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Toxoplasmosis and Pregnancy: Prevalence and Immune Profile among Aboriginal Pregnant Women in Two Departments of Congo

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Abstract

Introduction: Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii*, which can affect pregnant women with varying degrees of severity. This parasitosis has not been studied in native pregnant women in our country. The aim of this study was therefore, to investigate the immune profile of aboriginal pregnant women in the Republic of the Congo. **Patient materials and methods:** An analytical cross-sectional study was conducted in the departments of Lékoumou, Sangha and Brazzaville from 1 January to 30 September 2017. It involved aboriginal and Bantu pregnant women residing in the aforementioned departments. The ELISA technique was used for toxoplasma serology. Immunoglobulin G and M testing was combined with an immunoglobulin G avidity test. The statistical test was significant when $p < 0.05$. **Results:** We enrolled 97 aboriginals and 97 Bantus. The mean age of aboriginal and Bantu pregnant women was similar (27.7 years ± 7.9 vs 27.2 years ± 6.9). The seroprevalence of toxoplasmosis between the two groups was not statistically different (23.1% vs 30.9%; $p = 0.07$). The presence of immunoglobulins was Ig G = 20%, Ig M = 1% in the aboriginal vs Ig G = 7%, Ig M = 0 in the Bantus in the first trimester; Ig G = 33.3%, Ig M = 0% vs Ig G = 58.3%, Ig M = 1% in the 2nd trimester; Ig G = 46.7%, Ig M = 0% vs Ig G = 35%, Ig M = 0% in the 3rd trimester. The serological status of aboriginal pregnant women did not differ from that of Bantus: non-immune (53.6% vs 38.1); immune (30.9% vs 50.5); acute infection (15.4% vs 11.3). **Conclusion:** Toxoplasmosis is a reality in aboriginal pregnant women. Studies are needed to identify the risk factors associated with the occurrence of this parasitosis in native pregnant women.

Keywords

Immune Profile, Toxoplasmosis, Pregnant Women, Aboriginal People, Bantu, Republic of the Congo

1. Introduction

Toxoplasmosis is a cosmopolitan parasitic infection caused by *Toxoplasma gondii*, which infects mammals and humans [1] [2]. Maternal contamination is via the oral route from oocysts and cysts contained in raw or undercooked meat, inadequately cleaned fruit and vegetables and water containing Toxoplasma cysts [3]. Toxoplasmosis is a public health problem affecting nearly 211 million pregnant women a year, each of whom is at risk of complications such as miscarriage, foetal death, neurological and neurocognitive deficits, chorioretinitis and disability in the child [4]. Poor economic conditions, low levels of development, living in rural or urban areas and the presence of cats in the environment are all factors that play a part in the occurrence of the disease.

Foetal involvement indicates transplacental passage of the parasite. However, since parasitosis is generally asymptomatic, blood tests are needed to detect the disease using molecular biology and serology, especially in pregnant women [5]. During pregnancy, diagnosis is based on the detection of specific G and M type immunoglobulins (Ig) [6]-[9]. The seroprevalence of toxoplasmosis in pregnant women varies between 70 - 80% from one country to another and from one region to another [10]-[13]. Within the same country, seroprevalence may vary between urban and rural areas, especially in developing countries where rural areas do not always have health systems capable of managing pregnancy in accordance with the recommendations [14]. It is in these mostly disadvantaged rural areas that aboriginal peoples are found [3]. This is the case in the Republic of the Congo, where the aboriginal peoples are located in rural areas [15] [16]. Some studies have been carried out on toxoplasmosis in pregnant women of aboriginal and non-aboriginal peoples, showing on the one hand that the prevalence of toxoplasmosis is higher among aboriginal than among non-aboriginal, and on the other hand, that it remains the same. Studies carried out in Quebec and Indonesia reported seroprevalence of 50% and 10.9%, respectively among pregnant aboriginal [17] [18]. Studies carried out in Brazil revealed seroprevalences of between 14% and 30% [19]. In the Central African sub-region, particularly the Democratic Republic of the Congo and Congo, seroprevalence varies between 50 and 80% among Bantus [3] [6] [7] [15] [16]. However, toxoplasmosis has not been studied in aboriginal people, whose lifestyle could be an important determinant in its transmission in aboriginal pregnant women [20]. We therefore conducted this study to gain a better understanding of toxoplasmosis in aboriginal pregnant women, with the general aim of studying the prevalence and immune profile of toxoplasmosis in aboriginal pregnant women in the Republic of the Congo.

2. Patients, Material and Method

This was a cross-sectional analytical study, running from 1 January to 30 September 2017.

The study took place in the Republic of the Congo in two departments: Lékoumou, and Sangha. Samples were analysed in the Parasitology-Mycology Department of Brazzaville University Hospital Centre.

The Department of Lékoumou is located in the south of the country and covers an area of 20,950 km^2. It is located 317 km from Brazzaville, and is accessible by land and air. It has 5 districts and 71,248 inhabitants, including 11,456 aboriginal people.

The Department of Sangha is located in the north of the country, with a surface area of 12,266 km^2, 643 km from Brazzaville. It has 6 districts and 205,986 inhabitants, including 7885 aboriginal people. The study took place mainly in the Pokola urban community, which has a population of 1663, including 14,573 Bantus and 1490 aboriginal people.

Brazzaville is a department in the south of the country. It is the political and administrative capital of the Republic of the Congo, covering an area of 2639 km^2. It has a University Hospital Centre where the serological analyses were carried out in the Parasitology-Mycology Department.

The study population consisted of two groups: group 1, aboriginal pregnant women, and group 2, Bantu pregnant women. For each aboriginal pregnant woman, we associated a Bantu pregnant woman.

The aboriginal and Bantu pregnant women were recruited from the health centres providing antenatal consultations in the departments in which the aboriginal pregnant women lived. Consenting pregnant women, regardless of their gestational age, residing in the above-mentioned departments were included in the study. Pregnant women who did not live in the above-mentioned départements but were staying there, non-consenting pregnant women living in the départements and pregnant women whose sera had been haemolyzed after centrifugation were excluded. Each pregnant woman was investigated twice (epidemiologically and biologically) using a survey form.

The interviews were conducted in French, Lingala, Kituba and Bendjélé (the local dialect) with the assistance of an interpreter. Each pregnant woman was given an identification code and number to ensure confidentiality and anonymity.

The sample size was calculated according to the schlesselman formula considering P0 = proportion of exposed in group 1 = 31% = 0.3 [15] and P1 = proportion of exposed in group 2 = 60% = 0.6 [16]. $Z\alpha = 1.65$; $Z\beta = 1.28$. Thus, the sample obtained was 47 gestating females as the minimum size allowing statistical exploitation.

The selected aboriginal and Bantu pregnant women had 5 ml of blood taken from the elbow in a dry tube and transported to Brazzaville, to the Parasitology-Mycology laboratory, in cold accumulators for analysis of toxoplasmosis serology for immunoglobulins G and M.

The sera were analysed using BioMérieux's mini-Vidas automated system for the detection of Immunoglobulin G and M. The advantage of this technique is that it limits the interference classically associated with the determination of Ig M (natural Ig M, rheumatoid factors, anti-nuclear antibodies, Ig G/Ig M competition). After immunocapture, specific anti-toxoplasma Ig G and Ig M can be determined by immuno-enzymology using an alkaline phosphatase-labelled immunocomplex. Final detection of immunoglobulins is by ELFA (Enzyme Linked Fluorescence Assay) immunofluorescence. The Vidas system manages all the stages.

The Ig M results are obtained in the form of an index i which reflects the ratio between the fluorescent signal of the serum tested and the stored signal of the standard. Any value of $i < 0.55$ corresponds to a serum free of anti-toxoplasma Ig M; any value of $i > 0.65$ corresponds to a serum positive for specific Ig M.

IgG results are given in IU/ml. A titre of less than 4 indicates a serum free of anti-toxoplasma IgG. Any titre value above 8 indicates a positive serum for specific IgG.

A second sample was not taken when the immunoglobulin values were intermediate.

When at least one class of Ig G or Ig M corresponded to the threshold for the presence of immunoglobulins in the BioMérieux Vidas Toxo technique, the results were considered "positive".

The serological results were interpreted according to the following immunoglobulin Ig G and Ig M serological profiles:

- Non-immunised: Ig G−; Ig M−
- Immune: Ig G+; Ig M−
- Recent infection: Ig G+; Ig M− with an Ig G threshold greater than 300 IU/ml
- Active infection: Ig G−; Ig M+ or Ig G+; Ig M+

Ethical considerations

The study was authorised by the departmental directorates of the Ministry of Health and Population, and by the Ethics Committee of the Faculty of Health Sciences.

Statistical analysis

Statistical analysis of the database was carried out using IBM SPSS version 20 software. Qualitative variables were expressed as frequency and quantitative variables as mean ± standard deviation. The association between two variables was considered statistically significant when p value was <0.05.

3. Results

The socio-demographic characteristics and gestational age of aboriginal and Bantu pregnant women are given in Table 1.

The mean age of pregnant women was 27.7 ± 7.9 (14 - 41) in the aboriginal group and 27.2 ± 6.7 (15 - 40) in the Bantu group.

The majority of pregnant women were in the 20 - 25 age group, both Bantu (25.8%) and aboriginal (36.1%), with no significant difference (p = 0.304). Most

of the women in both groups were married (54.6% vs 88.6%). They were significantly unemployed in both groups; the majority of aboriginal pregnant women were farmers, whereas the Bantu women were vendors (p = 0.001).

Table 1. Socio-demographic characteristics and gestational age of aboriginals and Bantus pregnant women from 1 January to 30 September 2017.

	Aboriginal		Bantus		p
	n = 97	%	n = 97	%	
Tranche d'âge					0.304
14 - 19	17	17.5	22	22.7	
20 - 25	35	36.1	25	25.8	
26 - 31	25	25.8	21	21.6	
32 - 37	11	11.3	19	19.6	
38 - 43	9	9.3	10	10.3	
Marital status					0.138
Single	44	45.4	11	11.3	
Married	53	54.6	86	88.6	
Profession					**0.001**
Unemployed	51	52.6	49	50.6	
Civil servant	-	-	9	9.3	
Saleswoman	12	12.4	17	17.5	
Farmer	31	32.0	14	14.4	
Student	3	3.1	8	8.2	
Gestational age (trimester)					0.290
First	18	18.6	12	12.4	
Second	43	44.3	53	54.6	
Third	36	37.1	32	33.0	

In terms of gestational age, the second trimester of pregnancy was the most common age for both aboriginal and Bantu pregnant women, with no significant difference (p = 0.290).

The overall seroprevalence of toxoplasmosis was 54%, with 23.1% of aboriginal pregnant women versus 30.9% of Bantu pregnant women. The difference was not statistically significant (p = 0.07).

The toxoplasmosis serological status of aboriginal and Bantu pregnant women is given in Table 2. There was no significant difference between the toxoplasmosis serostatus of aboriginal and Bantu pregnant women.

Similarly, no profile was significantly more represented than the others. However, the majority of aboriginal pregnant women were not immune to toxoplasmosis (53.6%), whereas the Bantu pregnant women were (50.5%). *T. gondii* infection

was much more common in aboriginal pregnant women (14.4%) than in Bantu pregnant women (10.3%). One case of acute infection was found in both groups.

Table 2. Toxoplasmosis serological status of aboriginal and Bantus pregnant women from 1 January to 30 September 2017.

	Aboriginals		Bantus		p
	n = 97	%	n = 97	%	
					0.051
Not immune	52	**53.6**	37	38.1	
Immune	30	30.9	49	**50.5**	
Recent infection	14	14.4	10	10.3	
Acute infection	1	1.0	1	1.0	

Grouping by type of anti-toxoplasma immunoglobulin gave the following profiles:

- **In aboriginal pregnant women:**

  Ig G−/Ig M−: 53.6%

  Ig G+/Ig M−: 45.3%

  Ig G−/ Ig M+: 1.0%

- **In Bantu pregnant women:**

  Ig G−/Ig M−: 38.1%

  Ig G+/Ig M−: 60.8%

  Ig G−/Ig M+:1.0%

Ig M was equally prevalent in the aboriginal and Bantu populations, as shown in Figure 1. The frequency of Ig G was higher in Bantu pregnant women (61.9%) than in aboriginal women (44.3%).

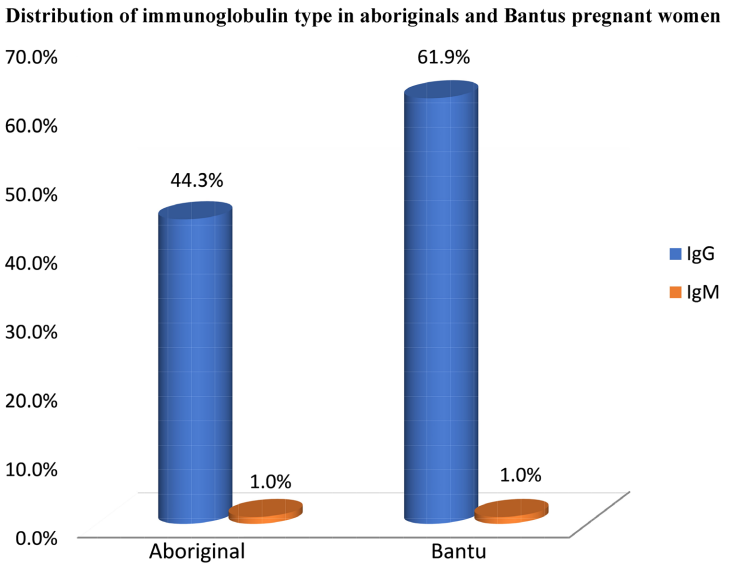

Figure 1. Distribution of immunoglobulin G and M in aboriginal and Bantu pregnant women from 1 January to 30 September 2017.

Figure 2 and Figure 3 show the distribution of Ig G and M according to gestational age in aboriginal and Bantu pregnant women.

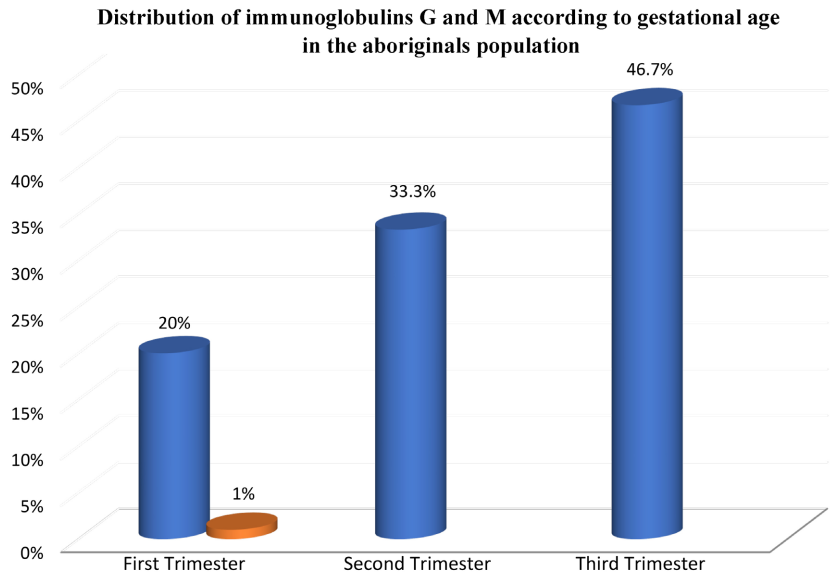

Figure 2. Type of immunoglobulin by gestational age in aboriginal pregnant women from 1 January to 30 September 2017.

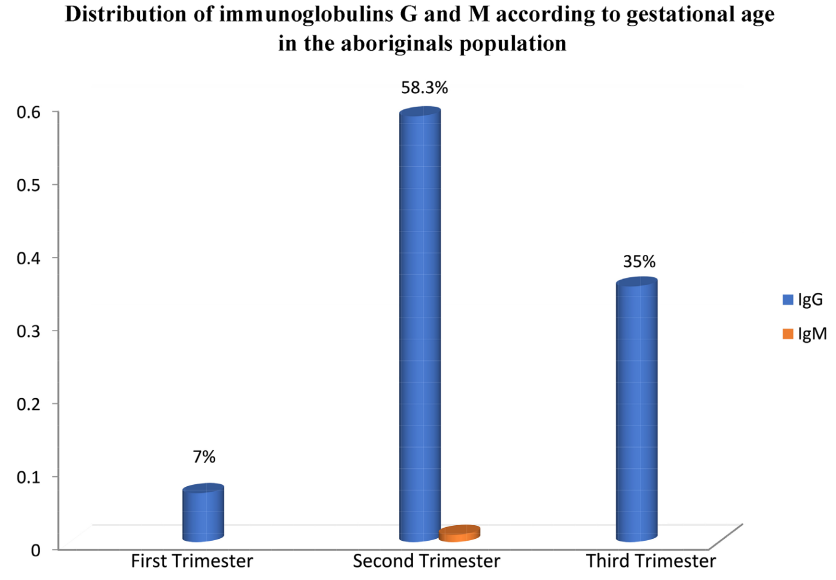

Figure 3. Type of immunoglobulin according to gestational age in Bantu pregnant women from 1 January to 30 September 2017.

The presence of Ig M was noted in the first trimester of pregnancy in aboriginal women and in the second trimester in pregnant Bantu women. The frequency of Ig G was higher in pregnant aboriginals in the third trimester, whereas it was higher in pregnant Bantus in the second trimester. There was also an increase in the frequency of Ig G according to the age of the pregnancy, from the first to the third trimester, in native pregnant women.

4. Discussion

The mean age of aboriginal pregnant women was 27.7 years compared with 27.2 years for Bantu pregnant women. There was no statistically significant difference in age between the two population groups. These results could be explained by the similarity of the average reproductive age [21] among women of childbearing age in the Congo, for both aboriginal and Bantu populations. In addition, the maximum reproductive age found in the Congo Demographic Health Survey was 24 years [22]. Furthermore, the average age of aboriginal pregnant women was similar to that of 27 years reported by Dwinata *et al.* in Indonesia [18].

The overall seroprevalence of toxoplasmosis in our study was 54%. These results may be explained by promiscuity, lack of hygiene, poor eating habits and, above all, ignorance of the disease and its mode of contamination among the aboriginal and Bantu populations, which would have allowed the population to be contaminated. This could also reflect the situation of toxoplasmosis in the general aboriginal population. As shown by Santos ALC *et al.* [23], who found a seroprevalence of toxoplasmosis of 66.9% in the aboriginal population of the Utiariti territory in Brazil The seroprevalence was 23.1% in aboriginal pregnant women compared with 30.9% in Bantu pregnant women. The difference observed was not statistically significant ($p = 0.07$). This overall seroprevalence is lower than that found by Yobi in Kinshasa, which was 80.3% [15]. This difference could be explained by the size of the sample, which is 7 times larger than ours. But it could also be due to the presence of cats in urban areas, combined with climatic conditions and humidity favourable to the development of *T. gondii*. Furthermore, the seroprevalence of toxoplasmosis in the aboriginal population in our study was higher than that found by Dwinata in Indonesia, where seroprevalence was 10.9% in a sample of 330 pregnant women [18]. The difference may be explained by the socio-economic level of the town where the study was carried out and the climatic conditions. This difference can be explained by the socio-economic level of the town where the study was carried out and the climatic conditions.

Ndassebe [17] in Quebec found a seroprevalence of 50% in Inuit aboriginal pregnant women. This is higher than our result. The variability of toxoplasmosis seroprevalence in different regions and the duration of the study (9 years) are two factors that could explain the difference.

One point to note about toxoplasmosis among aboriginal peoples is the difficulty of access and the remoteness of these peoples from major cities, which makes it fairly rare to obtain data on toxoplasmosis. As a result, comparisons with results obtained in other African studies cannot easily be made. This scarcity of data means that we are not in a position to draw up comparative analyses that could provide hypotheses for the African region.

In our study, the immune profile was established according to the presence or absence of anti-toxoplasma immunoglobulin G and/or M.

Non-immunised status was defined by the absence of anti-toxoplasma immunoglobulins G and M. This absence of anti-toxoplasma immunoglobulins was

noted in 53.6% of cases in aboriginal pregnant women compared with 38.1%. However, the difference observed was not statistically significant. These results could be explained by the fact that the pregnant women lived in rural, quasi-forest areas, where the presence of the cat was not effective among aboriginals in the Congo. We could also add to this the absence of drinking water for cleaning certain vegetables and raw vegetables consumed by the aboriginal populations, and the absence of modern toilets, which is a factor in the dissemination of *Toxoplasma gondii* oocysts contained in the faeces of contaminated people [14]. Bantu pregnant women, who live in environments that often contain cats with the potential for contamination of food and drinking water by *Toxoplasma gondii* cysts, could explain why they are in greater contact with the cysts and develop immunity to toxoplasmosis.

On the other hand, the presence of anti-toxoplasma immunoglobulins was noted in 30.9% of cases in aboriginal pregnant women compared with 50.5% in Bantu pregnant women, with a statistically significant difference. The high seropositivity in Bantu pregnant women can be explained by frequent contact with cats and the consumption of cold meats. Poor cooking of food and frequent contact with the ground are also significant factors.

Aboriginal pregnant women had a prevalence of recent infection of 14.1% compared with 10.3% of Bantu pregnant women, and the prevalence of acute Ig M infection was 1% in both aboriginal and Bantu pregnant women. The difference observed was not statistically significant. However, it should be noted that the presence of Ig M, indicative of an acute infection, may in some cases be due to the persistence of these immunoglobulins beyond 9 months [6] [7]. The presence of toxoplasma infection shows that the risk of congenital toxoplasmosis is present in both aboriginal and Bantu pregnant women. This represents a real danger for the foetus, although the seriousness of this risk depends on the term of pregnancy of the pregnant woman at the time of infection [24]. These results are similar to those of Ogouyemi *et al.* [12] in Benin (1.1%) and El Mansouri in Rabat, Morocco (1.8%) [25]. Pamatika CM *et al.* in Bossembelé in the Central African Republic also found a high frequency of Ig M toxoplasma infection [26]. However, our results are superior to those of Messerer in Algeria [11] who found a 2.6% incidence of progressive Ig M infection in pregnant Algerians. LO *et al.* [13] in Dakar found a prevalence, lower than ours, of 0.34% of progressive infection. These results clearly show that active toxoplasmosis exists, but its complications depend on the stage of pregnancy. However, they should be prevented in both aboriginal pregnant women in the first and second trimesters and in Bantu pregnant women.

5. Conclusion

Toxoplasmosis is a reality in both Bantu and aboriginal pregnant women. It is a public health problem. The predominant serological status was that of absence of immunisation. The existence of active infection highlights the importance of carrying out prenatal consultations of good biological quality in order to improve

screening for this parasitosis in pregnant women.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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