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### RESEARCH ARTICLE

#### SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF TOXOPLASMOSIS IN WOMEN RECEIVED IN ANTENATAL CONSULTATION AND DOMESTIC CARNIVORES (CATS AND DOGS) IN DAKAR CITY, SENEGAL

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#### Abstract

The present paper aimed to determine the seroprevalence and associated risk factors of *Toxoplasmosis* among pregnant women and domestic carnivores. A Cross sectional study was designed among 262 carnivores (141 cats, and 121 dogs) and 100 women attended antenatal care from August 2011 to February 2012 in health centers of Dakar. Venous blood was collected from each women and domestic carnivores. Sera were tested for anti-*Toxoplasma gondii* antibodies using modified agglutination test (MAT). Moreover, participants were interviewed using a structured questionnaire to identify the potential risk factors of *Toxoplasmosis*. The overall seroprevalence of *T. gondii* infection was 52.3±9.7%. Toxoplasmosis was around 53% among women having history of abortion (43%). After analysis of risk factors, only fresh milk consumption predisposes women to be contaminated. In carnivores, the prevalence of infestation was higher in adults than in young animals. Toxoplasmosis prevalence was 55.37±9% in cats with sex and age as risk factors, and 43.97±8% in the dogs with only age as risk factor. These results are therefore a signal to competent health authorities for a better awareness of the real incidence of this disease in Dakar.

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#### Introduction:-

Infectious and parasitic diseases are the second leading cause of death in humans worldwide (WHO, 2004) and approximately two-thirds of these are zoonoses (Cleaveland et al., 2001), diseases transmissible to humans by animals. Parasitic zoonoses are a major public health problem worldwide. Among them, toxoplasmosis is one of the main zoonoses in the world caused by *Toxoplasma gondii*, which infects almost all warm-blooded animals, including humans (Liu et al., 2012). Cats and other felines are natural hosts, the other species acting as intermediate hosts. In humans, infection is often benign but can lead to abortion, congenital diseases, and severe clinical signs in immune-compromised individuals (Dubey and Beattie, 1988). Most infections among humans occur by eating

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unpasteurized milk, raw or undercooked meat containing tissue cysts or by exposure to oocysts through ingestion of contaminated foods and drinks with cat faeces (Dubey JP, 2009).

This major parasitosis is cosmopolitan and it is estimated that around 30% of the world's human population is chronically infected with *T. gondii* (Montoya et al., 2008, Moncada and Montoya, 2012). The seroprevalence of toxoplasmosis ranged from 10 to 50% in developed countries and over 80% in developing tropical countries (Kaplan et al., 2009). In urban Africa, toxoplasmosis seroprevalence during pregnancy ranged from 31% in Burkina Faso (Bamba et al., 2012), 43.7% in Nigeria (Olusi et al., 1996), 50.6% in Morocco (Mansouri et al., 2007), 56% in Gabon (Mpiga et al., 2010), to 60% in Yopougon in Côte d'Ivoire (Adou-bryn et al., 2004) and in Central African Republic (Morvan et al., 1999).

In Senegal, *T. gondii* seroprevalence in population ranges from 31.15% to 46.03% among pregnant women and women of childbearing age (Faye et al., 1998, Ndiaye et al., 2011, Lo et al., 2012), cats are identified as *T. gondii* carriers in Dakar (Ndour APN, 2012). Despite its medical and health importance, this protozoonosis remained neglected compared to major African endemics as malaria, trypanosomiasis, and schistosomiasis. However, its impact on the health of the newborn, child and reproductive disorders is not negligible. In Dakar, there is little information on its prevalence and risk factors among women in prenatal consultation and domestic carnivores (dogs and cats) and there is an increase in unexplained abortions among pregnant women (personal survey). Indeed, these animals, whose numbers are constantly growing in Dakar, played a role in the epidemiology of this disease.

The aim of the present study was to assess the seroprevalence and associated risk factors of *T. gondii* infection among women received in antenatal consultation and domestic carnivores (cats and dogs) in Dakar, Senegal's capital.

## Material And Methods:-

### Study design, area, and period

A cross-sectional study was conducted from August 2011 to February 2012 in two of the most visited health centers of Dakar, Senegal's capital. These are the health posts Albert Ngor NDOUR (ANN) of Thiaroye and Malika, chosen randomly for this study (figure 1). This region has been chosen because it accounted for about 25% of the total population and the bulk of Senegal's economic pole, therefore constantly urbanizing due to a large rural exodus.



**Fig 1:-** Study area map. The two studied districts of Dakar are highlighted on the map. (<https://www.weather-forecast.com/locations/Pikine/forecasts/latest>). 15 June 2020.

### Human and Animals population

In this study, the human population consisted of women in antenatal consultation (ANC) followed in two health centers in Albert Ngor NDOUR (ANN) Thiaroye and Malika, randomly chosen. The sample size was estimated using the following formula:  $n = \frac{P(1-P)z^2}{d^2}$  (Thrusfield, 2005) with an expected *T. gondii* seroprevalence (p) ranges

from 31.15% to 46.03% among pregnant women and women of childbearing age in Senegal (Faye et al., 1998, Ndiaye et al., 2011, Lo et al., 2012). The confident level was 95% ( $Z = 1.96$ ) with an absolute precision (d) of 10%. The calculated sample size ( $n = 95$ ) was increased to 100 pregnant women for better accuracy.

Due to difficulties to catch carnivores, the animal population consisted of all occasional stray and domestic dogs and cats obtained in different districts of Dakar without distinction of sex, age, race and physiological state. A total of 262 animals (cats = 121 and 141 dogs) were sampled based on expected prevalence of 26% (Salle, 2010) for both cats and dogs at 8% absolute precision and 95% confidence level using the method recommended by Thrusfield (2005).

#### **Data collection**

Two investigation forms were designed with one intended for women and the other for animals. Socio-demographic characteristics and risk factors associated with *T. gondii* infection were collected for each study participant. Using a structured questionnaire, data on exposure to possible risk factors were documented, and included contact with cats and dogs, water source, consumption of market gardening produce, consumption of grilled meat, milk consumption, age group (year), maternity (primiparous, pauciparous, multiparous), education (schooled and unschooled) and history of abortion were collected as socio-demographic characteristics. Age was classified in three categories (< 25 years, 25-39 years and > 40 years). For animals, they were first identified, and their physiological status was assessed. Free and informed consent of women was necessary before any action. The interview was individual, always preceded by a brief statement on the reasons for the investigation and followed up on the sampling. Then, data were treated confidentially.

#### **Animal capture, restraint, and blood sampling**

In animals, sampling was always preceded by capture/containment. Domestic animals capture often required the presence of the owner. Despite this, restraint was done using a muzzle and tranquillization required an intramuscular injection of an anesthesia dose of Ketamine (Imalgene<sup>®</sup>) and Acepromazine (Calmivet<sup>®</sup>). The animals were marked to prevent them from being taken a second time. For each selected animal, blood sample was collected in glass tubes collected from saphenous vein, without anti-coagulant and stored in ice box. Sample were transported to parasitology and mycology laboratory of EISMV (*Inter States School of Science and Veterinary Medicine*) of Dakar, where sera were extracted by centrifugation at 3500 revolutions per minute for 25 min and stored at 20°C until they were tested.

Five milliliter (ml) of blood were also collected from each pregnant woman and sera collected in identified cryotubes, were preserved under -20°C until serological testing.

#### **Serological analysis**

For animals, the modified agglutination test (MAT) was used for the detection of anti-*T. gondii* antibodies with the commercial kit "Toxo-Screen DA" from Biomérieux, France (Reference 75481) following manufacturer's instructions. The kit had with sensitivity and specificity of 92% and 95%, respectively.

For pregnant women, serological tests were carried out by using two commercial kits to detect anti-*Toxoplasma* antibodies in sera, according to their manufacturer's instruction. First, samples were screened with the Toxolax kit [Ref. 1201002; sensitivity = 96.1% and specificity = 89.6%] of the SpinReact<sup>®</sup> laboratories. The positive tests were then certified by an indirect hemagglutination serodiagnosis with the Toxo-Hai kit [Ref. 5250; sensitivity = 99.2% and specificity = 97.7%] from Fumouze<sup>®</sup> Laboratories. Positive and negative control tests were done for each batch of test run to ensure kits are working properly and technical procedures are carried out correctly.

#### **Data Analysis**

These data were recorded using Epidata 3.1 software, and then was analyzed using the Rcommander 2.12.0 software. Univariate and multivariate analysis were performed to determine factors associated with toxoplasmosis seroprevalence by logistic regression. The odd ratio (OR) at 95% confidence interval (CI) were calculated to appreciate the strength of association of risk factor to the seropositivity. P-values less than 0.05 were considered as statistically significant.

### Ethical clearance

Before each sampling, the aim of the study was explained to women. Informed written consent was also obtained from each pregnant woman prior to involvement in the study. The agreement of the director of the hospital was also obtained. The institutional independent review board of Cheikh Anta Diop University gave its ethical clearance.

### Results:-

#### Sociodemographic characteristics

Table 1 showed sociodemographic characteristics of the study participants. Thus, a total of 100 pregnant women were included in the study. Most of the study participants (51%) were aged between 25-39 years. The age groups of < 25 years old and above 40 years old were represented respectively 25% and 24% of the studied subjects. Most of the participants (55%) had at least primary education. The survey also revealed that more than 88% of women had given birth at least once and 43% of them had reported having abortions in the first trimester of pregnancy (Figure 2). More than 79% of women aged above 40 years had abortion while this proportion was respectively 37% and 15% for women aged 25 to 39 years and under 25 years. In our study population, 44 women (44%) had contact with cats and only 3% had dogs at home. They also claimed they consume raw market-gardening produce (98%), grilled meat (84%) and raw milk (72%) with their families.

**Table 1:-** Socio-demographic characteristics of the study population.

	Categories	No. of women	Percent (%)
<b>Age group (years)</b>	< 20	25	25
	20-39	51	51
	≥ 40	24	24
<b>Education level</b>	<i>Unschool</i>	45	22
	<i>Schooled</i>	55	28
<b>Maternity</b>	<i>Primiparus</i>	12	3
	<i>Pauciparous</i>	43	21
	<i>Multiparous</i>	45	26
<b>History of abortion</b>	<i>Yes</i>	43	43
	<i>No</i>	57	57
<i>Pregnant women infection risk factors</i>			
<b>Presence of domestic cat</b>	<i>Yes</i>	73	73
	<i>No</i>	27	27
<b>Water source</b>	<i>Water supply</i>	77	77
	<i>Other**</i>	23	23
<b>Consumption of market gardening produce</b>	<i>Yes</i>	98	98
	<i>No</i>	2	2
<b>Consumption of grilled meat</b>	<i>Yes</i>	84	84
	<i>No</i>	16	16
<b>Raw milk consumption</b>	<i>Yes</i>	72	72
	<i>No</i>	28	28

#### Seroprevalence of *T. gondii* infection and Risks factors in women attending ANC

Seroprevalence of *T. gondii* infection in relation to demographic characteristics of the pregnant women is given in Table 2. The overall seroprevalence of *T. gondii* infection was 50±9.8% (50/100) (real prevalence = 52.3±9.7%). The infection prevalence among age groups <25; 25 – 39; and >40 was 40±19.2% (10/25), 47±13.7% (19/51), 66.6±18.8% (16/24) respectively. However, no significant association was observed between seroprevalence and age. Among women who had history of abortion 53.4±9.7% (23/43) were seropositive.

Logistic regression analysis of risk factors showed that raw milk consumption predisposed women to be contaminated ( $p < 0.05$ ; OR > 1). Pregnant women who consumed raw milk had 2.79 times more risk of toxoplasmosis than those did not ( $P=0.025$ ; OR = 2.79 95% CI [1.11 - 7] (Table 2). However, there was no statistically significant association between seropositivity of *T. gondii* infection and educational status, contact with dogs or cats, drinking water, consumption of market gardening produce, grilled meat, maternity (Primiparous, Pauciparous, Multiparous).

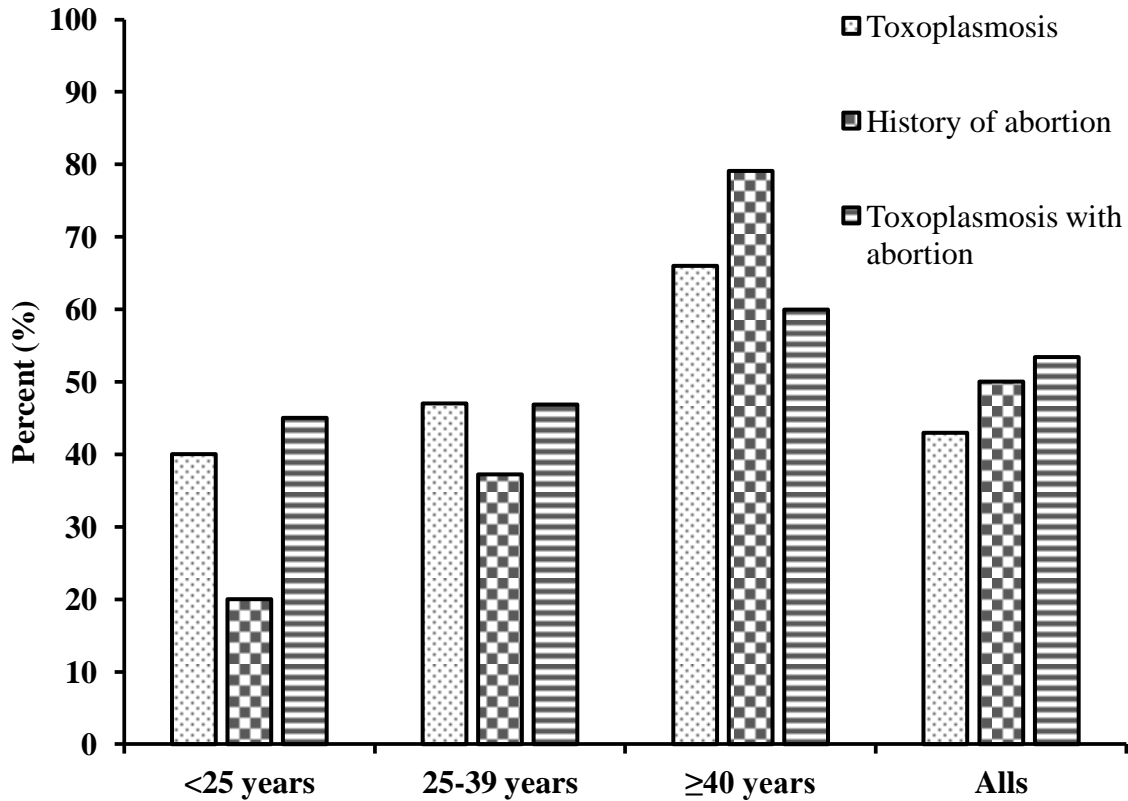


Fig 2:- Seroprevalence of toxoplasmosis according to the age and history of abortion.

**Seroprevalence and risk factors of toxoplasmosis in cats and dogs**

The apparent and real prevalence of toxoplasmosis in cats was 55.3 ± 9% and 57 ± 8.8% respectively (Table 3). According to multivariate analysis using logistic regression model, *T. gondii* seroprevalence was 2.09 times higher in females than in males. (OR: 2.09 95% CI: 0.92-4.91), and old cats (OR: 3.29 95% CI: 0.96-11.24). In dogs, the results of multivariate analysis of the associated factors showed that only gender had significant effect on the prevalence of toxoplasmosis (OR: 4.09 95% CI: 1.54-10.7). Oldest dogs had two times odds of being infected than young. The bivariate analysis on the race, lifestyle and rabies vaccination did not reveal any significant association between the serologic prevalence of *T. gondii* (p>0.05).

**Table 2:-** Logistic regression analysis of risk factors with *T. gondii* seropositivity among pregnant women attending antenatal clinics (ANC).

Risk factors		No. of women	No. of seropositive women	Seroprevalence of <i>T. gondii</i> infection			
				Seroprevalence (%)	P-value	OR	95%CI
Education level	Unschool	45	22	48.8	0.8	1.08	0.45-2.56
	School	55	28	50.9			
Maternity	Primiparus	12	3	25	0.11	2.53	0.58-10.99
	Pauciparous	43	21	48.8	Ref.	-	-
	Multiparous	45	26	57.7	0.07	0.67	0.27-1.68
Presence of domestic cat	Yes	73	37	50.7	0.91	0.95	0.42-2.18
	No	27	37	51.1			
Water source	Water supply	77	41	53.25	0.2	1.761	0.62-5.21
	Other**	23	9	39.13			
Consumption of	Yes	98	50	51.02	0.15	Infinite	0.18-Infinite

market gardening produce	No	2	0	0			
Raw milk consumption	Yes	72	31	43.06	0.025*	2.79*	1.11-7
	No	28	19	67.86			
Consumption of grilled meat	Yes	84	43	51.19	0.58	1.344	0.40-4.67
	No	16	7	43.75			

Primiparous= women with 1st pregnancy, Multiparous= women having more than 4 children, Pauciparous= women having 1 to 3 children. \* = Significant, Ref = reference factor, \*\*Other sources of water consumed= well and borehole, OR= Odds Ratio; p= p-value. CI= Confidence Interval, p= p-value, CI= Confidence Interval.

**Table 3:-** Analysis of risk factors related to *T. gondii* seropositivity in a population of cats and dogs.

Variables		Seroprevalence of <i>Toxoplasma gondii</i>									
		Dogs					Cats				
		Total number	Pos .	Pa (%) ±CI	p	OR (95%CI)	Total number	Pos .	Pa (%)±CI	p	OR (95%CI)
Race	Local	47	24	51±20	Ref.		121	67	55.4±9		
	Mixed	15	6	40 ±25	0.63	1.60 (0.45-5.66)	-	-	-	-	-
	Exotic	79	32	40.5±11	0.74	1.43 (0.68-3.02)	-	-	-	-	-
Sex	Female	60	26	43.3±12	0.89	1.04 (0.5-2.16)	76	37	48.6±11	0.05*	2.09*(1.2-4.91)
	Male	81	36	44.4±11			45	30	66.6±14		
Age	Youth	31	6	19.3±14	0.004*	4.07*(1.54-10.7)	18	6	33.3±22	0.05*	3.29*(1.6-11.24)
	Adults	110	56	50.9±9			103	61	59.2±9		
Lifestyle	P. stray.	6	6	100±0	0.05*	-	108	59	54.6±9	0.84	1.14(0.27-4.85)
	O. stray.	9	8	88.8±20			-	-	-		
	Domestic	126	48	38.1±8			13	8	61.5±7		
Deworming	Yes	99	39	39.3±10	0.09	1.92(0.88-4.15)	6	3	42.8±36	0.87	0.87(0.16-4.52)
	No	36	20	55.5±16			103	55	56.1±9		
Rabies vaccination	Yes	99	40	40.4±10	0.08	0.65(0.30-1.39)	8	4	50 ±34	0.29	1.18(0.25-5.56)
	No	37	19	51.3±16			102	59	54.1±9		
<b>Total</b>		<b>141</b>	<b>62</b>	<b>43.9 ± 8 (Pr =45±8.2)</b>			<b>121</b>	<b>67</b>	<b>55.3± 9 (Pr = 57±8.8%)</b>		

Pa= apparent prevalence; Pr= real prevalence; P. stray= Permanent stray; O. stray= Occasional stray; Pos= positive, OR= Odds Ratio, p= p-value, CI= Confidence Interval. \*= Significant, Ref= reference factor

### Discussion:-

The current study is one of the few studies carried out in Senegal to explore the seroprevalence of *T. gondii* infection among pregnant women in Dakar. The seroprevalence of *T. gondii* among pregnant women in the Dakar area (Thiaroye and Malika) was found to be relatively high (52%); like prevalence in Ghana 51.2% (Ayi et al., 2016), Central African Republic 50.6% (Morvan et al., 1999) and Morocco 50.69% (Mansouri et al., 2007). Unlike our result, lower prevalences were recorded in previous studies in Senegal 40.2% (Faye et al., 1998), 38.86% (Salle,

2010), 44% (Ndiaye, 2010), 32.5% (Allanonto, 2012). This current finding is also higher than the findings in Tanzania 30.9% (Mwambe *et al.*, 2013), Sri Lanka 30.27% (Iddawela *et al.*, 2017)<sup>[19]</sup> and China 18.1% (Cong *et al.*, 2015) but lower than the findings from Brazil 68.37% (Da silva *et al.*, 2015), Arbaminch, Ethiopia 79.3% (Yohanes *et al.*, 2017), Bench Maji, Ethiopia 85.3% (Abamecha *et al.*, 2016). Such differences of the prevalence of toxoplasmosis may be attributed to the serologic tests used, the size and the mode of sampling, the geo-climatic differences (humidity and heat favoring the survival of oocysts in environment). In addition, in these districts, living and environmental conditions was precarious and people had narrow and frequent contact with animals, particularly cats and sheep. Of the 43% of women who reported having had at least one abortion, 53% were positive for the toxoplasmosis test compared to 61.8% in Côte d'Ivoire (Adoubryn *et al.*, 2004). This difference could be due to the eating habits of women in both countries. Transmission would be more by consuming uncooked meat, badly washed raw vegetables and fresh milk contaminated by oocysts and by the propensity of Abidjanis to eat more often outside their homes than Senegalese women. Consumption of fresh milk was identified as a risk factor (OR = 2.79, CI: 1.11 - 7, P = 0.025) in our study. Similar findings were reported by other studies elsewhere like in London which revealed that women who drank raw milk would be more likely to be infected with toxoplasmosis. (Flatt and Shetty, 2013). In agreement with the results from Ethiopia (Zemene *et al.*, 2012), this study showed no significant difference between levels of education and the different occupations.

The prevalence also increased with gestation: 25% in Primiparous, 48.8% in Pauciparous, 57.7% in Multiparous. However, no link was found between seroprevalence and gestation (p = 0.11). To the contrary, in other study data indicated an increased risk from the 6th pregnancy. Indeed, Bittencourt *et al.* (2012) found that gestation was a risk factor for toxoplasmosis with a prevalence of Multiparous estimated at 66.7% (p = 0.004).

In cats, the current prevalence was 57±8.8%. This value was different from that obtained in Colombia (45.2%) (Dubey *et al.*, 2006), and Bangkok (75%) (Jittapalpong *et al.*, 2007) but very close to that obtained in France (58%) (AFSSA, 2005). This demonstrated variability in this prevalence around the world and could be explained by environmental conditions and the presence of sources of infestation, especially by stray cats. (Millan *et al.*, 2009). The link between presence and number of stray cats and the high prevalence of toxoplasmosis in intermediate hosts was demonstrated by Fajardo *et al.* (2013).

As revealed by most studies (Euzéby, 1997; Millan *et al.*, 2009), age of cat emerged as a risk factor multiplied by 3 in adults (p = 0.05, OR = 3.29, CI = 1.6-11.24). The prevalence of toxoplasmosis increased according to the age of animals because older animals have been more exposed to parasite oocysts compared to younger animals. Sex is also a risk factor multiplied by 2 in female's cats (p = 0.05, OR = 2.09, CI = 1.2-4.91). The same observation was made in other studies (Jittapalpong *et al.*, 2007). According to Euzéby (1997), even if toxoplasmosis is a disease seen in animals in both sexes, the physiological state of female may influence the sensitivity of the animal.

The level of dog infestation by *T. gondii* was 45±8.2%. It is close to that obtained in India (48.5%) (Dubey *et al.*, 2008). This author found an association between dog's age and seroprevalence status as demonstrated in our study. Moreover, a similar study in Nigeria (Kamani *et al.*, 2010) gave a lower prevalence (25%) with an influence on age; the adult dog was 2 times more exposed to toxoplasmosis than the young while this exposure was increased by 4 times in current study. These differences would be due to several factors such as sample size, environmental conditions. However, dog consumption is not a common cultural practice in Senegal, the level of antitoxoplasmic antibodies does not really have an interest in public health. Nevertheless, this prevalence appears to be a witness of a high parasitic load of meat products and the environment of a high seropositivity of women and cats.

### Conclusions:-

Toxoplasmosis is an endemic parasitosis in Senegal. The seroprevalence of *T. gondii* among pregnant women in Dakar area was high 52,3% compared to previous studies of Senegal. In this study, the recorded prevalence of toxoplasmosis was high, particularly for animals located in Dakar (cats 57% and dog 45%). Consumption of fresh milk was significantly identified as a risk factor for toxoplasmosis (p=0.02) in these areas. The data observed in this study should allow the country's competent health authorities to raise awareness of the real incidence of this disease in Dakar and to establish sensitization and prevention programs for all population to explain how to avoid contamination by the food, water and soil.

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