**Seroprevalence and risk factors of two abortive diseases, toxoplasmosis and neosporosis,
in small ruminants of the Mongo County, southern Gabon.**

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Abstract

In order to estimate the seroprevalence and to assess risk factors of *Toxoplasma gondii* and *Neospora caninum* in the province of Nyanga, in southern Gabon, a cross-sectional study was conducted in sheep and goats in the county of Mongo. Serological screening was performed using an indirect multi-species enzyme-linked immunosorbent assay and a commercial direct agglutination test to test serum samples for the presence of anti-*N. caninum* and anti-*T. gondii* Immunoglobulines (Ig) G antibodies, respectively.

From a total of 201 small ruminants, including 95 sheep and 106 goats, the overall anti-*N. caninum* and anti-*T. gondii* IgG seroprevalences were 31.3% (n=63) and 45.8% (n=95), respectively. Statistical analyses showed that adult small ruminants were 4 times more likely to be infected with *T. gondii* than young animals ($p<0.001$; OR=4.27; 95% CI: 2.07 – 8.81). The locality was significantly associated with *T. gondii* seropositivity ($p=0.001$). The Dilemba ($p<0.001$; OR=0.07), Moulengui-Binza ($p=0.023$; OR=0.05) and Rina-Nzala localities ($p=0.005$; OR=0.1) were not identified as risk localities associated with *T. gondii* infection. The seroprevalence of *N. caninum* was significantly higher in sheep (42.1%) than goats (21.7%). The species was not associated with *N. caninum* infection ($p=0.005$; OR=0.27; 95% CI: 0.11 – 0.68). In Bibora, small ruminants were almost 4 times more likely to get *Neospora* infection than the animals in the other localities ($p=0.04$; OR=3.98; 95% CI: 1.06 – 14.93). The serological prevalence of *N. caninum* and *T. gondii* could suggest a considerable impact on the reproductive process from these pathogens in sheep and goats within the Mongo County and a high exposure of humans to *T. gondii*.

Key words: *N. caninum*; *T. gondii*; seroprevalence; sheep; goats; Gabon

Introduction

Abortive diseases (AD) are infectious or parasitic diseases having direct effects on animals (Rekiki et al., 2005). AD in small ruminants can be induced by various pathogens including: bacteria such as *Brucella*, *Listeria*, *Coxiella*, *Chlamydia*, *Leptospira* or *Salmonella*; viruses, for example the Rift valley fever virus; and finally, protozoan, such as *Neospora* or *Toxoplasma*. AD directly affect animal productivity by reducing the natality rate (abortions), the production of milk, meat and derivatives (textile, oil...) which may have a global impact on the small ruminant industry. These diseases can also have indirect effects because of the costs of veterinary healthcare and the expenses related to livestock rebuilding. Finally, the trade in small ruminants and their products also merits consideration because sheep and goats may transmit zoonotic abortive diseases such as Rift Valley fever, brucellosis and listeriosis (Sherman, 2001). Thus the study of causal agents of AD has its importance in public and animal health, as in the case of *Toxoplasma* and *Neospora*.

Neosporosis is an AD due to the cosmopolitan coccidian parasite, *Neospora caninum* that mainly affects dairy cattle in which it induces 10-25% of abortions (Bowman et al., 2003; Dubey, 2003; Dubey et al., 2007). It can also cause infections in dogs and, occasionally in horses, deer, sheep and goats (Dubey, 2003). Several studies suggest that neosporosis is frequent in goats. Indeed, some cases of caprine neosporosis have been described in California (Barr et al., 1991), Pennsylvania (Dubey et al., 1992), Costa Rica (Dubey et al., 1996) and Brazil (Corbellini et al., 2001). In addition, studies have reported the presence of antibodies against *N. caninum* in goats in Sri Lanka (Naguleswaran et al., 2004), in Argentina (Moore et al., 2007), in Brazil (Faria et al., 2007) and in Poland (Czopowicz et al., 2011). Although neosporosis also occurs in sheep, this is less frequent and has been associated with weak offsprings, stillborn births and congenital infections. A weak relation between *N. caninum* infection in sheep and abortion has been suggested (Dubey et al., 1992; Dubey and

Lindsay, 1996; Corbellini et al., 2001; Howe et al., 2008; Dubey and Schares, 2011). In Gabon, the circulation of this AD is still under-documented despite the high prevalence of ADs.

Toxoplasmosis is also an AD, it is caused by the protozoan parasite *Toxoplasma gondii* which is able to infect many species of domestic and wild mammals (Ripert, 1996; Marié et al., 2009). In sheep and goats, toxoplasmosis is the major cause of infectious abortions, stillbirths, and weak offsprings (Innes et al., 2009). *T. gondii* is mainly transmitted by ingesting contaminated grass with feces from definitive infected hosts, domestic and wild felines such as cats (Innes et al., 2009). In some parts of Gabon, sero-epidemiologic studies showed high prevalence values of infection in humans (Duong et al., 1992; Mpiga Mickoto et al., 2010).

Considering the relevance of toxoplasmosis as a zoonosis and the lack of information concerning *N. caninum* in sheep and goats in Gabon, this study aims to determine the circulation of both *T. gondii* and *N. caninum* in sheep and goat populations in rural localities of the Mongo County, southern Gabon, and their transmission risk factors through a sero-epidemiological investigation.

Materials and Methods

Ethical considerations

For this study, verbal authorization was previously obtained from the local authorities, namely the heads of the villages. An oral consent was obtained from all breeders of small ruminants for blood sampling.

Study area

Nine localities of the Mongo County were selected based on the presence of small ruminants and livestock activities (Figure 1). The Mongo County (latitude: 3°26'S; longitude:

11°44'E) is located about 65 km from Tchibanga, capital of the Nyanga province at the south-west of Gabon. This area is a plateau facing the Mayombe mountain range filled with savannah and vast grasslands containing some small shrubs. There, the equatorial climate is warm and humid with a mean temperature of 25.3°C and an average rainfall of 1371 mm/year. The Mongo County houses the largest and most important cattle breeding (ranch Nyanga) of the country with a population estimated at 4,690 cattle heads.

Animals sampling

No preliminary census of livestock was done in this region. No specific inclusion criteria was defined. Healthy animals (presenting no clinical symptoms) were selected according to the ability of breeders to maintain them during the blood collection. Any clinical state was an exclusion criteria. No anesthetic or other chemical treatment was administrated before blood collection.

Blood and data collection

Animal whole blood was collected in 4-ml BD Vacutainer tubes (BD-Plymouth, UK) by puncturing the jugular vein. Serum and plasma were separated by centrifugation (3000 rpm for 3 minutes) and stored at -20°C until their shipment to the *Centre International de Recherches Médicales de Franceville* (CIRMF) for further serological analyses.

Data on health history (vaccination, last diseases, abortions and stillbirths in females), species, sex, age were collected using a standard questionnaire submitted to each breeder. Animals were classified as young or adult based on dentition (Russel, 1991).

Serological screening

The presence of anti-*N. caninum* IgGs was tested by indirect multi-species enzyme-linked immunosorbent assay (ELISA) on serum samples using the ID Screen® *Neospora caninum* Indirect Multi-species (IDVet Innovative Diagnostics, France) (Alvarez-Garcia et

al., 2013) following the manufacturer's instructions. ELISA plates were then read by spectrophotometry using the SUNRISE automate (Tecan Austria GmbH, Austria). The cut-off has been fixed at 50% (percentage S/P (S/P%)), following the manufacturer's instructions. The S/P ratio or Serum/Plasma ratio, is the ratio of the difference between the sample's optic density (OD) and the OD of the negative control on the difference of OD of the positive control and the OD of the negative control, or $S/P\% = [(OD_{\text{sample}} - OD_{\text{negative control}}) / (OD_{\text{positive control}} - OD_{\text{negative control}})] \times 100$. The results were considered positive if the S/P percentage was $\geq 50\%$ and all values $\leq 40\%$ were negative. As proposed by the manufacturer, all sera that react with S/P% values between 40% and 50% are considered questionable. The serum samples were analyzed for *T. gondii* specific IgG antibodies using a commercial direct agglutination test (Toxo-Screen DA, bioMérieux, France), following the manufacturer's instructions. Sera reacting at the 1:40 dilution were regarded as positive, according to the manufacturer.

Statistical analysis

The risk of seropositivity was assessed by logistic regression. P-value < 0.05 was considered for significant results. Odds ratios (OR) and 95% confidence intervals (CI) were presented as risk index. Univariate and multivariate models were built considering four variables (age, species, sex and locality). Analyses were performed using the R software version 3.1.0 (Development Core Team 2014, USA).

Results

A total of 201 small ruminants were sampled, including 106 goats and 95 sheep. Among them, 38% of the females in goats and 62% of the females in sheep had problems of abortions and/or stillbirths.

The results showed that the overall anti-*N. caninum* IgG seroprevalence was 31.3% (95% CI: 24.9 – 37.7). The seroprevalence of *N. caninum* was higher in sheep (42.1%) than goats (21.7%). Whatever the species, the young had a lower prevalence (28%) than adults (33.3%) (Table 1). Finally, the results showed that the seroprevalence of neosporosis in females (32.1%) was higher than males (28.9%). The table 2 showed specific anti-*N. caninum* IgG were found in 7 of the 9 villages investigated. The highest number of positive samples was collected from the locality of Bibora, where 21/38 (55.3%) tested samples presented IgG antibodies specific to *N. caninum*.

For *T. gondii*, a total of 92 individuals (45.8%; 95% CI: 38.8 – 52.6) were serologically positive (Table 1). Sheep had a higher seroprevalence (57.9%) than goats (34.9%). Moreover, whatever the species, young animals had a lower seroprevalence (26.7%) than adults (57.1%). Finally, females showed a higher serological prevalence (48.7%) than males (35.6%). Specific anti-*T. gondii* IgG were found in animals from 8/9 villages (Table 2). The highest number of positive samples was collected from the locality of Rina-Nzala, where 14/17 (82.4%) tested samples presented IgG antibodies specific to *T. gondii*. Mixed infections were found both in sheep and goats. Indeed, *T. gondii* and *N. caninum* specific IgG were found in 14.93% of the studied population, including 28.42% and 2.83% in goats and sheep, respectively. Analyses of the results showed that 66.6% (14/21) of the females which were reported in the questionnaire as having had an abortion and/or stillbirths were seropositive for at least one of the two abortive parasites studied (Figure 2).

The results of the univariate and multivariate logistic regression analyses are summarized in tables 3 (supplementary material 1) and 4 (supplementary material 2). The age of the small ruminants showed significant differences in regard to potential risk factors for *T. gondii* infection. The adults were 4 times more likely to be infected with *T. gondii* than young animals ($p < 0.001$; OR=4.27; 95% CI: 2.07 – 8.81) (Table 4). No statistically significant

association was found between age and seropositivity to *Neospora caninum* in sheep and goats. Concerning the locality, Bibora was significantly associated with *N. caninum* infection ($p=0.04$; OR=3.98; 95% CI: 1.06 – 14.93). No locality was significantly associated with *T. gondii* infection. The type of species was not identified as a risk factor for *N. caninum* infection. Likewise, no significant association of species with *T. gondii* infection was shown. Similarly, no significant association was found between the sex of individuals and seropositivity to *N. caninum* and *T. gondii* in small ruminants.

Discussion

This study highlights serological evidence regarding the circulation of two abortive pathogens, *T. gondii* and *N. caninum* in small domestic ruminants from southern Gabon, where abortions and/or stillbirths have been observed in sheep and goats.

Few studies have been led on seroprevalence of neosporosis in small ruminants. The overall anti-*Neospora caninum* IgG antibodies found here was 31.34%. This result was higher than those found in sheep and goats in China (8.726%) using an indirect ELISA test and an indirect fluorescent antibody test (IFAT) (Liu et al., 2015) ; in goats in Brazil (4.58%) using the indirect immunofluorescence test (Topazio et al., 2014); in dairy sheep in Argentina (3%) using an IFAT (Hecker et al., 2013); and in sheep and goats in Greece (12.4%) using an ELISA test (Anastasia et al., 2013). These observations could suggest that animals in this region of Gabon may be less sensitive to neosporosis or simply under-exposed to the parasite. However, differences between methodologies and the variability of the analyzed species make a tight comparison difficult.

The anti-*Toxoplasma gondii* IgG antibodies prevalence found in this study (45.77%) was much higher than those found in sheep and goats in China by Liu et al. (2015) and Yin et al. (2015), 25.6% and 20.3%, respectively; lower than that found in sheep and goats in Greece

(57.1%) (Anastasia et al., 2013); close to those found in many studies conducted in sheep and goats in Brazil (40.5% and 48.7%) (Rêgo et al., 2016), in sheep in the Ivory Coast (Kone et al., 2008) and Senegal (Dia, 1992; Pangui et al., 1993; Pangui, 2011), respectively 40% and 38.5-55%; and much higher than those found in South Africa (4.3% and 5.6%, respectively by ELISA and IFAT) (Samra et al., 2007). However, other studies reported lower seroprevalences in dairy sheep in Argentina (17.3%) (Hecker et al., 2013), in goats in Senegal (33.75%) (Dia, 1992), in goats in Myanmar (11.4%) (Bawm et al., 2016), except for Benkirane et al. (2015) who reported an overall serological prevalence of toxoplasmosis of 74% in sheep and goat herds in Morocco, using an indirect ELISA kit. Finally, as suggested by Gebremedhin et al. (2015), the differences observed in the seroprevalence of toxoplasmosis and neosporosis between this study and the aforementioned studies may be the result of serological techniques (Huang et al., 2010), sample sizes and sampling techniques (Halova' et al., 2012), type of breeding (wandering or confinement) (Dubey, 2009; Dubey, 2010), climatic variations and density of felines (Gharekhani, 2013; Heidari et al., 2013).

The study of risk factor associated with both infections showed that small ruminants from Bibora were almost four times more likely to get *Neospora* infection than animals from the others localities. This disparity between the localities could be due to the presence of stray dogs found in the locality of Bibora, as well as poor management of household refuse by rural human populations, creating dumps which are common sources of food for these stray dogs and the wandering small ruminants. According to Liu et al. (2015) poor hygiene conditions presented a greater risk of *N. caninum* infection in small ruminants in China. Otherwise, previous studies have already pointed out that contact with domestic dogs or the presence of dogs in farms were an important risk factor associated with neosporosis infection in sheep and goats (Abo-Shehada and Abu-Halaweh, 2010; Topazio et al., 2014; Liu et al., 2015). Moreover, the type of species was not identified as a risk factor for *Neospora* infection

although sheep seemed more likely to get *Neospora* infection than goats. This is supported by Dubey et al. (1990), who showed that sheep were more receptive to experimental inoculation of *Neospora caninum* than goats, and others recent studies in sheep and goats (Nasir et al., 2012; Anastasia et al., 2013; Liu et al., 2015). A previous study in Jordan in 339 sheep and 309 goats showed that the seroprevalence of *N. caninum* was significantly higher in sheep (63%) than goats (2%) (Abo-Shehada and Abu-Halaweh, 2010). Finally, seropositivity to *N. caninum* was not significantly associated with age and sex in sheep and goats. This is supported by Topazio et al. (2014) whose study showed no significant association between infection with *Neospora caninum* and age and sex in goats.

Only age was identified as a potential risk factor of infection with *T. gondii*. Indeed, age was significantly associated with *T. gondii* infection in small ruminants. In this-study, adults were 4.27 times more likely to be infected with *T. gondii* than young animals. According to other previous studies, the seroprevalence of *T. gondii* is also significantly higher in adults (Rossi et al., 2011; Hecker et al., 2013). The higher risk of infection to *T. gondii* in adults suggests that horizontal transmission by ingestion of sporulated oocysts in the environment seems to be the main method of transmission (Rossi et al., 2001) because young are fed exclusively on breast milk during the first months of their life, therefore their immunity would be reinforced by the colostrum. However, in their studies, Bawm et al. (2016) and Yin et al. (2015) did not identify age as a risk factor associated with *T. gondii* infection in goats and sheep. Moreover, even though the type of species was not pointed out as a risk factor associated with *T. gondii* infection (OR=0.39), sheep seemed more likely to get *Toxoplasma* infection than goats. The same result has been found by Rêgo et al. (2016) in Brazil. On the other hand, other studies reported that goats had higher *Toxoplasma* seroprevalence than sheep (Anastasia et al., 2013; Liu et al., 2015).

The high seroprevalence in some of the studied localities can be explained by a stronger presence of domestic cats in these areas, with which small ruminants were regularly in contact.

Finally, regarding gender, although females showed a higher serological prevalence (48.7%) than males (35.6%) as shown in previous studies (Van der Puije et al., 2000; Hecker et al., 2013; Bawm et al., 2016), but in contrast to those reported in other studies (Soares et al., 2009; Ueno et al., 2009; Yin et al., 2015), a significant association between gender and *T. gondii* seropositivity was not found in either sheep or goats. This result is supported by Yin et al. (2015) and Rêgo et al. (2016) who showed that gender was not a significant risk factor for sheep. Otherwise, Rêgo et al. (2016) showed that gender (female) was a significant risk factor for goats.

Co-infections with both *N. caninum* and *T. gondii* were found in some localities where we investigated both sheep and goats. This has previously been reported by several studies (Rossi et al., 2011; Hecker et al., 2013; Liu et al., 2015).

The results of the investigation with the breeders may suggest that these pathogens could have been the cause of the abortions or stillbirths observed in females. However, the detection of IgG antibodies does not reflect current or active infections. Moreover, the research of potential abortive agents was not conducted exhaustively. Therefore, it is too early to draw conclusions on the involvement of *N. caninum* and *T. gondii* in these abortions and stillbirths. The case of the remaining 7 females which had problems of abortions and/or stillbirths, in which we did not find IgG antibodies against the two abortive pathogens, can be explained by the fact that these females were not infected with the pathogens researched.

Conclusion

The serological prevalences of *N. caninum* and *T. gondii* reported in this study could suggest a considerable impact on the reproductive process of sheep and goats, particularly within the Mongo county. To better understand the impact of these two abortive agents and others (*Brucella*, *Salmonella*...) on animal health in Gabon, further investigations are required on larger ruminant populations in other regions and on a bigger number of cases of abortions or stillbirths to provide detailed information on reproductive losses and the responsible causative agents. In addition, continuous monitoring and implementation of integrated control strategies (control of stray dogs and cats, public awareness campaigns...) of *N. caninum* and *T. gondii* infections are needed to develop livestock in Gabon and prevent human infections.

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Figure captions

Figure 1. Location of the study area. The red ellipse represents the nine selected villages of the Mongo County.

Figure 2. Identification of *N. caninum* and *T. gondii* IgGs in females with problems of abortion/stillbirth.

Table 1. Presence of IgG anti-*N. caninum* and anti-*T. gondii* in small ruminants of the Mongo County.

Parasites	Species	N of IgG-positive sera (n)		Sex				Age			
				Male		Female		Young		Adult	
		n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
<i>N. caninum</i>	Sheep	40/95	42.1	7/17	41.2	33/78	42.3	11/28	39.3	29/67	43.3
	Goats	23/106	27.7	6/28	21.4	17/78	21.8	10/47	21.3	13/59	22.0
	Total	63/201	31.3	13/45	28.9	50/156	32.1	21/75	28.0	42/126	33.3
<i>T. gondii</i>	Sheep	55/95	57.9	5/17	29.4	50/78	64.1	7/28	25.0	48/67	71.6
	Goats	37/106	34.9	11/28	39.3	26/78	33.3	13/47	27.7	24/59	40.7
	Total	92/201	45.8	16/45	35.6	76/156	48.7	20/75	26.7	72/126	57.1

N : number of samples tested

Table 2. Seroprevalences of IgG antibodies against *N. caninum* and *T. gondii* in small ruminants from the villages in the Mongo County.

Villages	N° of IgG positive sera/N° tested		Prevalence (%)	
	<i>N. caninum</i>	<i>T. gondii</i>	<i>N. caninum</i>	<i>T. gondii</i>
Pc Nyanga	3/31	9/31	9.67	29.03
Moulengui-Binza	9/27	17/27	33.33	62.96
Dilemba	8/33	22/33	24.24	66.66
Doumanga	5/17	8/17	29.41	47.05
Rina-Nzala	6/17	14/17	35.3	82.35
Bibora	21/38	9/38	55.3	23.68
Bayadi	11/24	12/24	45.8	50
Mabotsa	0/9	0/9	0	0
Mibamba	0/5	1/5	0	20

Figure 1

Figure 2