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### RESEARCH ARTICLE

#### SEROPREVALENCE OF TOXOPLASMOSIS IN PREGNANT WOMEN AT AVICENNA MILITARY HOSPITAL IN MARRAKESH

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#### Abstract

Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* which can have serious consequences for the fetus in case of pergravidicseroconversion. The present work is analytical and descriptive complementary studies: prospective (1-09-2015 to 31-12-2015) and a retrospective study (01-01-2010 to 31-12-2014) performed in the service of Parasitology - Mycology Marrakesh. The seroprevalence of pregnant women immunized against toxoplasmosis in our study is 35%, including women from rural and illiterate are proven to be the most immune. The main risk factors for disease transmission are the lack of knowledge about toxoplasmosis, the modes of transmission and means of prevention, and the low level of hygiene. We also raised a blatant lack of regular serological monitoring in seronegative pregnant women who received only one serological test in 66%. The present study confirmed that more than half of pregnant women are not immunized against *Toxoplasma gondii* and deserve monthly monitoring for early detection of possible seroconversion and implement effective therapeutic management.

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#### Introduction:-

Toxoplasmosis is an anthroozoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite that infects most species of warm-blooded animals, including humans .[1]

Toxoplasmosis is generally benign in the immunocompetent, but it can be formidable in the fetus and immunocompromised.

The objective is to evaluate the seroprevalence of toxoplasmosis in pregnant women at the military hospital Avicenna of Marrakesh and to approach the perception of this parasitosis by the pregnant ones by studying the risk factors predisposing to the development of toxoplasmosis

#### Methodology:-

These are two complementary analytical and descriptive studies: A retrospective over a period of 5 years from 2010 to 2014 and a cross-sectional epidemiological field study over a period of 4 months (1-9-2015 to 31-12-2015) whose

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serological examinations were carried out at the laboratory of parasitology -Mycology of the military hospital Avicenna of Marrakesh.

### **Study Population**

110 pregnant women living in the city of Marrakesh and the surrounding area were the subject of our prospective study and 4591 patients for our statistical study.

### **Procedure**

These are two studies at the Military Hospital Avicenna of Marrakesh: a retrospective over a period of 5 years and to complete the information on pregnant women, we conducted a field study over a period of 4 months specifying the demographic-sociocultural and educational data.

The study population comprises 4701 pregnant women. For this, we used the exploitation of medical records, registers and databases and interviewed participants using a pre-established form record. Women whose files were incomplete and who did not express their consent were excluded.

### **Prospective study**

To carry out our study, an exploitation sheet was prepared to gather the necessary information from the pregnant women who took their blood samples at the parasitology-mycology analysis laboratory in Marrakesh.

### **The diagnostic procedure followed**

The blood samples were taken on a dry tube and then centrifuged at 3000 rpm for about 10 minutes, and the serum was separated and aliquoted.

### **Etude des anticorps IgG Anti-Toxoplasma gondii**

#### **IgG Anti-Toxoplasma Gondii Antibody Study**

The detection and titration of anti-T. gondii IgG antibodies in human serum were made by a solid-phase immunoenzymatic method called indirect ELISA technique (PlateliaTMToxo IgG test) by the Evolis Twin plus (Bio-RAD).

T.gondii antigen is used to sensitize the microplate. A monoclonal antibody labeled with peroxidase and directed against human gamma chains (anti-IgG) is used as a conjugate. The antibody concentration in the sample is determined from a calibration curve.

1. A titer greater than or equal to 9 IU / ml is considered a positive
2. A titer strictly less than 6 IU / ml is considered negative
3. And a titer greater than or equal to 6 IU / ml and strictly less than 9 IU / ml is considered doubtful.

### **Detection of IgM Anti-Toxoplasma Gondii Antibodies**

Detection and titration of anti-T. gondii IgM antibodies in human serum were performed by an immunoenzymatic method with solid-phase IgM immunocapture

Anti-human  $\mu$  chain antibodies are used to sensitize the microplate. A mixture of T.gondii antigen and peroxidase labeled anti-antigen T.

Gondii monoclonal antibody is used as a conjugate.

The results for a given sample are expressed as a ratio

1. The ratio greater than or equal to 1.00 = result is positive.
2. The ratio strictly lower than 0.80 = result is negative.
3. A ratio greater than or equal to 0.80 and strictly less than 1.00 = result is doubtful

This result must be confirmed by a new sample taken at least three weeks after the date of the first examination

### **Detection of the avidity of IgG antibodies against Toxoplasma gondii**

This test is performed using the PlatellaTM TOXO IgG AVIDITY kit used with the PlateliaTM TOXO IgG kit.

The principle of this technique is based on measuring the avidity of IgG antibodies for *T.gondii* antigen. Using an agent dissociating the antigen/antibody binding in parallel with the usual IgG antibody measurement technique makes it possible to compare the optical density (OD) obtained after the dissociating agent has acted with the OD obtained without the action of this agent.

Low avidity is the case where the antigen/antibody binding is dissociated easily. Conversely, we speak of high avidity in the case where the antigen/antibody binding is weakly dissociated. Only samples with a titer greater than or equal to 9 IU / ml with the Platelia TM TOXO IgG test can be tested

Then the greed index is calculated by the automaton:

An avidity index of less than 0.40 is rather in favor of a recent primary infection of fewer than 20 weeks. However, such a result does not confirm this diagnosis with certainty.

An AI greater than or equal to 0.40 and strictly less than 0.50 corresponds to an intermediate greed zone

An avidity index greater than or equal to 0.50 is rather in favor of an old infection of more than 20 weeks. However, such a result does not allow excluding with certainty a recent primary infection of fewer than 20 weeks

In case of suspicion of recent infection or the case of an intermediate avidity index, a second sampling may be performed at two weeks intervals.

### **Retrospective study**

This is a study that we carried out by exploiting the available medical records, registers, and databases of the laboratory of parasitology-mycology in Marrakesh.

### **Inclusion criteria:**

Pregnant women of any term of pregnancy who have applied for serology for toxoplasmosis at the Military Hospital in Marrakesh were selected and their consent was obtained. No other special criteria were taken into consideration.

### **Exclusion criteria**

Pregnant women who did not give their favorable opinion to participate and be included in the study, and patients whose files are incomplete or unavailable, were excluded.

### **Statistical tools**

Statistical analyzes were entered on Microsoft Office Excel 2013 and exploited using SPSS version 11 software. These are the KHI two tests.

### **Results:-**

The test results from 4591 pregnant women from 2010 to September 2014 were filed and 110 pregnant women from September 2015 to December 2015.

The vast majority of women, 63%, had only had one serology. The prevalence found is 33%.

We carried out a prospective study specifying demographic, socio-cultural, and educational data to complete the information on pregnant women.

The average age of our patients was 27 years, with extremes ranging from 17 to 45 years, and 58% of pregnant women were between 20 and 30 years old.

Among all pregnant women, 42% were primiparous, while 21% of women were multiparous.

The bivariate analysis of risk factors revealed a statistically significant association between a positive serology and some risk factors, particularly geographical origin, socio-economic level, and certain eating habits. Thus in our study, 88 women were of urban origin and 22 of rural origin. Among the 22 pregnant women of rural origin, 59.09% are immunized, while 43, 18% of women of urban origin are (**Table 1**).

The economic level and the level of education significantly influenced the toxoplasma seroprevalence with a p-value of 0.0001 and 0.01, respectively. (**Table 2**).

### **Discussion:-**

According to the literature data, the results of epidemiological studies in humans differ from one study to another. In fact, the prevalence varies from one geographical region to another and within the same population.

It should also be remembered that the sampling methods used, the diagnostic techniques, and their proposed thresholds of specificity are of great variability.

Thus, the heterogeneous nature of the protocols used and the populations surveyed suggest caution in interpreting and comparing serology results between studies.

In our country, few studies have been published on this topic. The main goal was to evaluate the toxoplasmic seroprevalence in pregnant women in the city of Marrakesh and try to establish a causal link between this prevalence and some risk factors by our prospective study.

### **The seroprevalence of toxoplasmosis**

Comparison of our seroprevalence results with those of other studies.

The prevalence of toxoplasmosis varies widely across geographical areas. These variations may have several explanations, including weather conditions, eating habits, and the level of hygiene.

The prevalence of toxoplasmosis found in our study is 35%. This value remains slightly lower than that found by the other studies; this can be explained by the very different sampling and the improvement of the level of hygiene.

The El Moussaoui study in the coastal city of Tetouan[2] showed the influence of the ocean climate on the increase in the number of cases of toxoplasmosis. The same observation was made earlier by Nejmi and Alami[3], following the comparison of the prevalence of toxoplasmosis in coastal towns and cities far from the sea.

Contrary to our study, which is located in Marrakesh, city of the interior, those of Mekouar, [4] El Mansouri and al [2], Errefaiy[5], were realized in cities of the sea respectively Rabat, Essaouira where the degree of hygrometry is higher. Only the results of Nejmi and Alami corroborate those of our study.

Although climate plays a key role in determining the frequency of toxoplasmosis, we notice a difference between different cities belonging to the same climatic region.

### **Risk factors**

Geo-climatic factors are not enough to explain the prevalence of this parasitosis in a population, so other factors intervene in the contamination. Their control helps prevent the disease, in this case, the socio-economic, socio-cultural level, educational and eating habits.

### **Sociodemographic factors**

#### **Age**

Our study found a statistically significant positive correlation between age and seroprevalence with a  $P = 0.045$ . The figures are between 46.8% and 47.2% respectively in the age group 20- 30 years and 30-40 years, and those over 40, 50% were immunized

Seroprevalence of toxoplasmosis increases with age, according to several studies. An Indian study published by Singh showed that there is a linear increase with age. Between 20-25 years, the seroprevalence was 38.5%, and it was 77.8% among those between 35-39 years [6]. Similarly, in France, the prevalence of toxoplasmosis among pregnant women of French nationality increased with age. [7] Studies were done in Turkey [8], and Saudi Arabia [9] joined this.

El Mansouri has also reported an increase in seroprevalence with age. Thus, among pregnant women under the age of 20, the prevalence was 32.4%, while among those over 40 years of age was 63.8% [2]

**Place of residence**

Bivariate analysis of risk factors revealed a statistically significant association between positive serology and some risk factors; geographical origin, socio-economic level, and some eating habits. Thus in our study, 88 women were of urban origin and 22 of rural origin. Of the 22 pregnant women of rural origin, 59.09% are immunized, while only 43.18% of urban women are immunized with a  $P = 0.03$ .

These ties in with studies conducted in Saudi Arabia [9] and Egypt [10], where they found a significant difference in seroprevalence between women from the Middle East. Rural areas and urban areas.

**Parity**

On the other hand, in our study, parity was not identified as a predictor of toxoplasmic immunization ( $p = 0.2$ ). We could not compare this data with similar studies for lack of information.

**Economic level**

We tried to correlate seroprevalence with socioeconomic level. After data analysis, we found that quality of life and hygiene measures have a statistically significant influence on the serological status of recruited women.

We found that 70.05% of economic-level women are seropositive of toxoplasmosis and that 42.35% are middle-level only 3.75% higher-level ( $p = 0.0001$ ).

This is in line with the Egyptian study, which concluded that 56.96% of seropositive people are low economic level, while the middle and high level represent 40.8% and 4.2%, respectively. [10] Similar results are reported by a study in India, which showed that seroprevalence is high among a group of women with low socio-economic status (33%) compared to the high-level group (22%) [11]

**Level of study**

In this study, we noted that the level of education also plays a role in the immune status of pregnant women. According to the analysis, the difference was significant ( $P = 0.01$ ), ranging from 33.33% for women with a university-level to 78.57% for illiterate women.

The same findings were made in Safi-Essaouira [5] and Rabat [2], and Saudi Arabia [9]. A study in Brazil concluded that a higher level of education is a protective factor against *T. gondii* infection [12]

**Behavioral factors****Eating habits**

Regarding the relationship between some eating habits of pregnant women and their immune status, we noted that these factors are highly associated with parasite transmission; the consumption of poorly cooked meat plays a role in the transmission of the disease. In fact, half of the seropositive women consume undercooked meat (50%). This risk factor comes up most often in the different studies.

The study carried out in the Safi-Essaouira region is consistent with our results, unlike the results of El Mansouri, which did not find undercooked meat as a potential risk of acquiring toxoplasmic antibodies [2].

Kapperud's study found that eating undercooked or undercooked meat was associated with a high risk of infection [13] as well as Buffolano, who noted that the consumption of smoked pork or raw meat, at least once a month, multiplied by three the risk of toxoplasmic infection [14].

Regarding the relationship between cheese or raw milk consumption and seroprevalence, we found a slight increase in seroprevalence in women who do not eat cheese or raw milk compared to those who consume it. Regarding the relationship between cheese or raw milk consumption and seroprevalence, we found a slight increase in seroprevalence in women who do not eat cheese or raw milk compared to those who consume it. (46.91% vs 44.82%) This difference is not statistically significant ( $p = 0.1$ ) on toxoplasmic seroprevalence.

**Contact with the cat**

The presence of a cat in the home is also a factor associated with the spread of toxoplasmosis. This ties in with Errifaiy's study in the Safi-Essaouira region. [5] The consumption of undercooked meat and the presence of cats in

the outbreak appear as potential risks of acquiring anti-toxoplasma antibodies. Similar results were reported in the Algerian study by Chouchane and al 2005 [15]. Also, studies in France have retained the possession of a cat as a significant risk factor [16]. A prospective Norwegian study of control cases has found that cleaning cat litter is associated with a high risk of toxoplasmic infection [17].

In other epidemiological studies, contact with the cat is not considered to be an important risk factor. Cook and al; reports on a multicentre control case study, which included 252 cases of seroconversions or recent toxoplasmic infections, that contact with cats is not a risk factor for infection [18]. This was also found in the Moroccan study of El Mansouri [2]

### **Contact with the earth**

The contact with the soil appears in our study as a potential risk of acquiring anti-toxoplasmic antibodies ( $p = 0.034$ ). In fact, 68.42% of candidates who contact the earth have been immune to parasitosis. Same result in the Safi-Essaouira region. [5]

The contact with the soil appears in our study as a potential risk of acquiring anti-toxoplasmic antibodies ( $p=0.034$ ). In fact, 68.42% of candidates who contact the earth have been immune to parasitosis same result in the safi-Essaouira region.

Other studies reveal the causal link between contact with the soil and toxoplasmic infection in Egypt [10] and Iran [19]. El Mansouri found that only 54% of immunized women have permanent contact with the earth, while 44.6% of this same women category does not have this contact. This difference remains statistically significant [2]

### **Level of knowledge**

The lack of knowledge about toxoplasmosis remains the most important factor in the immunization of Moroccans. Indeed, only 34 women (31%) know about toxoplasmosis in this work, and 76 (69%) have never heard of the disease. In our series, 60.52% of women who have never heard of toxoplasmosis are HIV-positive, while 14.7% of immune women have some level of knowledge with a  $p = 0.003$ .

Among this category 38%, their source of information is their doctor and 11% their entourage. This shows some failure of communication and awareness on the part of health professionals.

### **Serological follow-up**

On the other hand, our study showed that 63% of all women presented only one serology application for toxoplasmosis, which is insufficient (out of a total of 4591), 98% did not perform a pre-conception serology, and 61% tested for the first time in the first quarter and 28% in the second quarter. This shows the absence of toxoplasmic serology in the management of pregnancy and the legal vacuum that exists at this level.

The most important finding, which deserves a great deal of attention, is that this study has focused on a failure to monitor toxoplasmosis and monitor seronegative pregnant women. The same observation is made in the Safi-Essaouira region [5] and Mohammed V Rabat's Military Hospital.

In France, toxoplasmosis has been the subject of mandatory prenatal screening programs since late 1970, as part of a policy currently governed by Articles L. 2122-1 to 2122-5 of the Public Health Code (CSP), within the framework of a policy currently governed by Articles L. 2122-1 to 2122-5 of the Public Health Code (CSP), Decree No. 92-143 of 14 February 1992 on mandatory prenatal, pre- postnatal by setting the content. The serological screening of toxoplasmosis during pregnancy is currently part of an algorithm requiring serologies to be performed regularly and every month during pregnancy and at birth in seronegative pregnant women so as not to disregard late seroconversion. In Morocco, the decree of the Minister of Health No. 2519-05 of 30 Chaabane 1426 (September 5, 2005) sets the conditions and episodes of medical monitoring of pregnancy, childbirth, and its aftermath. Indeed, Article 4 of this decree sets the additional examinations that must be prescribed during a consultation, including toxoplasmosis serology. Still, it does not set the terms of the follow-up. In addition, no text obliges systematic screening for toxoplasmosis before marriage; it is a void that strict laws must fill.

**Conclusion:-**

Toxoplasmosis is a parasitosis most often benign in the immunocompetent subject. However, a maternal toxoplasmic primary infection during pregnancy can be very dangerous for the unborn child.

The data obtained from this work allowed us to better understand toxoplasmosis in the Marrakesh region in terms of seroprevalence in pregnant women and to identify the main risk factors related to the contamination.

**What is already known on this topic**

1. Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*, which can have serious consequences for the fetus in case of pergravidicseroconversion
2. A major failure in serological monitoring and surveillance of seronegative pregnant women
3. Informing expectant mothers plays a key role in preventing these infections

**What this study adds**

1. The experience of the Avicenna military hospital in Marrakesh in the care of pregnant women seronegative of Toxoplasmosis
2. This experience will help to emphasize collaboration between health professionals to educate pregnant women and systematically determine their immune status.
3. Regular monitoring of non-immunized pregnant women to detect possible seroconversion and manage it as early as possible, thus avoiding the worst in unborn children.

**Conflicts of interest**

No conflict of interest.

**Authors' contributions**

All the authors contributed to the conduct of this work. All authors also declare that they have read and approved the final version of the manuscript

**The table**

**Table 1:-** Toxoplasmosis seroprevalence distribution of pregnant women according to their economic level and level of education.

**Table 2:-** Toxoplasmosis seroprevalence distribution of pregnant women according to their eating habits.

**Table 1:-**

		Number	Toxoplasmosis Seropositive (IgG)	%	P-value
Economic level	High	8	3	3,75	<b>0,0001</b>
	Way	85	36	42,35	
	Low	17	12	70,05	
level of study	Illiterate	14	11	78,57	<b>0,01</b>
	Primary	13	6	46,15	
	Middle School	30	13	43,33	
	High school	23	11	47,82	
	University	30	10	33,33	

**Table 2:-**

		Number	Toxoplasmosis Seropositive (IgG)	%	P-value
Consumption of undercooked meat	Yes	4	2	50	<b>0,05</b>
	No	106	49	46,22	
Consumption of cheese or raw milk	yes	29	13	44,82	<b>0,1</b>
	No	81	38	46,91	

Contact with the cat	yes	17	12	70,58	<b>0,029</b>
	No	93	39	41,19	
Contact with the land	yes	19	13	68,42	<b>0,034</b>
	No	91	38	41,75	
Washing vegetables and fruits with bleach	Yes	37	17	46,57	<b>0,09</b>
	No	73	34	45,94	

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