM IgG (DIASORIN) chemiluminescent tests were performed at the Hassan II University Hospital and Moulay Ismail Hospital laboratories respectively. The other tests were carried out in the region's private laboratories.

Data recording and statistical analysis: The data collected were entered into Microsoft Office Excel 2013 and processed using EPI-info software version 7.0. The tests used were: ANOVA, t-test, Fisher's exact test and Chi-square test. The significance level was set at 5%.

RESULTS

A total of 285 pregnant women from the Fès-Meknès region were included in this study. The mean age was 30.18±12.34 years, with extremes of 18 and 44 years. The association between age and current serology was statistically significant (P= 0.0003). In women aged under 30, seroprevalence was 38.64%, contrasting with a prevalence of 58.39% in those aged 30 and over (p = 0.001). The majority of women were from urban areas (84.21%), compared with 15.79% from rural areas. Geographical origin was also significantly related to serology. Patients from urban areas were 1.45 times more likely to have positive serology than those from rural areas (p = 0.04). Most women had an average economic level of 49.12%, 48.77% of patients had secondary education and 17.54% were illiterate. The correlation between literacy and serology was significant (p =0.01), so illiteracy multiplied by 1.46 the risk of having a positive serology among the women in our sample.

The majority of subjects had no occupation (80.70%). Employed women accounted for 19.30%. With regard to the eating habits of the women surveyed, 251 women (88.07%) consumed raw or undercooked vegetables, 52 women (18.25%) consumed untreated water, 167 women (58.60%) consumed undercooked meat, but none consumed raw meat. 113 patients (39.65%) consumed unpasteurized milk or cheese. 119 patients (41.75%) frequently ate out. (Figure 1) Consumption of raw or undercooked vegetables was significantly associated with positive serology (p < 0.0001). Women who consumed raw or undercooked vegetables were 3.65 times more likely to have a positive serology than those who did not (Figure 2).

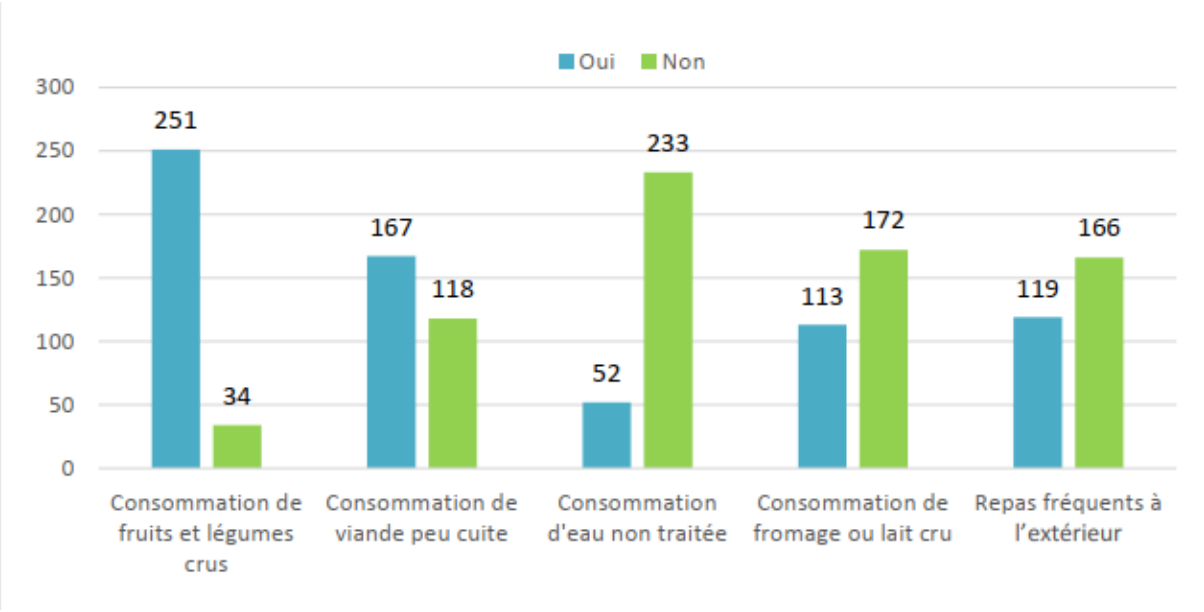

Figure 1: Distribution of women by eating habits

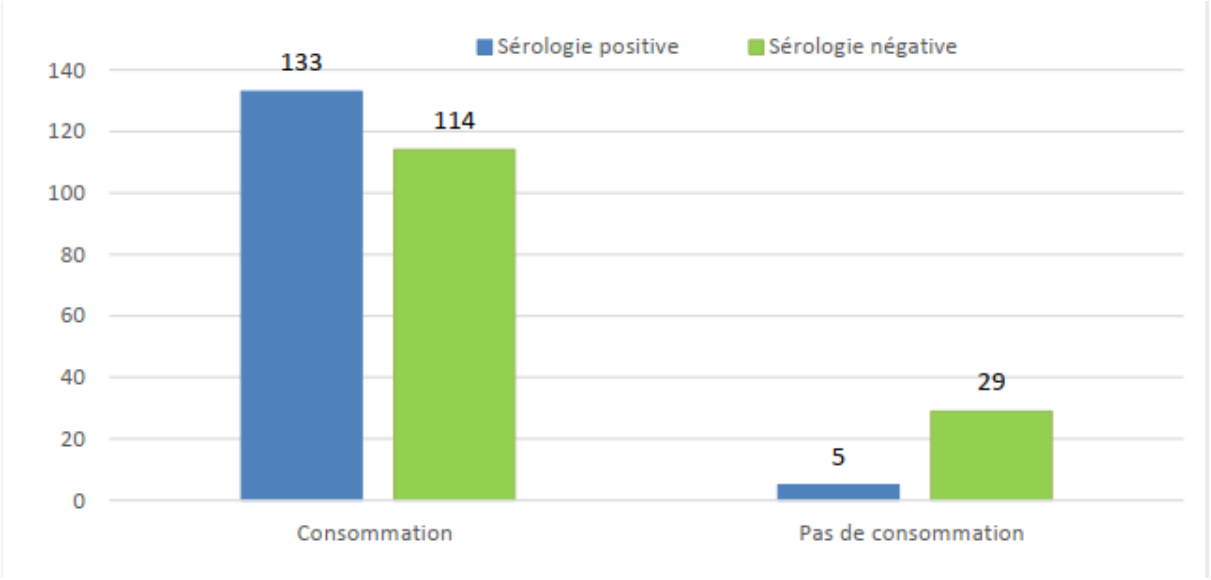

Figure 2: Distribution of serologies according to consumption of raw or undercooked fruit and vegetables

41 women out of 285 (14.39%) had some notion of contact with cats, while 74 patients (25.96%) had some notion of contact with the earth, either as part of an agricultural activity, or as part of leisure activities (gardening, picking). In our study population, 143 women had no knowledge of toxoplasmosis, versus 142 who had heard of it. 77 women knew the modes of contamination, 10 women the clinical signs, and 21 women the fetal complications. Lack of information about fetal complications was significantly correlated with positive serology (p = 0.016). (Figure 3) 65 women were familiar with preventive measures. Knowledge of protective measures against the parasite was significantly correlated with negative serology (p =0.003). In fact, women who knew these measures had a 41% reduction in the risk of having a positive serology, compared with those who didn't know how to prevent infection (Figure 4).

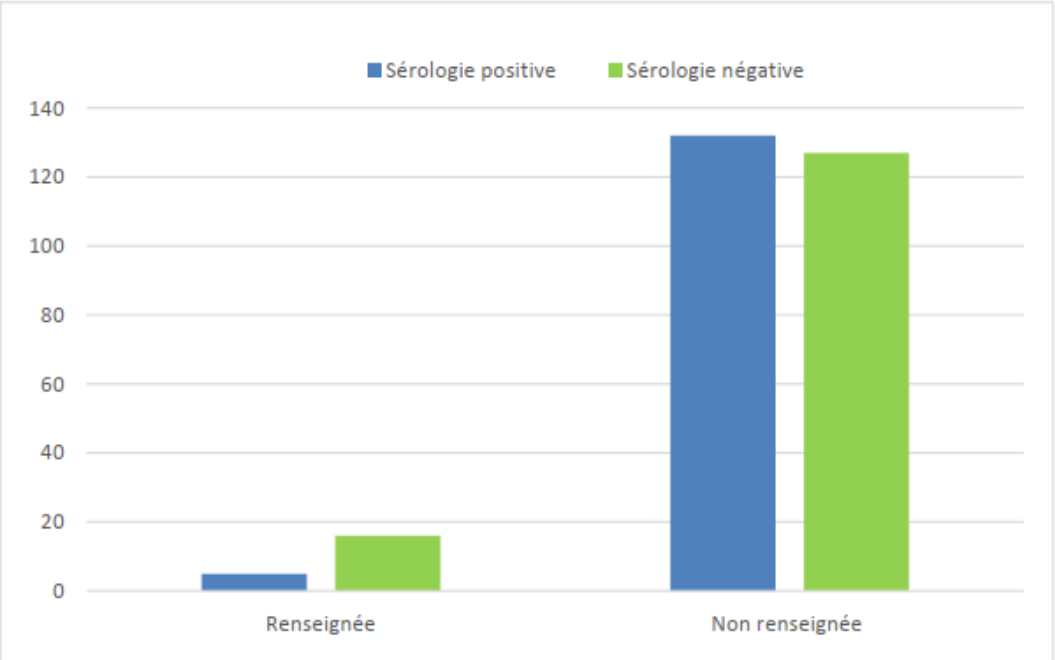

Figure 3: Distribution of serologies according to knowledge of fetal complications

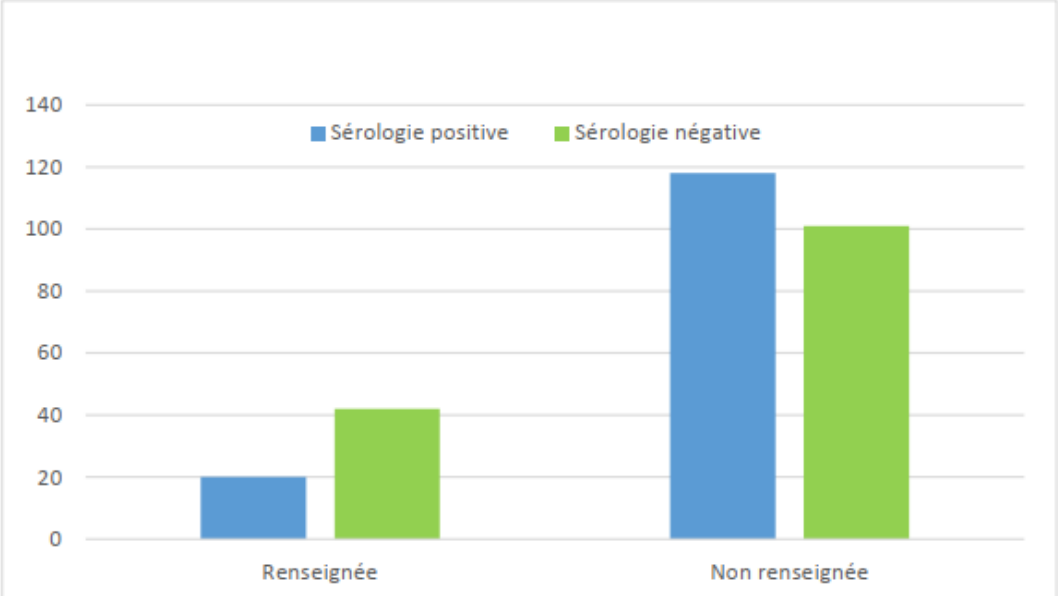

Figure 4: Distribution of serologies according to knowledge of preventive measures

In the present study, toxoplasmosis seroprevalence was 49.11%. Of the 285 women tested, 138 were immune, 158 women had already had toxoplasmosis serology in previous pregnancies, 3 women had had an anti-T. gondii antibody test before conception, and 133 women had had at least one previous toxoplasmosis serology in the current pregnancy. 5.92% of women had their first serology before the end of the 1st trimester, compared with 46.05% in the 2nd trimester and 48.03% in the 3rd trimester. The association between previous serology results during the current pregnancy and current serology was significant ($p < 0.0001$). A serological shift during pregnancy was observed in only one pregnant woman, representing a seroconversion rate of 0.35%. The patient, who had previously had negative serology with IgG at 0.10 IU/ml (below the cut-off value of 3 IU/ml provided by the laboratory), was well monitored with monthly toxoplasmosis serological testing. Seroconversion occurred during the 2nd trimester of pregnancy, when serology results showed positive IgG and IgM levels, with respective values of 93.20 IU/ml (above 3 IU/ml) and 3.96 VT (above the threshold value of 0.65 VT). IgG kinetics were marked by a multiplication of the latter (604.7 IU/ml) on the sample taken 4 weeks apart. IgM kinetics showed a drop to 2.98 VT at the end of the 4th week. As soon as IgG was positive, the patient was started on spiramycin SPIRALIDE® 3 M U three tablets a day, and underwent two ultrasound scans in the month following seroconversion, with no abnormalities detected. The

patient was then referred to a professor of gynecology for amniocentesis.

DISCUSSION

Primary toxoplasma infection is benign or clinically inapparent in over 80% of cases, which justifies the importance of serology as the basis for prevention through screening and diagnosis of toxoplasmosis. By identifying non-immune pregnant women, the risk of contamination during pregnancy can be limited by food hygiene measures [5]. Human contamination occurs through horizontal transmission, via ingestion of tissue cysts present in the muscle and nervous system of contaminated intermediate hosts (cattle, sheep, goats, poultry, etc.), and via ingestion of oocysts present to varying degrees in the environment, contaminating plants and water intended for human consumption. Congenital vertical transmission: in the case of T. gondii infection in a seronegative mother, tachyzoites can cross the placenta and contaminate the fetus. Gestational age during primary infection is a crucial factor in the frequency and severity of this transmission. Indeed, in the first trimester, the risk of transmission is relatively low (<20%), but if it does occur, serious consequences, such as hydrocephalus, mental retardation and spontaneous abortion, are observed, while in the last trimester, the risk of transmission increases to nearly 80%, but the majority of cases are subclinical [2]. The prevalence of toxoplasmosis fluctuates, with underdeveloped countries having a higher incidence than developed ones. High prevalences are recorded in Latin America, tropical Africa, parts of Central and Eastern Europe, and parts of the Middle East. Moderate prevalences (30-50%) are noted in some Central and Southern European countries. Low seroprevalences (10 to 30%) are observed in North America, Southeast Asia, Northern Europe and the African Sahel [3]. Our study included 285 pregnant women aged between 18 and 44 who underwent serological testing. The results showed IgG antibodies to T. gondii in 138 patients, representing a seroprevalence of 49.11%. This high prevalence is comparable to those found in other regions of the kingdom, notably El Mansouri in Rabat (51%) [6], Guessous in Casablanca (51.5%) [7], and the Agadir-Inzegane region (47.33%) [8]. This is close to the overall Moroccan seroprevalence estimated at 51% by Mekkouar in 1972, but differs from those found in the Marrakech-Safi region, Nador, Tétouan and Kenitra, which were 62.1%, 43.3%, 42.6% and 36.7% respectively [9].

Compared with other regions of the Maghreb, our results are close to those found in the department of Annaba in Algeria, with a prevalence of 47.8% among pregnant women [10], in the southern provinces of Tunisia (47.7%) [9], and in Benghazi in Libya (47.7%) [9]. The Fès-Meknès region is characterized by a temperate Mediterranean climate, with a relatively marked continental aspect. This corresponds to the Csa type of the Köppen-Geiger classification. Average annual rainfall is between 500 and 700 millimeters. These climatic characteristics make the region an ideal environment for oocyst sporulation and thus for the sexual reproduction of T. gondii, contributing to the high prevalence determined in our study [11].

In our study, a high seroprevalence (58.39%) was observed in women aged 30 or over, while in those under 30 it was only 38.64%. This correlation is statistically significant (p = 0.001), which could make age a factor favoring T. gondii infection. Our results are in line with those found in other national studies. In the study carried out by El Mansouri in Rabat, there was a significant link between age and the prevalence of toxoplasmosis. The percentage of seropositivity was 32.4% in pregnant women aged under 20, 52% in those aged between 20 and 39, and 63.8% in those aged over 40 [6]. In a survey carried out in the Essaouira-Safi region, too, prevalence increased linearly with age, with women over 30 being more immune (64.5%) than those under 30 (35.5%) [12]. The same conclusion was reached in the Agadir-Inzegane region [8].

Internationally, several studies support the association between age and the prevalence of toxoplasmosis. In a study carried out in the USA, seroprevalence climbed from 9.3% in subjects aged 20 to 29 to 14.8% in those aged 40 to 49 [13]. In India, it ranged from 38.5% in women aged 20-25 to 77.8% in those aged 35-39 [14]. Age was also identified as a risk factor in surveys in France [15], Turkey [16], Saudi Arabia [17] and Ethiopia [18]. This finding could be explained by a longer duration of exposure to sources of infection, which makes it necessary to educate women of childbearing age about toxoplasmosis, emphasizing the importance of primary prevention. A study of the variation in serology according to geographical origin showed a statistically significant association between these two variables (p = 0.04), with patients from urban areas more often positive (51.69%) than those from rural areas (35.56%). This result, although different from those obtained in other regions of Morocco (Essaouira-Safi [33] and Agadir-Inzegane [8]) where rural women were more often seropositive, is not new. Indeed, Bouratbine in Tunisia [19], Mwambe in Tanzania [20], and Sissinto-Savi de Tove in Benin [21], all reported significantly higher seroprevalences of toxoplasmosis in urban than in rural areas. This correlation could be explained by the diversity of lifestyle and dietary behavior between these two environments, notably the frequency of meat consumption, the nature of the meat consumed (cattle, sheep, etc.) and the consumption of food products imported from high-prevalence regions. Food cooking methods also come into play, with microwave ovens, widely used in cities, being less effective at eliminating pathogens [22]. Several experimental studies have concluded that these insects are capable of carrying infective oocysts on their surfaces, eliminating them in their droppings and

disseminating them to their surroundings [19]. Analysis of risk factors related to the dietary habits of parturients revealed a statistically significant association between consumption of raw vegetables and positive serology ($p < 0.0001$). Such consumption has also been identified as a risk factor for toxoplasmosis in France [23] and Italy [24]. In Norway, it is said to be the most important risk factor for infection in pregnant women [25]. This mode of transmission reflects a farming environment highly contaminated with oocysts, either through direct exposure to cat faeces or after irrigation with contaminated water. Oocysts can persist for several days on the surface of vegetables, thanks to the cold and humidity of the latter [26]. In our series, 14.39% of women had a history of contact with cats, with no statistical association between this factor and serology. This result is in line with those of numerous national and international studies, which have not considered contact with cats as a risk factor for infection [27-29]. Some countries on the American continent have high prevalences of toxoplasmosis in cats. In Europe, these prevalences are often intermediate [30]. To our knowledge, no study to date has examined the prevalence of toxoplasmosis in felids in Morocco. In order for a cat to be a contaminant, it must be infected; however, domestic cats are usually non-immune, due to their diet based on table scraps, canned food and kibble [26]. Pregnant women must also come into contact with faeces containing sporulated oocysts. This is the case, for example, when she cleans a litter box that has not been emptied for several days, a situation that has been identified as a risk factor in France [23], but which is not preponderant in our context. The risk of intrauterine infection of the fetus, the risk of clinical manifestations and the severity of congenital toxoplasmosis depend on gestational age at the time of maternal infection, the mother's immunological competence, and the number and virulence of parasites transmitted to the fetus. In the first trimester, the risk of vertical transmission after maternal primary infection is 14%, but when it occurs, its effects on the fetus are the most severe, whereas in the last trimester, the risk of fetal infection increases to around 59%, but its consequences are less severe [4]. Toxoplasma antibody testing is preferably prescribed during a preconception consultation, and before the end of the 1st trimester of pregnancy at the latest**.** The aim of serological monitoring is to identify non-immune pregnant women, in order to limit the risk of infection during pregnancy by observing preventive measures, and to diagnose maternal seroconversion as early as possible in order to institute appropriate management. The techniques most commonly used for this purpose are enzyme immunoassay, immunocapture and immunofluorescence [36]. In France, toxoplasmosis serology is compulsory at the prenuptial examination or at the time of pregnancy declaration. This has contributed to the decline in the number of cases of congenital toxoplasmosis over the years, and to the virtual disappearance of its severe forms [31]. In Morocco, there are no regulations on this subject, which explains why in our study only 1.05% of patients underwent preconception serology, and 21.71% underwent serology before the end of the 1st trimester. The serologies in our sample were all performed by automated enzyme-linked immunosorbent assays, including Liaison Toxo IgG (45.58%) and Architect Toxo IgG (26.15%) chemiluminescence tests, available from the laboratory at Moulay Ismail Hospital in Meknes and Hassan II University Hospital in Fez, respectively. These tests are currently the most widely used worldwide, offering high sensitivity and specificity, reproducibility and rapid, accurate measurement of IgG and IgM antibody levels, even in small quantities of serum [32].Interpretation of results: IgG and IgM negative: The absence of detection of specific IgG and IgM antibodies indicates the absence of immunity to T. gondii, which means that monthly serologies should be carried out until delivery, and the pregnant woman should be informed of the hygienic and dietary measures to be taken [31]; IgG negative and IgM positive: If IgG is not detected, but IgM is, a second serology test is recommended within 1 to 2 weeks. The appearance of specific IgG proves toxoplasmic seroconversion. On the other hand, if IgG is absent after two serological controls, this may be due to non-specific natural IgM or interference, and continued serological monitoring is recommended [31]; IgG and IgM positive: The presence of IgG and IgM can be related to both old and recent immunity, hence the importance of measuring IgG avidity in the absence of previous serology. High avidity indicates an old infection, and a stable IgG level at 3-week intervals confirms this hypothesis, so the risk of primary infection is nil and follow-up is unnecessary. On the other hand, intermediate or low avidity does not eliminate the hypothesis of a recent infection, and measurement of antibody kinetics at 3-week intervals is mandatory for dating, as a stable IgG level points to an infection probably more than 2 or 3 months old. Conversely, a doubling of IgG means that the infection is less than 2 to 3 months old [31]; IgG positive and IgM negative: In the absence of previous serological tests, the presence of IgG and the absence of IgM should prompt a control serology at 3 weeks. A stable IgG level is observed in cases of long-standing infection. A rising level indicates that an avidity test should be performed. A high avidity is found in cases of serological reactivation of an old infection, while a low avidity cannot exclude a recent infection, and management will then depend on gestational age [36]. Monitoring of toxoplasmosis in the facilities hosting our study was mainly based on IgG testing. 50.89% of our study population was IgG negative. These patients were all prescribed toxoplasmosis serology to be presented to their GP at the next monthly consultation. 49.11% of our parturients were IgG positive. The appropriate course of action was to compare these results with previous serologies, if available, and to request a second serology 3 weeks after the first in order to compare IgG levels.

CONCLUSION

Toxoplasmosis is a worldwide parasitic infection whose prevalence varies considerably from one country to another. Usually latent, it must be monitored during pregnancy, as it can take on serious forms in the event of primary infection of the pregnant woman, with transmission to the fetus. Our work has given us a better understanding of the characteristics of T. gondii infection in our Fès-Meknès region, enabling us to determine the seroprevalence of toxoplasmosis (49.1%) and identify the main risk factors involved in positive serology. This study highlights the role of toxoplasma serology in pregnant women as a key to screening and surveillance. Our results also underline the importance of educating women of childbearing age about toxoplasmosis, and non-immune pregnant women about the need to comply with preventive measures. To this end, toxoplasmosis should no longer be the subject of recommendations alone, but rather a national prevention and screening program should be set up to ensure better management of this disease during pregnancy.

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