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Current Status of Toxoplasmosis in Breeding Sites, Slaughterhouses and Cats in Brazzaville, Republic of Congo

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A B S T R A C T

Toxoplasma gondii is a protozoan responsible for infections in humans and animals. Human contamination by *Toxoplasma gondii* occurs through the consumption of parasitized and undercooked farm animal meat containing cysts, as well as fruit, fresh vegetables and water contaminated by oocysts which are eliminated by cats in the environment. In order to assess the prevalence of toxoplasmosis of animal origin in Brazzaville, Republic of Congo, a total of 300 animals were sampled from slaughterhouses and breeding sites, as well as 100 cats, from March 2023 to November 2023. Analyses were carried out on a total of 400 samples, including 300 blood samples for the detection of anti-toxoplasma antibodies using the cypress diagnostic latex agglutination method and the Toxo Meddiff immunochromatographic test, and 100 cat stool samples for the detection of oocysts using the Dubey sucrose flotation method. The results showed that 191 of the 300 blood samples were positive, giving a seroprevalence of 63.66%. However, when evaluated by animal type, the highest seroprevalence was observed in cattle with a seroprevalence of 76.42%, followed by sheep with a seroprevalence of 60.90% and pigs with a seroprevalence of 34%. Coprological tests on cats showed that 6 of the 100 stool samples taken were positive, i.e. a prevalence of 6%, with 7.5% in Diata, 6.66% in M'PILA and 5% in Moungali. This study shows a strong presence of toxoplasmosis in the animal population, correlated with the presence of oocysts found in cat feces, demonstrating that environmental contamination is a source of animal contamination. This study provides us with a better understanding of the dynamics of toxoplasma transmission to humans, demonstrating the importance of sanitary and hygienic-dietary measures in both humans and animals.

Introduction

Toxoplasma gondii is a protozoan responsible for infections in humans and animals. Infection is usually asymptomatic in immunocompetent individuals. On the other hand, severe forms are observed during congenital transmission (abortions, malformations) or in immunocompromised people (Sara, Mlle, 2018). The

parasite can infect all warm-blooded animals (Makuwa *et al.,* 1990). The definitive host of toxoplasma is the domestic cat (*Felis catus*) and felids, which disseminate oocysts via feces. Mammals and birds act as intermediate hosts.

In addition to transplacental transmission, other routes of contamination include consumption of undercooked meat (containing tissue cysts) or water (contaminated with oocysts).

Human contamination by *Toxoplasma gondii* occurs through the consumption of parasitized and undercooked farm animal meat, unpasteurized goat's milk, fresh fruit, vegetables and plant products, as well as water contaminated with oocysts (Nguyen, 2017). The World Health Organization (WHO) has estimated that around 22% of human *T. gondii* infections are caused by meat (Ouchetati *et al.,* 2021).

In Europe, meat consumption has been associated with 30 to 63% of toxoplasmic infections in pregnant women (Cook *et al.,* 2000; Tenter, 2009).

Toxoplasmosis affects livestock and is a major cause of abortion in cattle and sheep (Abu- Dalbouh *et al.,* 2012; Bamba *et al.,* 2012; Villena *et al.,* 2012). The resulting financial losses can be substantial for agro-pastoral developing countries. Worldwide, it is estimated that around 3 million dollars in losses each year, the consequences of infecting 500 million farm animals with *Toxoplasma gondii* (Villena *et al.,* 2012; Da Silva *et al.,* 2013; AFSSA, 2005; Berger-Schoch *et al.,*2011; Dubey, 2010).

Numerous epidemiological surveys point to meat as a major source of human contamination, but there are no data in Congo Brazzaville to enable us to estimate this risk.

In Congo Brazzaville, a seroprevalence of toxoplasmosis of 60% was reported among pregnant women by Makuma *et al.,* over a 5-year period from 1986 to 1990 (Makuma *et al.,* 1990). In Brazzaville, the figure was 47.2% in 144 pregnant women in 2016 (Sekangue *et al.,* 2016).

However, the lack of sanitary monitoring of animals intended for consumption and good culinary habits could be at the root of this contamination. The present study aims to assess the seroprevalence of toxoplasmosis in animals intended for human consumption, notably cattle, sheep and pigs, and the coprological prevalence of toxoplasmosis in cats, to determine whether animal carriage is comparable to that in humans.

Materials and Methods

Biological material

Blood samples were collected on dry tubes from 140 cattle, 110 sheep and 50 pigs.

Cat stool samples were collected in sterile jars..

Type of study

Sampling site

This study was carried out over a period from March 2023 to November 2023 in Brazzaville at the farms of the Ecole Nationale Supérieure. of agronomy and forestry, Lycée technique agricole Amilcar Cabral, MAYANGA and MADIBOU, M'pila slaughterhouse, Moungali and Diata.

Inclusion criteria

All beef, pork and sheep meat.
Cats.

Exclusion criteria

All meats other than beef, pork and sheep.

Considérations ethical

All samples were taken after receiving research authorization from the Direction de l'agriculture et de l'élevage de la Mairie de la commune de Brazzaville N°057/CB/M/SG/DRH-SFCGPEC

Blood sampling in animals

On cattle and sheep farms, blood was drawn from the jugular vein. Blood is drawn from the jugular vein using an epicranian adapted to a syringe. To highlight the jugular vein, the animal is placed in a lateral decubitus position, well restrained, and the hair on the neck is shaved; the neck is stretched and pulled back. Once the vacutainer needle and syringe have been fitted, the vein

is pierced and the blood sample taken. The blood is then transferred to the dry tube, on which the outbreak number, sex and species name are recorded. After sampling, the animal is left in a quiet, lateral position.

In cattle and sheep at slaughterhouses, blood is drawn from the jugular vein after the animal has been tied up and its throat cut. The blood is then collected in a dry tube, on which the sex, species and origin are noted. In pigs, the sample was taken from the ears.

Stool Sampling in Cats

Faeces were collected from the rectum using forceps and placed in sterile vials in cats at veterinary surgeries. For apartment cats we collected feces early in the morning after defecation on the clean floor or in the litter box.

For stray cats, we collected faeces in the stores on campus, at the rectorates in Diata and in the stores at the Moungali market, where they came to feed, or we placed a litter box in the stores and collected the cats' faeces early in the morning.

They were then transported in a cool box to be stored at +4°C in the laboratory refrigerator while awaiting analysis.

Assay Method

After centrifugation and collection of the serum, the samples were analyzed in the bacteriology and parasitology department of the Luiz biomedical analysis laboratory in Congo Brazzaville, using the latex agglutination technique consisting of latex sensitized with a mixed total toxoplasmic antigen and the Kit toxo mediff France. This is an immunochromatographic test based on the detection of immunoglobulin M (IgM) and immunoglobulin G (IgG).

When an adequate volume of sample and diluent has been deposited in the deposition zone, the sample migrates by capillary action along the cassette. *Toxoplasma gondii* IgM, if present in the sample, will bind to the *Toxoplasma gondii* conjugates, and the immune complex will be captured on the membrane by the pre-coated anti-human IgM, forming a violet line on the M-band, indicating that the result is positive for anti-*Toxoplasma gondii* IgM. Anti-*Toxoplasma gondii* IgG, if present in the sample, will bind to the *Toxoplasma gondii* conjugates, and the immune complex will be captured on the membrane by the pre-coated anti-human IgG. and form a purple line on the G band, indicating a positive result for anti-*Toxoplasma gondii* IgG.

The absence of coloration in the M et G bands suggests a negative result. The test is equipped with an internal control system (C band), which should produce a violet coloration in the presence of the immune complex of control antibodies, independently of the coloration of the test bands (G and M). If the C band does not react, the test is invalid and the sample must be retested with a new cassette.

Faeces Observation Method

The method used to test stools for oocysts is Dubey's sucrose method (density 1.15).

A 10 g sample of stool is suspended in 20 mL of sucrose solution (density 1.15, obtained by mixing 53 g of powdered sugar in 100 mL of demineralized water). The resulting mixture is then filtered through a tea strainer and gauze over a beaker. The filtrate is pipetted into two dry 10 mL tubes.

After centrifugation at 1180 g for 10 min, a drop of supernatant is observed between slide and coverslip at objective 40. If oocysts are observed, 5 to 6 ml of each tube are removed and resuspended in 5 volumes of distilled water. After centrifugation at 1180 g for 10 min, oocysts are concentrated in the pellet.

Data Analysis and Statistical Methods

Data were entered into Excel and analyzed using R software. The 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. L'intervalle de confiance à 95 %, avec P= prévalence observée dans l'échantillon et N= nombre total des prélèvements examinés.

$$(IC) = P \pm 1{,}96\sqrt{p(1-p)/N}$$

Results and Discussion

This serological survey is the first to report *T. gondii* infection in cattle, sheep and pigs in Congo Brazzaville.

The serological studies showed an overall seroprevalence of 63.66% in animals, with a seroprevalence of 76.42% (107/140) in cattle, a seroprevalence of 60.90% in sheep and a seroprevalence of 34% in pigs at the different sites (table 2) (figure 1).

100 stool samples were collected from cats, 6 of which, corresponding to 6%, were positive for toxoplasmosis (Table 1). 40 samples were obtained in Diata, 3 of which, corresponding to 7.5%, were positive for toxoplasmosis, 30 samples were collected in M'Pila, 2 of which, corresponding to 6.6%, were positive for toxoplasmosis (Table 2).

Toxoplasmosis, 20 samples were obtained in Moungalie, of which 1 representing 5%, were positive for toxoplasmosis, while the 10 samples obtained in Mayanga were negative for toxoplasmosis

The serological results of our survey in Congo showed a seroprevalence in cattle of 76.42% this result is very close to that conducted in Spain by Garcia-Bocanegra *et al.,* (2013) which had given a seroprevalence of 83. 3%, however it is higher than that found in France by Gilotfromont *et al.,* in 2009 which was 7.8%, in Nigeria by Onyiche and Ademola in 2015 which was 13.9%, it was 13% in Senegal in a study conducted from 2011 to 2013 by Davoust *et al.,* and 44% in sudan by Dubey *et al.,* in 2009. Seroprevalence was 60.90% in sheep, a result similar to that of Rahman *et al.,* in Bangladesh, who found a séroprévalence de 69.9 % and Katzer *et al.,* in Scotland, who found a seroprevalence of 56.6%.

In Belgium, it was 87.4% in a study by Verhelst *et al.,* in 2014, and 87.05% in Gabon in a study by Barthelemy Ngoubangoye in 2007.

The serological data can be correlated with the diet of these animals and their drinking water, which comes from stagnant water (puddles, lakes, wells) conducive to the development or sporulation of oocysts, moist soil and favorable temperatures, as well as the presence of cats in the breeding environment (farm), whose faeces could contaminate the water and grasses dedicated to their consumption. They are also likely to ingest soil or water containing sporulated oocysts.

In villages, many cats live in the same concessions as humans and animals. The high prevalence observed in this study is a wake-up call for public health officials, especially in rural areas. Cats, of which there are many, both in villages and on farms and breeding sites, are reservoirs of toxoplasmosis.

Sheep are the most contaminated species of domestic ruminant.

We can assume that their contamination is due to ingestion of water or food contaminated with oocysts from cat faeces.

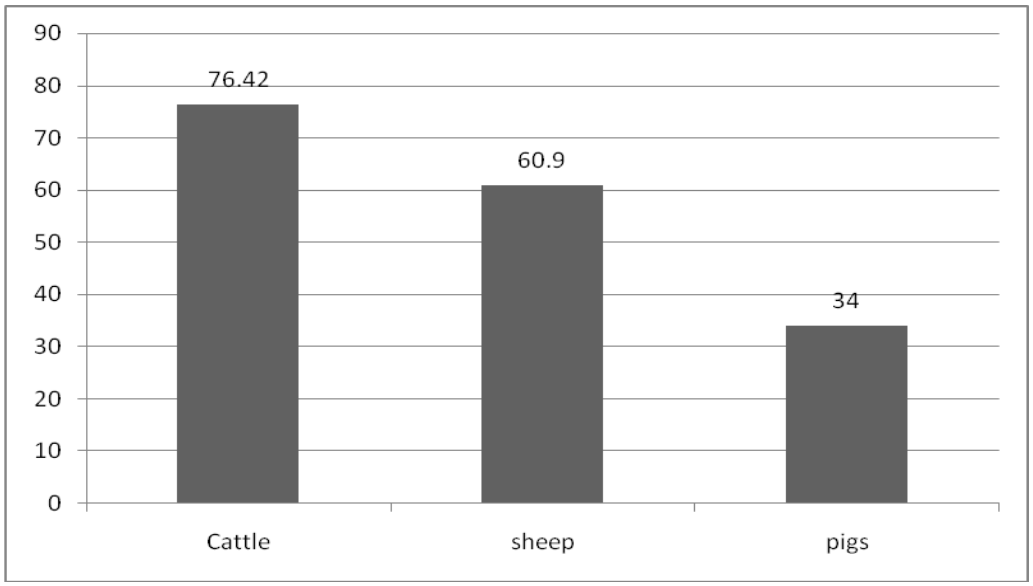
Table.1 Frequency of toxoplasmosis in cats by neighborhood

Neighborhood	Workforce	Number of positive cases	Prevalence (%)
Diata	40	3	7.5
M'PILA	30	2	6.66
Poto poto	20	1	5
Mayanga	10	0	0
Total	100	6	6

Table.2 Animal prevalences by site

SITE	SEROLOGY											
	CATTLE				SHEEP				PIGS			
	Serology positives		Serology negative		Serology positives		Serology negative		Serology positives		**Serology negative**	
	number	%	number	%	number	%	number	%	number	%	number	**%**
Slaughter houses M'PILA	99	82.5	21	17.5								
Breeding sites LYCEE CABRAL AMILCAR	8	40	12	60	13	65	7	35	0	0	0	**0**
Slaughter houses MOUNGALI RUE MBAKA	0	0	0	0	24	60	16	40	0	0	0	**0**
Breeding sites ENSAF	0	0	0	0	0	0	0	0	5	15.62	27	**84.37**
Slaughter houses Marche DIATA	0	0	0	0	0	0	0	0	12	66.66	6	**33.33**
Breeding sites MAYANGA	0	0	0	0	19	63.33	11	36.66	0	0	0	**0**
breeding sites MADIBOU	0	0	0	0	11	55	9	45	0	0	0	**0**
Total	**76.42%**		**23.57**		**60.90%**		**39.09**		**34%**		**66%**	

Figure.1 Prevalence of toxoplasmosis per animal



We found a seroprevalence of 34% in pigs, very close to that found in Finland by Jokelainen *et al.,* in 2012, which was 33%, and by Franco Hernandez *et al.,* in 2015, which was 30%. In Nigeria, it was 29.1% in a study conducted by Onyiche and Ademola in 2015.

This study shows a high prevalence of toxoplasmosis in the animal population, which correlates with the presence of oocysts found in cat faeces, demonstrating that environmental contamination is a source of animal contamination, with meat being the source of human contamination.

Thus, despite the small size of our sample, we can say that Brazzaville's cats are infested with toxoplasmosis and that they excrete oocysts into the environment at a given time.

In addition, knowledge of the seroprevalence of toxoplasmosis in cattle and the degree of contamination of meat are essential epidemiological parameters in an individual or collective prevention approach. This study provides us with a better understanding of the dynamics of toxoplasma transmission to humans, demonstrating the importance of hygienic-dietary measures for both humans and animals. Future studies will enable us to genetically characterize and determine the similarities of strains from various origins.

Author Contribution

Martinien Mayela: Investigation, formal analysis, writing—original draft. Jespin Akinikossou: Validation, methodology, writing—reviewing. Moyen Nanikaly:—Formal analysis, writing—review and editing. Rachel Moyen: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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