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Current Status of Congenital Toxoplasmosis in Brazzaville, Republic of the Congo

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Abstract

Toxoplasmosis is a parasitic infection caused by *Toxoplasma gondii*, which is transmitted by contact with animals carrying the parasite, or by eating contaminated food such as undercooked meat or raw fruit and vegetables. Toxoplasmosis is often benign, but can be serious in pregnant women. In order to assess the prevalence of congenital toxoplasmosis in Brazzaville, Republic of Congo, 464 women aged between 16 and 42 were surveyed in 3 health facilities, including one public hospital (Blanche Gomez) and two private medical centers (OCH medical and ultrasound practice and Luiz laboratory). A total of 1868 samples were taken and analyzed using the ELFA method on mini vidas, an enzyme-linked immunosorbent assay for fluorescence detection. The results showed that out of 1392 samples obtained from 464 pregnant women, 693 samples from 231 pregnant women were positive, *i.e.* a frequency of 49.78%. Analysis of the immunological profile showed 48.70% IgG and 1.07% IgG associated with IgM. The different frequencies varied from one health facility to another: 53.96% at Blanche Gomez Hospital, 53.76% at Luiz Laboratory and 31% at OCH. Seroprevalence by age group showed 57.14% in pregnant women aged 16 to 25, 52.77% in pregnant women aged 26 to 35 and 21.62% in pregnant women aged 36 to 42. A frequency of 52.65% was observed in pregnant women in contact with soil and 49.78% in pregnant women consuming vegetables, while 66.66% of women in contact with cats were positive for toxoplasmosis. IgG avidity assays showed a frequency of 74% for high avidity and 3.75% for low avidity. This study enabled us to diagnose 9 cases of seroconversion, including 6 cases of congenital toxoplasmosis. Among the 6 cases, 4 children showed toxoplasmosis with sub-clinical signs of IgG persistence at one year of

age, including one with thrombocytopenia associated with jaundice and 2 others with hepatomegaly associated with hypertransaminase and hyperbilirubinemia. The maternal-fetal transmission rate was 66.66%, with an incidence of congenital toxoplasmosis of 1.29 per 464 births.In conclusion, the results of this study show the impact of toxoplasmosis on public health, and demonstrate the importance of quarterly pregnancy monitoring and compliance with hygienic and dietary measures, which are very important for prevention, early detection and management of contaminated pregnant women during prenatal monitoring, thereby reducing the consequences for newborns.

Keywords

Congenital Toxoplasmosis, Pregnant Women, Frequency, Incidence, Brazzaville

1. Introduction

Toxoplasmosis is a major public health problem. It is transmitted from mother to fetus via the transplacental route when acquired during pregnancy. Maternal-fetal transmission increases with gestational age, and the mode of contamination is influenced by lifestyle, the presence of felids in the environment, and the different dietary habits of each individual.

This condition, contracted in utero, can lead to potentially severe clinical sequelae in the fetus, such as cerebral complications (intracranial calcifications; hydrocephalus), ocular complications (chorioretinitis; optic atrophy) or visceral complications (hepatomegaly; jaundice) [1].

The overall prevalence of Congenital Toxoplasmosis observed in France is around 2.6 per 10,000 births, and the prevalence of Congenital Toxoplasmosis cases diagnosed at birth is 1.35 per 10,000 births [2].

Worldwide, an estimated 3.5 billion people are affected by toxoplasmosis, the prevalence of which depends on geographical, climatic and socioeconomic conditions [2]-[4].

However, the size of the cat population, with infection rates ranging from 5.5% to 86%, the survival of oocysts in humid tropical climatic conditions, dietary habits and hygiene conditions, mean that human prevalence is highest in Africa and Latin America. In Africa, prevalence (60 to 80%) varies according to humid and forested areas (>60%) and dry or desert areas (<50%) [5]-[7].

The worldwide incidence of Congenital Toxoplasmosis is estimated at 190,100 cases [7]. In France, since 2007, the CNR (Centre National de Référence de la Toxoplasmose), in collaboration with the InVS (Institut de Veille Sanitaire), has set up nationwide surveillance of this disease, with notifications of cases of Congenital Toxoplasmosis. In 2013, 204 cases of Congenital Toxoplasmosis were diagnosed in France.

In the Democratic Republic of Congo, seroprevalence among pregnant women

was 80.3% in 2014 [8].

Toxoplasmosis affects livestock and represents a major cause of abortions in cattle and sheep [9]-[11]. The resulting financial losses can be substantial for developing countries with agro-pastoral activities. Worldwide, the consequences of infecting 500 million farm animals with *Toxoplasma gondii* are estimated to cost around $3 million a year [9]-[11].

In the United States, the estimated incidence of congenital toxoplasmosis ranges from 1/3000 to 1/10,000 births, and may reach 1.5 to 2.5/1000 births in certain parts of Africa [12].

In humans, horizontal contamination occurs either through ingestion of oocysts on soiled food, soiled kitchen equipment or in drinking water, or through cysts in raw or undercooked meat [13]-[15]. Vertical, transplacental contamination occurs when tachyzoites infect the fetus during primary infection in pregnant women [16].

Transplantation or blood transfusion from infected donors, and accidental inoculation via the cutaneous-mucosal route are also routes of contamination [17].

In the Republic of Congo, several studies have been carried out on the prevalence of toxoplasmosis in pregnant women, including one by M. Makuwa *et al.* over a 5-year period from 1986 to 1990. This study revealed that the prevalence of toxoplasmosis in the Congo among 2897 women examined was 60% [18]. Another study conducted at Brazzaville University Hospital on the seroprevalence of toxoplasmosis in pregnant women by Sekangue *et al.* ranging from 2015 to 2016 showed a seroprevalence of 47.2% [19] However, we have no information on the effect that this parasite has on unborn children during toxoplasmosis in pregnant women in Congo Brazzaville, so we set ourselves the goal of assessing the prevalence of congenital toxoplasmosis in Congo Brazzaville.

2. Material and Method

2.1. Material

Biological Material

Blood samples were taken on a dry tube from pregnant women and newborn babies of women.

2.2. Method

The type of study is prospective longitudinal analytical study.

2.2.1. Sampling Site

This study was carried out over a period from April 2021 to November 2023 in Brazzaville in the obstetric gynecology and maternity departments of Blanche Gomez Hospital, at Dr DONGUI's OCH Medical and Ultrasound Office and at the Luiz Biomedical Analysis Laboratory.

2.2.2. Sampling Size

The sample size was estimated by the prevalence find in CHU in 2016 by Sekangue *et al.* with a margin of error of 5% and a studie power of 80% the stata program

proposes us a minimum of 545 patients to enroll globally.

Ethical considerations

All samples were taken after receiving research authorization from the Faculty of Science and Technology of Marien Ngouabi University No. 395 and from the Brazzaville City Hall Directorate No. 057/CB/M/SG/DRH-SFCGPEC and for the approval of the ethics committee of the Congolese foundation for medical research (FCRM) No. 047/CEI/FCRM. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki concerning good practice in clinical research. Subjects enrolled in the study were voluntary individuals who had voluntarily given written informed consent. The enrolment of any minor participant in the study was done after obtaining her assent and the consent of her parents or legal representatives.

2.2.3. Inclusion Criteria

All pregnant women coming for prenatal diagnosis or prenatal follow-up at one of the above mentioned sites.

2.2.4. Exclusion Criteria

Non-pregnant women and women not living in Brazzaville, or those whose presence in Brazzaville could not cover the project period were excluded.

Women who don't give their consentement were excluded.

2.2.5. Variable Studied

Data were collected by interview soon after blood was collected by using standard questionnaire translated into the local language. The questionnaire contained Age, habitat, consumption of cooked or undercooked meat, contact with cats, and consumption of raw vegetables.

2.2.6. Blood Sampling

In pregnant women and newborns:

5 mL of blood is collected in a dry tube from the elbow crease, the blood is then centrifuged at 5000 rpm for 5 minutes and the serum is stored at −20˚C before processing at the Luiz laboratory.

2.2.7. Assay Method

All sera were tested for anti-*Toxoplasma gondii* IgM and IgG antibodies on mini vidas (**Figure 1**) using the ELFA method by Kit vidas toxo IgG II and toxo IgM. After a serum dilution step, IgM or IgG is captured by the polyclonal antibody present on the cone wall and detected specifically by a toxoplasmic antigen itself revealed by an alkaline phosphate-conjugated murine anti-toxoplasmic monoclonal antibody. In the final revelation step, the substrate is captured and expelled into the cone. The enzyme in the conjugate catalyzes the hydrolysis of this substrate into a product whose fluorescence is measured at 450 nm. The value of the fluorescence signal is proportional to the concentration of antibody in the sample.

An IgM assay result will be read as negative when its titre is <0.55 IU/mL and

positive when it is greater than or equal to 0.65. Results between 0.55 and 0.65 are equivocal.

An IgG assay result will be read as negative when its titre is <4 IU/mL and positive when its value is >8 IU/mL.

IgG avidity was measured in women who had seroconverted during pregnancy or in whom we suspected contamination during pregnancy.

Figure 1. Mini vidas.

2.2.8. Data Analysis and Statistical Methods

Data were entered into Excel and analyzed using R software. Prevalences and confidence intervals were calculated using the following formulas:

Prevalence (P) = n/N*100 where n = number of positive samples and N = total number of samples examined.

The confidence interval, with P = observed prevalence in the sample and N = total number of samples examined.

3. Results

Surveys in the health facilities showed an overall prevalence of toxoplasmosis in pregnant women of 49.78%.

The results showed that 693 blood samples from pregnant women were seropositive for toxoplasmosis, *i.e.* a prevalence of 49.78% out of a total of 1392 samples, with frequencies of 53.93%, *i.e.* 267 positive samples out of 495 blood samples from pregnant women at the Blanche Gomez hospital, 53.76% at the Luiz biomedical analysis laboratory, *i.e.* 321 positive samples out of 597 blood samples, and a frequency of 35%, *i.e.* 105 positive samples out of 300 blood samples from pregnant women at the OCH office (Table 1, Figure 2) The immunological profile found was IgG alone in 48.70 (226/464) of cases and IgG associated with IgM in 1.07% (5/464) of cases Table 1. The average age of pregnant women was 30.

Seroprevalence of toxoplasmosis according to socio-demographic characteristics was highest among women aged 16 to 25 (57.14%), followed by women aged 26 to 35 (52.77%) (Figure 3), 62.5% of women with anti-toxoplasmic antibodies had been in contact with soil, compared with 37.5% of seronegative women (Table 2).

Table 1. Prevalences obtained among pregnant women by hospital establishment.

SITE	Positive serologies		IgM+/IgG−		IgM−/IgG+		IgM+/IgG+		Negative serologies
	Nbre	%	Nbre	%	Nbre	%	Nbre	%	Nbre
BLANCHE GOMEZ	89	53.93	0	0	87	52.72	5	1.21	76
LABORATOIRE LUIZ	107	53.76	0	0	104	52.26	4	1.50	92
CABINET OCH	35	35	0	0	35	35	0	0	65
Total				231 49.78%					233 50.22%

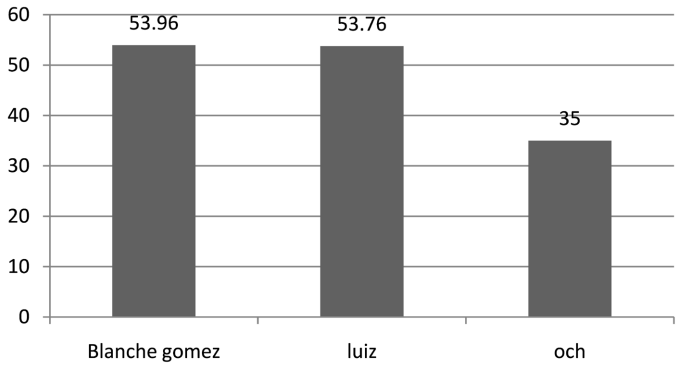
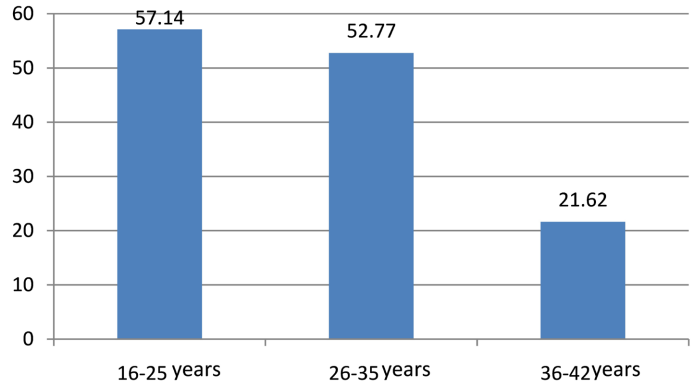


Figure 2. Prevalences obtained among pregnant women by hospital establishment.

Table 2. Risk factors.

Risk factors	Seropositives women (%) n = 231	Seronegatives women (%) n = 233
Contact with the ground		
Presence	50 (62.5)	30 (37.5)
Absence	181 (47.43)	203 (52.86)
Well cook vegetables		
Presence	231 (49.78)	233 (50.21)
Absence		
Consumption of well-cooked meat		
Presence	198 (52.65)	178 (47.34)
Absence	33 (37.5)	55 (62.5)
Knowledge on toxoplasmosis		
Presence	3 (33.3)	6 (66.66)
Absence	228 (50.10)	227 (49.89)
Contact with cats		
Presence	12 (66.66)	6 (33.33)
Absence	219 (49.10)	227 (50.89)



Toxoplasmosis prevalences in pregnant women by age group.

Figure 3. Prevalences obtained by age group.

Among toxoplasmosis-seropositive pregnant women, 52.65% were meat eaters, while 66.66% of women in contact with cats were toxoplasmosis-positive, as shown in **Table 2**. Frequency among pregnant women occupation shows that 43.29% were shopkeepers followed by students 35% as shown in **Figure 4**.

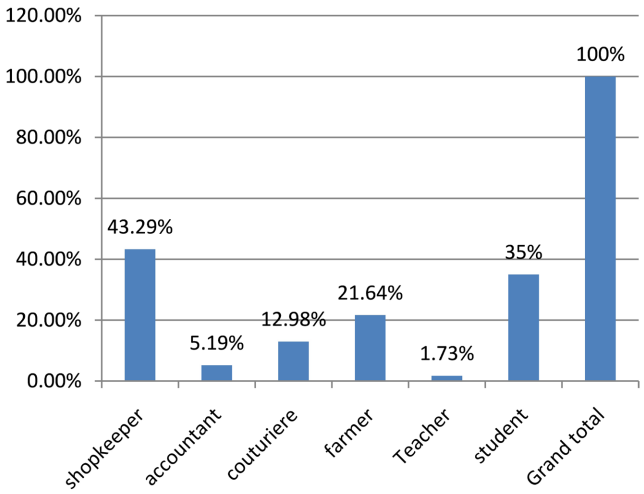


Figure 4. Frequency by occupation among pregnant women.

Of the 9 cases of seroconversion, we diagnosed 6 cases of congenital toxoplasmosis, corresponding to an overall maternal-fetal transmission rate of 66.66% in our study.

we had a total of 6 cases of congenital toxoplasmosis with subclinical forms showing IgG persistence at one year of age, four of whom had thrombocytopenia associated with jaundice, and two others with hepatomegaly associated with hyper transaminase and hyperbilirubinemia. Among women with a negative IgM level, 74% had a high avidity, indicating an old infection, and 3.75% had a low avidity, indicating a recent infection likely within 4 months. In women with a positive IgM level, 3.75% had a low avidity, **Table 3**.

Table 3. Avidity index of IgG.

	Avidity index	IgG		Total
		Negative	Positive	
IgM negative	Recent infection (<0.200)	0	3	3 (3.75%)
	Equivocal (0.200 - 0.300)	0	0	0
	Old infection (>0.300)	0	74	74 (92.5%)
IgM Positive	Recent infection (<0.200)	0	3	3 (3.75%)
	Equivocal (0.200 - 0.300)	0	0	0
	Old infection (>0.300)	0	0	0

Index avidity < 0.200 Recent infection, index avidity > 0.300% = Old infection and index avidity 0.200 - 0.300% Equivocal.

4. Limitation

Of the 545 women to be enrolled, only 464 were able to be followed through to delivery. 81 women could not be included because some of them moved during the study period.

5. Discussion

An overall seroprevalence of 49.78% was found in our study at the three sites, it is lower than that found by Wakuma, which was 60% [18] and close to that found by Sekangue *et al.*, which was 47.2% at the CHU de Brazzaville [19], the mean age in our study was 30 years, close to the 27 years found by Sekangue *et al.* [19]. This may be explained by the longer study period for Wakuma, the different analysis methods and the greater number of pregnant women included in the study, but also by preventive measures and better monitoring of pregnant women with systematic prenatal diagnosis in our health facilities today. It is higher than that found in Dakar in a study conducted by Ibrahim Mahamat Salle [20] which was 38.96%, it was 32.8% in a study conducted in Senegal in Saint Louis in 2012 but similar to that of Coulibaly in 2012 in Dakar which was 50% among women [21] and by Andrée Prisca Ndjoug Ndour in 2012 in Dakar 43.8%. The seroprevalence observed in France by Berger in 2003 was 44% [22].

Among women with negative IgM and positive IgG, 74% had high avidity, indicating a long-standing infection, while 3.75% had low avidity, indicating a recent infection likely within 4 months. Among IgM-positive women, 3.75% had low avidity. These results are contrary to those found in Madagascar by Randriamahazo *et al.* [23], who found that 40% of IgM-negative and IgG-positive women had high avidity, and 16.21% of IgM- and IgG-positive women had low avidity.

The seroprevalence of toxoplasmosis was highest among women aged 16 to 25 (57.14%), followed by women aged 26 to 35 (52.77%) (**Figure 3**). This may be explained by the socio-economic level of these women, their lack of knowledge and lifestyle hygiene, and their dietary habits, which tend to gravitate towards embers. 62.5% of women with anti-toxoplasmic antibodies had been in contact with

soil, compared with 37.5% of seronegative women. The presence of cats in the environment may be responsible for this result. Among toxoplasmosis-seropositive enceintes women, 52.65% were meat eaters, whereas 66.66% of women in contact with cats were toxoplasmosis-positive, contact with host animals, contact with uncooked meat and vegetable was a risk factor of contamination who where also mentioned by Al-Jebouri in Irak [24] and AlRashada in Saudi Arabia among female students [25]. This may be explained by the strong presence of stray cats in the neighborhoods, which are responsible for contaminating the soil, water and vegetables, and by the consumption of undercooked meats, particularly cold cuts and charcoal.

The percentage of seroconversion in our study was 1.07%, which is lower than that found by Sekangue *et al.* (2.8%), and by Wakuma (5.6%).

Out of 9 cases of seroconversion, 6 cases of congenital toxoplasmosis were confirmed, corresponding to an overall maternal-foetal transmission rate of 66.66% observed in our study. This result is lower than that found by Cyrille Wakieu between 2004 and 2011 [26], which was 15 cases of congenital toxoplasmosis, also found by W. Ferguson *et al.* [27].

6. Conclusions

This study of pregnant women in Brazzaville confirms the prevalence and activity of *Toxoplasma gondii* in the city. Unlike other studies based solely on seroprevalence in pregnant women, our study enabled us to diagnose cases of congenital toxoplasmosis linked to seroconversion in pregnant women during pregnancy, thus demonstrating the impact of contamination by the parasite on unborn children. The data obtained from this study have enabled us to gain a better understanding of toxoplasmosis in pregnant women, and to identify the main risk factors linked to contamination, which underlines the vital importance of serological over-surveillance of pregnant women, enabling us to detect and monitor non-immune women and progressive toxoplasmosis as early as possible, so that contaminated children can be cared for.

Congo Brazzaville is a low-income country whose socio-economic status is not conducive to better prenatal care. A diet that is more focused on undercooked meat, ember and dibiterie, which are recipes for dishes that are not well cooked, and the high presence of cats in our environment are risk factors that have an impact on the transmission of congenital toxoplasmosis. It therefore appears that many pregnant women with no anti-Toxoplasma antibodies are highly exposed to the risk of infection during pregnancy in this work 50.22% of women are at risk of primary infection. It is important to advise them to avoid eating undercooked meat, unclean water, unwashed market garden produce and contact with ruminant offspring, cat faeces and soiled soil.

Future studies will enable us to set up a method for diagnosing early congenital toxoplasmosis, in particular, the western blot during maternal and child check-ups, and to genetically characterize strains isolated during maternal and congenital

toxoplasmosis.

Author Contribution

Martinien Mayela	Investigation, methodology, formal analysis, writing—original draft.
Fréderic DONGUI	Validation, methodology, writing—reviewing.
KIPOUNGA Francelvie	Formal analysis, writing—review and editing.
Moyen Nanikaly	Formal analysis, writing—review and editing.
Rachel Moyen	Investigation, writing—reviewing.

Conflicts of Interest

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

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