

Original paper

High toxoplasmosis seroprevalence among young pregnant women in Medea, Algeria

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ABSTRACT. Toxoplasmosis is a parasitic disease with worldwide distribution and a major public health problem. In fact, the zoonotic pathogen *Toxoplasma gondii* infects humans and almost all warm-blooded animals. One of the most common sources of human *T. gondii* infection is the ingestion of tissue cysts in raw or undercooked meat. The current epidemiological study was carried out in Medea (Algeria), from January to June 2017, using Enzyme Linked Immunosorbent assay (ELISA). Antibodies against *T. gondii* were determined in sera of more than one thousand pregnant women. The different age groups were: 15–20, 20–25, 25–30, 30–35, 35–40 and 40–45. Among 1,012 pregnant women analyzed, 252 (25%) were found seropositive, 49 among them showed a IgG+/IgM+ profile, and 760 (75%) were seronegative exhibiting IgG-/IgM-. Toxoplasmosis prevalence's were 44.5% and 39.5% in 15–20 and 20–25 age categories, respectively. A long-term prevention program is needed and strategies should be focused on the serodiagnosis of toxoplasmosis, especially in pregnant women. Since the consumption of raw or undercooked meat is not a part of the culinary culture in that area of study, cat exposure remains the principal factor of human infection.

Keywords: ELISA, toxoplasmosis, seroprevalence, pregnant women

Introduction

Toxoplasmosis is one of the most common human infections, affecting one third of the world's population. It is caused by *Toxoplasma gondii* through the ingestion of contaminated meat, food or water as well as through the contact with cat faeces, from soil [1]. The transmission may also occur through organ transplant from infected donors, or from mother to the fetus (congenital infection in the baby) [2]. The gestational age remains controversial since during such a time of pregnancy, the parasitemia seems high and there is a risk of congenital toxoplasmosis [3]. In fact, if the infection takes place shortly before conception or during the first trimester of pregnancy, infected women are then capable of transmitting the acquired parasite to their fetuses [4]. On the other hand, the latent toxoplasmosis also contributes to the global distribution of infection [5].

In the neonate, manifestations of congenital toxoplasmosis might include hydrocephalus, microcephaly, intracranial calcifications, retinochoroiditis, strabismus, blindness, epilepsy, psychomotor and mental retardation, petechiae due to thrombocytopenia, and anemia [6].

The incidence of the infection was described to be higher in lower-, middle-income countries and, lower in industrialized regions. The most affected areas of the globe are Latin America, parts of Eastern/Central Europe, the Middle East, and parts of South-East Asia and Africa [7].

In Algeria, prophylaxis of congenital toxoplasmosis is part of a national surveillance program for pregnant women. In addition, medical care is guaranteed in case of toxoplasmic seroconversions or evolutive toxoplasmosis. Human toxoplasmosis seroprevalence was estimated at 51.56% [8]. Furthermore, we recently demonstrated that stray cats from Medea (Algeria) harbored *T. gondii* type 2

Table 1. Sample reference intervals were expressed in IU/ml (international unit per milliliter)

Interpretation	IgG	IgM
Positive	≥150.0	≥15.0
Equivoque	(100.0–150.0)	(10.0–15.0)
Negative	<100.0	<10.0

[9]. However, there are no data of this disease in humans from that region. The aim of this study was to investigate for the first time the pregnant women toxoplasmosis status in Medea, using a commercial Enzyme Linked Immunosorbent assay (SERION ELISA *classic* kit).

Materials and Methods

Study site and period

The study was conducted at Zitouni medical laboratory, in the city of Medea, Algeria. Medea is a province with a surface of 8,775.65 km². It is an agropastoral area, located in the center of the country, in the heart of the Atlas Tellian. Sera were collected during 2017.

Study population

The epidemiological study included all pregnant women who had come at the immunoserology service. A total of 1,012 pregnant women were sampled. The inclusion criteria were the availability of information concerning the consent of participants and their age. The exclusion criteria were the absence of those parameters. Patients whose characteristics were not available were further excluded from this study.

Sera samples collection

An aliquot of blood was withdrawn from each woman by a professional clinical laboratory technician. Sera were then separated by centrifugation and stored at –20°C until analysis. Each blood sample was analysed for the presence of *T. gondii* by identifying serum IgG and IgM antibodies to *T. gondii* using an enzyme-linked immunoassay test.

Serological testing

Testing for *Toxoplasma gondii* serology (IgM and IgG) was performed using a commercial Enzyme Linked Immunosorbent assay (SERION ELISA *classic* kit provided by Institut Virion/Serion

GmbH, Würzburg, Germany), which has a *Toxoplasma gondii* IgG sensitivity and specificity of 98.2% and > 99%, respectively; and *Toxoplasma gondii* IgM sensitivity and specificity of >99% and 97.2%, respectively. The different age groups were as follows: 15–20, 20–25, 25–30, 30–35, 35–40 and 40–45. The interpretation of SERION ELISA results were made according to Table 1.

Ethical clearance and informed consent

The purpose and procedures of the survey were explained to all participants. All the subjects gave their written informed consent for their inclusion before they participated in the study. If not, they were not included. All data were analyzed anonymously throughout the study.

Statistical analysis

The analysis took place in two stages; descriptive stage where the quantitative variables were expressed in means ± standard or median deviation, and qualitative ones were expressed in percentage. Statistical analyses were performed using SPSS Statistics 20.0. The statistical significance between seroprevalence and age or sex was evaluated by the χ^2 test. These analyses were considered significant when $p < 0.05$.

Results

Of 1,012 pregnant women included in our study, 252 (25%) were found seropositive by ELISA, and 760 (75%) were seronegative; 49 had (IgG+/IgM+) serology, 16 had (IgG-/IgM+), 187 had (IgG+/IgM-), and 760 had (IgG-/IgM-) (Table 2).

A total of 20 (44.5%) pregnant women belonging to the age category of 15–20 were found seropositive by Elisa, followed by 39.5% of 20–25 category age, and 25.3% of 25–30 years old. A total of 35 (14.6%) and 13 (10.3%) were found seropositive concerning the 30–35 and 35–40 age categories, respectively (Fig. 1). Statistically, a very significant difference ($p < 0.0005$) was found between ELISA results and the different ages groups.

IgM+/IgG+ profile was found in 17.8% in the age group of 15–20, 10.3% in the age group of 40–45, 6.3% in the age group of 30–35; 5% and 4.2% were found in 25–30 and 35–40, respectively. IgM+/IgG- profile was found in 4.5% in the age group of 20–25, and 3% in the 25–30 age group. IgM-/IgG+ results were recorded in 30.9% in 20–25

Table 2. Summary of anti-*Toxoplasma* specific IgM and IgG antibodies distribution according to age groups

Age group	No. Patients sampled	Antibodies		IgM+/IgG+	IgM+/IgG-	IgM-/IgG+	IgM-/IgG-
		ELISA + (%)	ELISA - (%)				
15-20	45	20 (44.5)	25 (55.5)	8 (17.8)	0 (0)	12 (26.6)	25 (55.5)
20-25	243	96 (39.5)	147 (60.5)	10 (4.1)	11 (4.5)	75 (30.9)	147 (60.5)
25-30	336	85 (25.3)	251 (74.7)	17 (5)	10 (3)	58 (17.3)	251 (74.7)
30-35	239	35 (14.6)	204 (85.4)	15 (6.3)	0 (0)	20 (8.4)	204 (85.3)
35-40	120	13 (10.3)	107 (89.2)	5 (4.2)	0 (0)	8 (6.7)	107 (89.2)
40-45	29	3 (10.8)	26 (89.6)	3 (10.3)	0 (0)	0 (0)	26 (89.6)
Total	1,012	252 (25)	760 (75)	49 (4.8)	16 (1.6)	187 (18.5)	760 (75)

age group, 26.6% in 15-20 age group, 17.3% in 25-30 and 8.4% in 30-35. Concerning the IgM-/IgG- profile, 89.6% and 89.2% recorded in 40-45 and 35-40, respectively; while, 85.3%, 74.7%, 60.5% and 55.5% were found in 30-35, 25-30, 20-25 and 15-20 age groups, respectively.

Discussion

Toxoplasmosis is a widespread cosmopolitan affection, generally benign in immunocompetent individuals, but may be responsible for severe clinical forms depending on the immune status of the host and strains involved. Serious forms can be found in the fetus and immunocompromised patients. The seroprevalence of this disease may correlate with the culinary habits and way of life of the population [10]. This is the first study aiming the identification of toxoplasmosis serology in pregnant woman from Medea, Algeria. The situation of human toxoplasmosis in Algeria is unknown.

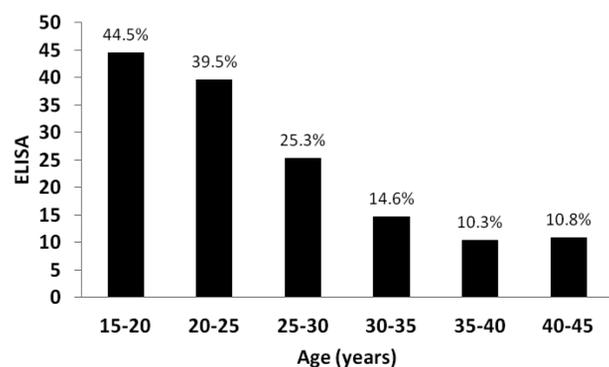


Figure 1. Frequency distribution of positive serology by age of pregnant women

Indeed, we do not have any data from either surveys or publications allowing us to have an idea about this condition. Up to now, few epidemiological studies have been performed in the framework of the activity report of the Pasteur Institute of Algeria [11].

The main aim of our work was to evaluate toxoplasmosis seroprevalence in pregnant woman in the region of Medea, an area not so far from Algiers where *T. gondii* type 2 is prevalent in cats [9]. Our studies were performed on more than one thousand pregnant women.

Our data revealed that the toxoplasmosis seroprevalence in the total pregnant women from Medea (north Algeria) was 25%, while prevalence values were 44.5% and 39.5% in 15-20 and 20-25 age categories, respectively. In Annaba in the eastern of Algeria, where a study had been conducted, a seroprevalence of 47.8% was found in pregnant women [12]. Human toxoplasmosis prevalence in the center of the country was 57.7% in 1981, 40.7% in 1993, 46.6% in 2001 and 47.9% in 2010 [12]. A study conducted in Constantine (northeastern Algeria) from September 1995 to July 1996, showed a seroprevalence of 50.1% (data from the Institute Pasteur d'Alger). Therefore, the seroprevalence of toxoplasmosis in Algeria is globally around 50% [12].

In the eastern of Algeria, a study was conducted in Setif on women of childbearing age, during a period from March 2005 to March 2007, and seroprevalence was around 60.9%, the risk factor found was the consumption of raw vegetables [13].

The prevalence of human *Toxoplasma* infection varies in different parts of the world, and had been

reported to be up to 75% [7]. In the Maghreb countries, very few studies have been focused on the seroprevalence of toxoplasmosis, these studies generally concerned toxoplasmosis in pregnant women. Follow-up data available from Tunisia showed many regional studies with different seropositivity rates. In 2001, a study revealed that the prevalence rate in Tunisia was 47.7% [14]. In another study performed 10 years later, a higher rate (58.4%) was reported indicating that *T. gondii* infection may be growing up [15]. However, in other countries there are no follow-up data available. The rates in Morocco and Libya were 50.6 and 63.3%, respectively [16]. In Egypt, a seropositivity rate of 46.1% was found among women with first trimester miscarriage [17]. Another study evaluating prevalence among pregnant women in Egypt showed a rate of 72.61% [18]. In Sudan, Elnahas et al. [19] reported a prevalence of 34.1% among pregnant women. In Senegal, 40.2% of the surveyed procreative women had antibodies of toxoplasmosis in 1998 [20]. In 2013, a human toxoplasmosis seropositivity rate of 27% was found in Mali [21]. Similarly in Niamey, Niger, 18.2% of the 400 undifferentiated people, were found seropositive [22].

In Europe, human toxoplasmosis seroprevalence is variable, 25.7% in Sweden [23], 29.5% in Greece [24], and relatively high in France (43.6%) [25]. In Spain, 2,623 pregnant women were studied, of whom 21% was seropositive [26]. In Portugal, *T. gondii* overall seroprevalence decreased from 47% in 1979–1980 to 22% in 2013 [27].

In United States, the overall *T. gondii* antibody seroprevalence among persons ≥ 6 years of age in 2009–2010 was 13.2% [28]. The seroprevalence of *Toxoplasma* infection in pregnant migrant and refugee women from Myanmar attending antenatal care in Thailand was 31.7% [29].

In our study, we found a statistically significant positive correlation between age and toxoplasmosis seroprevalence. In fact, rates ranging 50%–10% were found in age groups of 15 to 40. The ages 15 to 25 represent the period with the highest rate of marriage of girls in this region. Medical analyses are requested, and serology against toxoplasmosis is included, which explain the high level of toxoplasmosis seroprevalence, in these categories; this can reflect the reality about toxoplasmosis seroprevalence among pregnant women in this area.

A study performed in France and published by Berger [30] showed a linear increase with age,

patients under 20 years, their seroprevalence was 31% and it was 58.2% among those over 30 years. This correlation may be explained by the increased duration of risk of exposure to other risk factors with age, which highlights the importance of educating young women of childbearing age about risk of toxoplasmic infection. According to Remington et al. [3] a positive relation between increasing age and seroprevalence of *T. gondii* has been already established, due to previous longer exposure periods to the parasite. Such patients may retain a steady level of anti-*Toxoplasma* IgG in serum for years.

Globally, in pregnant women, the seroprevalence of *T. gondii* infection ranges from 14% to 77% [2]. It is then critical to determine the prevalence of the disease among pregnant women to be able to apply the suitable primary attention measures to susceptible women, and to administrate adequate therapies able to reduce fetal sequelae [31].

Generally, variations in seropositivity results can occur because of different diagnostic methods on diverse patient categories with variable obstetric history. Several commercial enzyme immunoassays are currently available to detect anti-*T. gondii* antibodies [32]. Since the outcome of epidemiological studies is determined by the reliability of diagnostic tests applied, their validation is important to correctly establish prevalence [33].

The information on diagnostic methods may be provided according to the patient's background [34]. It is well known that ELISA is a fast, easy and commonly used screening method for the detection of toxoplasmosis. However, this technique exhibits some drawbacks such as false-positive and false-negative results, or the variable stability of immunoglobulins recognized by antigens [34]. The suitable selection of antigens may increase the accuracy of the tests, increasing their sensitivity and decreasing costs [35].

Currently, the capacity to identify *T. gondii* infection remains based on serological assay detection of IgM, IgG, and IgA levels. IgMs primarily appear, usually one week after the infection. Their levels rise to a peak after 1–3 months. A slow decrease then occurs over the next nine months until the negativization [36]. Toxoplasmic IgG is detected after two weeks of infection with a peak three months later. Therefore, it remains at a plateau level during six months. After one year, it starts to slowly decrease. The kinetic of

IgA production seems similar to those of IgM. In fact, the peak of IgA is observed later than IgM. In addition, the IgA antibodies persist over three or four 4 months through the infection [37].

Moreover, many risk factors, such as cat contact, handling or eating raw or undercooked meat and drinking unfiltered water are known to have an influence on *T. gondii* transmission [3]. In Algeria generally, and in the study region specifically, culinary culture is a North African Maghreb culture, where all dishes with or without meat are well cooked. Medea is a rural area; people rely heavily on agriculture and livestock to meet their daily needs. Cats are present; they are still used as rodents' hunters in the houses. Exposure to cats could be the principle factor inducing human infection, and seropositivity.

The climate plays also a role in determining the frequency of toxoplasmosis, there are differences among regions belonging to the same climatic region [34]. It is clear that other factors are involved in the contamination and prevalence of this parasitic pathology. The control of such risk factors may help to manage the disease.

In conclusion, more than one thousand pregnant women were analyzed and a quarter of them was found seropositive, exhibiting an IgG+ profile. Toxoplasmosis prevalence's were highest in 15–20 and 20–25 age categories.

A long-term prevention program is needed. In addition, required strategies should be focused on the serodiagnosis of toxoplasmosis, especially in pregnant women. More serological studies are necessary and further identification and molecular genotyping of the circulating *Toxoplasma* strains in this area would be epidemiologically very useful.

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