

Full Length Research Paper

Seroprevalence and risk factors of neosporosis in women attending antenatal care and in domestic carnivores in Dakar city, Senegal

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The objective of the study was to estimate the seroprevalence of neosporosis due to *Neospora caninum* as well as the associated risk factors in women attending antenatal consultations and in domestic carnivore in the Dakar region, Senegal. For this purpose, 100 blood samples among women in Ante Natal Clinics (ANC), 141 in the dog's group, and 113 among the cats were collected and were examined by using ELISA. Regarding risk factors acquisition, a questionnaire was associated with each sample, and obtained data were compiled. The R Commander software was used for statistical analysis. In women attending ANC, overall seroprevalence rate was $17 \pm 7.3\%$. Forty-three percent (43%) had reported an abortion and 14% of them were positive of neosporosis. In multivariate analysis, only the consumption of grilled meat predisposed to neosporosis (OR = 3.38 and $p = 0.021$). Among the animals' group, the serological test showed a prevalence rate of $42.55 \pm 8\%$ in dogs and $60.1 \pm 9\%$ in cat's groups. More research on *Neospora caninum* need to be explored and its relationships with pregnant women particularly those with repetitive miscarriages.

Keywords: Neosporosis, seroprevalence, risk factors, pregnant women, domestic carnivores, Senegal.

LIST OF ABBREVIATIONS

ANC: Ante Natal Clinics
CI: Confidence Intervals
ELISA: Enzyme Linked Immuno Sorbent Assay
OR: Odds ratios
p : p value
Pa: apparent prevalence

INTRODUCTION

Neosporosis is a cosmopolitan disease caused by *Neospora caninum* (Dubey et al., 2007) and its definitive

host is canine. It was described for the first time by Bjerkas in 1984 in puppies with progressive paralysis following encephalopathy and myositis in Norway. Essentially abortive in cattle, it causes severe neurological disorders and often fatalities in dogs. It is also suspected of causing abortions in other domestic and

wild ruminants, as well as in the equine species (Pitel *et al.*, 2010). Neosporosis is a recently identified protozoa disease in dogs and cattle (Dubey *et al.*, 1988). At its discovery, it is first confused with toxoplasmosis because of the likeliness of their structures and life cycles, until 1988 when Dubey *et al.*, (1988) differentiated them on the ultra-structural and antigenic level. The prevalence observed in various dog populations is around 20% worldwide. In Dakar (Senegal), the prevalence is estimated at 14.2% (Kamga-Waladjo *et al.*, 2009).

Despite its high morbidity with economic and health repercussions (Kamga-Waladjo *et al.*, 2008), this protozoa disease is phylogenetically very closed to toxoplasmosis but remains neglected because of its mild symptoms. In Dakar, there is very limited information on its prevalence. As a result, this study was carried out to determine the seroprevalence of neosporosis and to identify the risk factors associated both in pregnant women attending Ante Natal Clinics (ANC) and domestic carnivores.

MATERIALS AND METHODS

Type of study

A cross-sectional study was conducted in 2012 at Abass Ndao Hospital in Dakar.

Study population

Human population

In this study, women of various origins who came to antenatal care and registered at the Abass N'Dao hospital were investigated. The questionnaire on the potential risk factors of neosporosis was administrated only to those who consented to participate in the study. The questionnaire included the following topics: sociodemographic factors (ethnic group, age, profession, schooling, origin), obstetrical history, possession of domestic carnivores as well as cooking and eating habits (in particular the consumption of unpasteurized fresh milk and / or local grilled meat "dibiterie", the type of water and meat consumed. For an expected prevalence of 10% and a risk of error of 6%, the sample size required was 100 women (Win Episcope 2.0 software). Blood sample was taken at the median vein of the elbow crease.

All sera were tested by the indirect Enzyme Linked Immuno Sorbent Assay (ELISA) technique for IgG titration for all species. The LSIVET *N. caninum* Blocking Kit ELISA [Ref.: 5-Vetneo-001 Se = 89% and Sp = 99%] and ELISA Thermo Scientific Multiskan © type reader were used for the treatment and analysis of samples taken according to the LSIVET laboratories prescriptions. All analysis was performed in accordance with LSIVET laboratory requirements.

Population of domestic carnivore

For an expected prevalence of 10% and a risk of error of 6%, 100 of each domestic carnivore were sufficient for

the study (Win Episcope 2.0 software). One hundred and thirteen (113) cats and 141 dogs were captured in different quarters of Dakar and from which blood samples were collected. Samples were collected from saphenous vein. A questionnaire was filled for each animal. It consisted of two parts: the first relating to the animal's identification and the second part to the animal health status. As for women, the diagnostic test used was ELISA. After getting the samples, all the animals were released.

Data Management and Statistical Analysis

Epidata © software was used for data entry. Statistical analysis were performed using R commander © version 2 and SAS version 9.2 software. In descriptive analysis, percentages with 95% confidence intervals (CI) were estimated. Univariate and multivariate analysis were performed in order to determine factors associated with neosporosis seroprevalence. Odds ratios (OR) were seek to determine association with 95% CI.

RESULTS

Prevalence and factors of variation of neosporosis in women attending antenatal care

A total of 100 women were surveyed and blood samples were collected from all of them. The apparent and real prevalence of neosporosis were $17 \pm 7.3\%$ and $18.1 \pm 7.5\%$, respectively (Table 1). This prevalence varies with age, occupation, consumption of meat, fresh milk and / or local grilled meat "dibiterie". The bivariate analysis of risk factors showed that consumption of local grilled meat "dibiterie" predisposes women to be infected with neosporosis (Table 2).

Prevalence and risk factors of neosporosis in domestic carnivores

In dogs, the apparent prevalence of neosporosis is $42.5 \pm 8\%$ while the real prevalence was $47.21 \pm 8.21\%$. No factor was statistically associated to neosporosis in multivariate analysis. The apparent and real prevalence of neosporosis were $60.1 \pm 9\%$ and $67.25 \pm 8.6\%$, respectively for cats. Sex, deworming and vaccination were considered as associated risk factors after various analyses (Table 3).

DISCUSSION

In women

The overall prevalence of neosporosis in this study was 17%. It is higher than the one obtained in Egypt (7.5%) (Hany *et al.*, 2009). This difference could be explained by climatic conditions that influence the survival of the parasite in the environment (Dubey and Schares, 2011).

Table 1. Seroprevalence of neosporosis and abortion prevalence among pregnant women by age group.

Age range	Numbers	abortions		Seropositive to <i>N. caninum</i>				
				All women		Women having aborted		p
(years)	Total	P	Pa± CI(%)	P	Pa± CI(%)	P	Pa± CI(%)	
< 25	25	5	20±15.7	3	12±12.73	0	0	0.7
25-39	51	19	37.2±13.2	9	17.6±10.4	2	10.5±8.41	
≥ 40	24	19	79.1±16.2	5	20.8±16.2	4	21.1±16.3	
Total	100	43	43±9.7	17	17±7.3	6	14±7.3	

CI = Confidence Interval P = Positive.
 Real prevalence (Pr) = 18.1 ± 7.5%.

Table 2. Risk Factors of Neosporosis in Women attending ANC.

Risk factors		N	Seroprevalence (Positive)				
			n	Pa%	p	OR	Clat 95%
Cats in the house	Yes	73	12	16.4	0.80	0.86	0.24-3.50
	No	27	5	18.5			
Profession	Active	58	11	19	0.53	1.399	0.42-5.06
	Unemployed	42	6	14.3			
Level of Education	Illiterate	45	9	20	0.47	0.68	0.20-2.22
	Educated	55	8	14.5			
Maternity	Nulliparous	12	3	25	0.43	0.48	0.099-2.37
	F.p.deliveries	43	6	14	Ref.		
	multiparous	45	8	17.8	0.89		
Dog in the house	Yes	3	1	33.3	0.44	0.39	0.02-24.75
	No	97	16	16.5			
Contact with cat	Yes	44	9	20.4	0.67	1.25	0.43-3.60
	No	47	8	17			
Presence of sheep	Yes	76	12	15.8	0.56	0.71	0.20-2.92
	No	24	5	20.8			
Water consumption	SDE water	77	14	18.2	0.56	1.476	0.35-8.81
	Mixed**	23	3	13			
Consumption of market garden products	Yes	98	17	17.3	0.51	-	0.03-Infinite
	No	2	0	0			
Consumption of fresh milk	Yes	72	15	20.8	0.10	3.38	0.7-32.68
	No	28	2	7.1			
Degree of cooking of the meat consumed	Well cooked	56	12	21.4	0.18	0.473	0.12-1.60
	Little cooked	44	5	11.3			
Grill (oil)	Yes	84	14	16.6	0.83	0.86	0.19-5.36
	No	16	3	18.7			
Consumption of local grilled meat "dibiterie"	Yes	75	9	12.0	0.021*	3.45*	1.15-10.28
	No	25	8	32.0			

F.p. deliveries = few previous deliveries ** = water SDE + mineral water and / or well water * = Significant Ref = reference factor N: Numbers, n: positive, Pa: apparent prevalence; p: p-value, CI: Confidence Interval.

Of the 43% of women who had abortions, 14% were *Neospora caninum* positive. This was similar (13.5%) with a study conducted in Kaolack by Adje in 2012 (Adje, 2012). *Neospora caninum* might be one of the causes of unexplained abortions in antenatal clinic women in Dakar, although according to Dubey *et al.* (2007) its zoonotic character remains to be proven (McAllister, 2017)].

Moreover, anti-*Neospora* antibodies have been found in patients (Lobato *et al.*, 2006). For Hany *et al.*, (2009), investigations should be made to remove doubt. Consumption of "dibiterie" grilled meat was shown as a risk factor (p = 0.02, OR = 3.45 and CI = 1.15-10.28). "Dibiterie" is a local barbecue grilled meat that is very popular with Senegalese women, and one of the sources

Table 3. Risk factors for neosporosis and their prevalence in domestic carnivores.

Variables		DOG			P	OR	CI	CAT			p	OR	CI
		N	n	Pa(%)±CI				N	n	Pa (%)±IC			
Race	Local	47	20	42.5±14	Ref.0.4440.30	0.71 1.26	0.20- 2.47 0.59- 2.66	113	68	60.1±9	-	-	-
	crossed	15	8	53.3±25				-	-	-			
	exotic	79	32	40.5±11				-	-	-			
Sex	Females	60	29	48.3±13	0.23	1.5	0.7- 2.9	71	50	70.4±10	0.003*	3.1*	1.4- 7.0
	Males	81	31	38.2±10				42	18	42.8±15			
Age	Youth	31	12	38.7±17	0.71	1.16	0.51- 2.61	14	11	78.5±21	0.15	0.37	0.09- 1.43
	Adults	110	48	43.69				99	57	57.5±10			
Way of life	P.Wanderers	6	3	50±40	0.51	0.	0.07-	101	66	65.3±9	0.96	-	-
	O.Wanderers	9	2	22.2±27	Ref.	28	1.81						
	household	126	55	43.6±9	0.41	0.36	0.03- 2.69	12	2	16.6±21			
dewormed (internal)	Yes	99	42	42.4±10	0.15	0.92	0.42- 1.98	6	0	0 ±0	0.003	0.04	0.002- 0.79
	No	36	16	44.4±16				103	68	63.5±9			
	Unknown	06	02	33.3				04	0	0 ±0			
Vaccinated (rabies)	Yes	99	42	42.4±10	0.14	0.84	0.39- 1.81	4	2	50 ±49	0.001	0.03	0.002- 0.65
	No	37	17	45.9±16				102	66	64.7±9			
	unknown	05	01	2				07	0	0 ±0			
Total		141	60	42.5±8	Pr= 47.21±8.21			113	68	60.1±9	Pr= 67.25±8.61		

O. Wanderers: Casual Wanderers P. Wanderers: wanderers permanent * = Significant OR: Odds Ratio N : Numbers. n: positive. Pa: apparent prevalence ; p : p-value. CI: Confidence Interval.

of *Neospora* infection is the consumption of undercooked meat (such as barbecues), which corroborates our diagnosis. Precautions and awareness of the population on the risks of eating such types of meat (“dibiterie”, barbecue) should be considered.

In the animals

In dogs, neosporosis prevalence was quite 42.5%. It is higher than that obtained in other studies in Europe (Dubey and Schares, 2011, Schüle *et al.*, 2015), Australia (Ash *et al.* 2015, Sloan *et al.*, 2017), Africa (Asmare *et al.*, 2014) and even Senegal by Kamga-Waladjo *et al.* (2009), who obtained 14.2%. The study conducted in Dakar, Senegal did not find any association between risk factors and positivity rate (Kamga-Waladjo *et al.*, 2009).

Despite the higher prevalence observed in our study, no variables were associated with seropositivity. This finding is consistent with the result reported a few years ago by Kamga-Waladjo *et al.* (2009). Several studies illustrated the global distribution of the parasite, but variations observed in seroprevalence have not yet been justified. However, they may reflect the maintenance of infection risk and exposure of intermediate hosts, particularly in cattle. Moreover, high prevalence observed by Kamga-Waladjo *et al.*, (2008) in local cows in Dakar (71.43%) associated with that of dogs, would likely be a factor of human exposure to *N. Caninum* in Senegal.

In cats, the prevalence of *N. caninum* was 60.1± 9%. It is distinctly superior compared to other studies: 0.6% in Hungary (Hornok *et al.*, 2008), 6.8% in Spain (Millan *et al.*, 2009), 10.3% in Australia (Silaghi, *et al.*, 2014), 24.5% in

Brazil (Bresciani *et al.*, 2007), and 32% in Algeria (Dubey and Schares, 2011). These data also showed a global distribution of neosporosis in cats, but for the time being there is no explanations for the different variations observed in the prevalence rate. Sex appears as an associated risk factor ($p = 0.003$, OR = 3.1, CI = 1.4-7.0). In fact, females were 3 times more likely to be infected. This may be due to pregnancy stress in females that may stimulate reactivation of encysted tissue forms thus contributing to the release of tachyzoites into detectable blood. However, deworming and vaccination can be considered as protective factors to some extent.

CONCLUSION

In Dakar, the observed prevalence may reflect the risk that women become more exposed to the parasite. With the uncertainty to date on public health importance of neosporosis according to some studies (Dubey *et al.*, 2007, Lobato *et al.*, 2006, McAllister, 2017), it would be wise to invest more in research on *N. caninum* and its relationships with women during pregnancy particularly those with repetitive miscarriages to remove controversy.

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