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Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in non-human primates at a primate center at Franceville, Gabon

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Toxoplasma gondii **can cause fatal disease in both humans and non-human primates.** ***Neospora caninum*** **can also cause economic loss and disease to livestock. The distribution of antibodies against these parasites in non-human primates bred at the CIRMF Primate Center in Franceville, Gabon was determined. For their annual medical examination,** ***T. gondii*** **antibodies were identified using a modified agglutination test (MAT). Twenty-one percent were positive with antibody titers varying from 1:40 to 1:4000.** ***Pan troglodytes*** **(*n*=38; 42.1%) had the highest seroprevalence followed by** ***Mandrillus sphinx*** **(*n*=139; 16.5%). Only one** ***Gorilla gorilla*** **out of the four examined and one** ***Cercopithecus solatus*** **out of 12 were positive. At the same time, the general seroprevalence of** ***N. caninum*** **determined by competitive enzyme linked immunosorbent assay (ELISA) was 68.67% in the four species tested.** ***M. sphinx*** **(*n*=139; 66.12%),** ***P. troglodytes*** **(*n*=38; 16.75%),** ***C. solatus*** **(*n*=12; 4.75%), and** ***G. gorilla*** **(n=4; 1.10%) had the highest prevalence. Co-infection was noted in 24.07% of the positive cases. This study suggests that these primates may constitute different reservoirs for** ***T. gondii*** **and** ***N. caninum*** **in the cystic form and high distribution of these parasites in this environment.**

Key words: *Toxoplasma gondii*. *Neospora caninum*, antibodies, old world monkeys.

INTRODUCTION

Toxoplasma gondii is a Protozoa Apicomplexa intracellular parasite that infects most homeothermic animals (Nicolle and Manceau, 1909). This zoonosis can cause significant economic loss, as well as a significant public

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health problem (Dubey and Schares, 2011; Gharekhani, 2013). Some of the clinical features induced by *T. gondii* are abortion in humans and animals, neurological involvement, fetal malformation, and encephalitis in immunocompromised individuals (Sahwi et al., 1995; Israelski and Remington, 1988). The definitive host is felid. Different animals are infected via oocysts in the soil-contaminated oocyst (Frenkel et al., 1975) or ingestion of infected meat with cysts or bradyzoites. *T. gondii* is responsible for substantial mortality in New World primates (Central and South America) (Dubey et al., 1985). The disease is characterized by pulmonary involvement, diarrhea, and hypothermia (Salant et al., 2009; Carme et al., 2009). Old World primates (Africa, Southeast Asia) seem to be asymptomatic (Dietz et al., 1997; Dubey, 1986), and it is not clear whether they are resistant or susceptible but clinically asymptomatic.

Neospora caninum is a parasite discovered in 1984 (Bjerkas et al., 1984) and identified in 1988 (Dubey et al., 1988). It shares general morphological traits with *T. gondii* and clinical manifestations such as abortion in cattle (Dubey and Lindsay, 1996). The definitive hosts are dogs and coyotes (Canidae) and intermediary hosts are mainly cattle, but many warm-blooded animals have been reported to be infected (Shannon et al., 2015). The parasite is acquired congenitally or when carnivores ingest tissues containing bradyzoites; other animal species may be infected by ingesting food or drinking water containing sporulated oocysts (Dubey et al., 2007). Perinatal mortality has been reported in axis deer (Basso et al., 2014). However, some species seem resistant to *N. caninum* tachyzoites (Uillians et al., 2013).

In general, recent reports have shown that *T. gondii* and *N. caninum* are present in wild birds (Darwich et al., 2012), dogs (King et al., 2012), kangaroos (Mayberry et al., 2014), and rodents (Meerburg et al., 2012), but a recent review did not mention any report on old world primates (Shannon et al., 2015). Although there are recent reports of *T. gondii* in new world primates (Pires et al., 2012), there is no recent report on African non-human primates. In Gabon, timber exploitation has reduced the size of the primate habitat. Human-animal contact has therefore become frequent because of the use of common water sources and forest resources for such uses as agriculture, fishing, hunting, timber, and ecotourism. This situation will increase zoonotic disease prevalence or transmission. Many non-human primates are endangered species due to human activities or sometimes disease outbreaks. Many disease outbreaks of environmental origin have been reported in humans, specifically *T. gondii* (Carme et al*.,* 2002) and in Brazil (Vaudaux et al., 2010).

The outbreak of these diseases has not been reported in Gabon, suggesting that the distribution and the mode of dispersion of the pathogenic agent in nature need investigation. The conservation of biodiversity has become an urgent necessity. Due to difficulties in accessing wildlife primates, the International Centre for Medical Research of Franceville (CIRMF) primate center offers the opportunity for studies to be conducted on the distribution and dispersion of certain pathogens because it has animals in a semi-free-ranging enclosure natural forest and in cages. The prevalence of *T. gondii* and *N. caninum* among endemic primates in Gabon is not known. Therefore, the aim of this study was to provide preliminary results to make this information available.

MATERIALS AND METHODS

Sociodemographic characteristics of animals

*Gorilla gorilla*s are ground-dwelling herbivorous animals living in the forest of central Africa; males weigh 180 kg and females weigh 110 kg, and they are about 1.7 to 1.8 m tall. They live in groups. Females become mature at 10 to 12 years of age and males at 11 to 13 years of age; their lifespan is about 35 to 40 years.

Pan troglodytes (chimpanzee) live naturally in the forests and savannas of Central and Western Africa, weighing 40 to 65 kg; females are 1.3 m tall and males are 1.6 m tall. They reach puberty at 8 to 10 years of age and enjoy a 50-year lifespan. They live in a group of 15 to 150 members and are mostly frugivorous.

Cercocebus torquatus are also called red-capped mangabey and live in the coastal forest from Nigeria to Gabon, where they are limited geographically by the Ogooué river in Gabon. They live in groups composed of 14 to 23 members. *Cercopithecus solatus* are located only in Gabon; characterized by their “sun tail”, are generally frugivorous and live in groups of 10 to 20 members.

Mandrillus sphinx lives in the rainforest in central West Africa from Cameroon to Gabon and Congo Brazzaville. They are omnivorous. Two of the most common traits of these species are that they are endangered species due to the destruction of evergreen forest and commercial bush meat hunting. Two others species present in primatology center are: *Macaca mulatta*, also called macaque rhesus monkeys. They move naturally from Afghanistan to India, measuring 64 cm and weighing 5.3 to 7.7 kg with a 30-cm-long tail, and generally live in arid areas or uncovered areas.

They are herbivorous and live in groups of up to 180 individuals. *Chlorocebus pygerythrus,* also called vervet monkeys, live in southern and eastern Africa, they are herbivorous, males are 50 cm tall and females 40 cm, males weigh 3.9 to 8 kg and females 3.4 to 5.3 kg. They live in groups.

Five out of the seven species analyzed were endemic to the region: *M. sphinx* (*n*=139), *P. troglodytes* (*n*=38), *C. solatus* (*n*=12), *G. gorilla* (*n*=4) and *C. torquatus* (*n*=2); two were imported species: *C. pygerythrus* (vervet monkey, *n*=4) and *M. mulatta* (rhesus monkey, *n*=1). Animals of both genders were tested. Their mean age was 26.06 years, ranging from 1 to 30 years. No clinical signs suggested that *T. gondii* or *N. caninum* infection were recorded during a retrospective analysis of the animals’ medical file.

Primate population and sample collection

The primatology center houses primates either in cages or in a semi-free-ranging enclosure, with potential contact with free-living animals (Figure 1). Food and water are provided to those in cages (Figure 1, numbers 1 to 6) or the aviary (Figure 1, label Q and B), while those in the forest (Figure 1, number 7) use food available in their environment, complemented by fruits and cakes stocked daily by a guardian at different fixed points in the forest. The colonies are formed by animal founders brought to the CIRMF 30 years ago and animals born at the CDP since then. No outbreak of toxoplasmosis

Figure 1. Primatology Center of CIRMF (CDP). The figure shows the map of the CDP with cages (numbered from 1 to 6), aviary (Q and B), and natural forest surrounded by fence (number 7).

or neosporosis has been observed in the colony. During their annual health check-up, the animals were bled under anesthesia, and plasma or serum was obtained after centrifugation of total blood at 3000 rpm for 10 min at room temperature. Aliquots of plasma were kept at -20°C or used immediately in the modified agglutination test (MAT) or cELISA for *N. caninum* testing. No data existed before this study on the animals.

Ethical approval

This study was conducted in accordance with normative procedures as defined by the National Ethics Committee of Gabon, reference PROT N°0005/2013/SG/CNE, and Gabonese government authorization, reference RG/MINEF/00468.

Modified agglutination test for *T. gondii*

The Toxoscreen DA MAT (Biomerieux, Lyon, France) was performed on samples from animals diluted up to 1/4000 as recommended by the manufacturer, in 96-well plates containing formalin-treated *T. gondii* trophozoite and 0.2 M of 2-mercaptoethanol and left for incubation at room temperature overnight. A positive sample was characterized by agglutination of the *Toxoplasma* in a mat covering half of the well base, while negative samples were characterized by sedimentation of *Toxoplasma* in a button.

Competitive ELISA for *N. caninum* (cELISA)

To detect *N. caninum* antibodies, a competitive ELISA was performed according to the manufacturer's protocol (LSI Vet, Lissieu, France). Briefly, 50 µl of test serum from primates and controls was incubated in 96-well plates pre-sensitized with *N. caninum* antigen. After 1 h incubation at room temperature, the plate was washed with 300 µl of a wash solution and a monoclonal anti-*N. caninum* combined with peroxidase was added. After 20 min incubation, the plate was washed again three times with 300 µl wash solution followed by the addition of 50 µl of chromogenic substrate. The reaction was stopped by addition of 50 µl of stop solution. The intensity of the coloration was measured on a spectrophotometer at 620 nm. A lack of coloration or weak coloration indicated the presence of *N. caninum* antibodies. The measurement was validated when the optical density (OD) of the negative control was within 1.6 to 0.600 and the percentage of inhibition of the positive control was greater than 40%. Negative and positive control was included in each plate. The percentage of inhibition of each sample was determined using the formula:

%inh = OD negative control - OD tested sample/OD negative control × 100

Samples were considered positive when the percentage of inhibition was 30% or greater.

Statistical analysis

These analyses were done by comparing median values of groups of data using the Mann-Whitney U-test, the Chi-square test for groups of samples, and the Pearson correlation for relationships between data. These analyses were carried out using SPSS software. Results with a probability less than or equal to 0.05 were considered significant.

RESULTS

Seroprevalence of anti-*T.gondii*

Table 1 shows that 21% of animals were positive for IgG

Table 1. Seroprevalence of *Toxoplasma gondii* in the primates at the Franceville Centre of Primatology.

Primates spp.	Number	Positive	Prevalence	CI (%)
Mandrillus sphinx	139	23	16.5	11-23
Pan troglodyte	38	16	42.1	16.7-68
Cercopithecus solatus	12	1	8.3	-
Gorilla gorilla	4	1	25	-
Cercocebus torquatus	2	1	50	-
Chlorocebus sabaeus	4	0	-	-
Macaca mulatta	1	0	-	-
Total	200	42	21	23.83-21.16

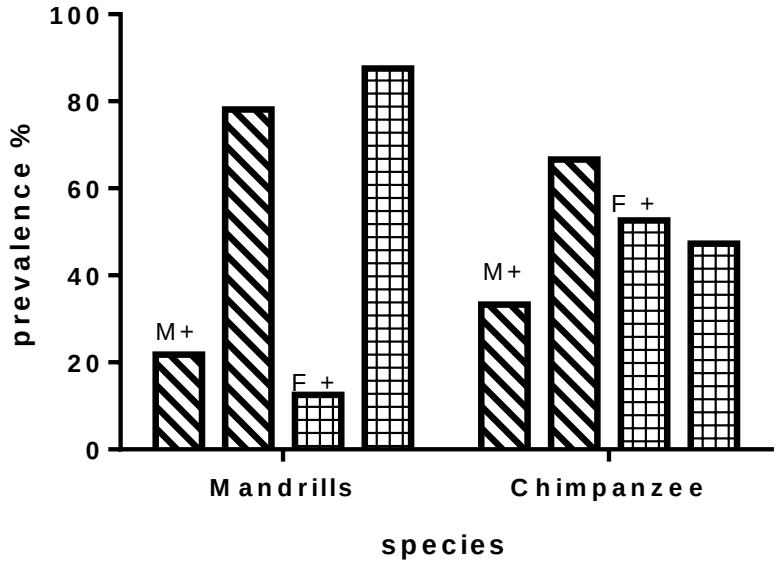

Figure 2. Distribution of *T. gondii* according to gender and animal species. *T. gondii*-positive animals were recorded as prevalence (%); this prevalence was plotted on a histogram for positive males (M+) and positive females (F+) compared to their negative counterparts.

against *T. gondii* (42/200). Seroprevalence according to species (Figure 2) shows that *P. troglodytes* had the highest prevalence (42.1%; 16/38) followed by *M. sphinx* (16.5%; 23/139), with a significant difference between the two species (42.1% versus 16.5%; p=0.001). Seroprevalence according to gender (Figure 3) showed that the prevalence was affected by gender in *M. sphinx*, with males being more infected than females (23.4% versus 10.6%; p = 0.043). However, in *P. troglodytes*, despite high prevalence (50%) in females compared to males (33.3%), this difference was not significant (p = 0.229). Furthermore, in *M. sphinx* the carriage of anti-*T. gondii* was not affected by age (Figure 3); although this carriage was high between 5 and 10 years, there was no correlation between age and seroprevalence (r = −0.534; p = 0.237). In contrast, the age of *P. troglodytes* (Figure3) affected the seroprevalence with increasing seroprevalence as age increased (r = 0.839; p = 0.037).

Seroprevalence did not vary according to habitat (Table 2), as shown by the distribution of IgG among the main species studied: *M. sphinx* (p = 0.214) and *P. troglodytes* (p = 0.513). It was noticed however that caged mandrills had no anti-*T. gondii* antibodies but only forest-dwelling animals (free-ranging) had these antibodies. The antibody titer rose to 1/4000 for all positive animals.

Seroprevalence of *N. caninum*

A total of 83 primates were tested and an overall prevalence of 68.67% was observed. A detailed distribution shows that *M. sphinx* (66.12%; 41/62), *P. troglodytes* (75%; 12/16), *C. solatus* (75%; 3/4), and one *G. gorilla* out of one (100%; 1/1) were the most infected

Figure 3. Progression of prevalence of *T. gondii* according to age. The prevalence of positive animals (%) per group of animals from 0–5 years up to 30–35 years recorded and plotted against prevalence (%) on a histogram for each species.

Table 2. Seroprevalence according to habitat.

Species	Habitat (*n*)	Positive (%)	Negative (%)
Mandrillus sphinx	Free-ranging (126)	23	103
	Cage (8)	0	8
Pan troglodyte	Cage (25)	10	15
	Aviary (9)	4	5

Table 3. Distribution of *N. caninum* among some primate species and coinfection with *T. gondii.*

Species	Number	Positive	Prevalence (CI 95%)	Co-infection (%)
Mandrillus sphinx	62	41	66.12 (54.34-77.9)	6 (14.63)
Pan troglodyte	16	12	75 (53.78-96.22)	6 (50)
Gorillagorilla	1	1	100 (-)	1 (100)
Cercopithecussolatus	4	3	75 (32.57-117.43)	0
Total	83	57	68.67 (58.69-78.65)	24.07

(Table 3). *T. gondii* and *N. caninum* co-infection was seen in 24.07% of the entire population of primates examined, that is, the percentage of individuals positive for both *T. gondii* and *N. caninum* (14/57 individuals). This was most prevalent in *P. troglodyte* (50%; 6/12), *G. gorilla* (100%; 1/1), and *M. sphinx* (14.63%; 6/66). No co-infection between these two parasites was seen in *C. solatus* (Table 3).

DISCUSSION

This study is the first to present seroprevalence of *T. gondii* and *N. caninum* in non-human primates in Gabon. Many reports are from outside Africa, with few coming from Africa (Ekanayake et al., 2004; Garcia et al., 2005; Sedlàk and Bàrtovà, 2006; McConnell et al., 1973). Primates are known as indicators of outbreaks of some

zoonoses in humans, such as Ebola (Georges et al., 1999). It is therefore necessary to study these parasites in primates, since these parasites might be a threat for human health or wildlife in general. Gabon is geographically well located for such studies (with the world's second largest forest).

The present study has shown that both *T. gondii* and *N.caninum* are prevalent among non-human primates but not distributed uniformly among different species of primates nor their habitats. In both cases, mandrills living in contact with the wild had more anti-*T. gondii* and anti-*N. caninum* antibodies than those in cages, suggesting the existence of these species of parasites in the wild. The prevalence of co-infection (24.07%) of these parasite species seems to indicate that both are distinct species despite certain biological and clinical similarities. In addition, they do not necessarily follow the same dynamics in their evolution and dispersion. Furthermore, it was shown that *T. gondii* and *N. caninum* differed in their host and transmission strategies (Reid et al., 2012). While age seems to be an important criterion for seropositivity in chimpanzees, in mandrills it was noted that these animals aged between 5 and 10 years had a very high prevalence of *T. gondii*. It was also observed that males had a higher prevalence than females. The prevalence of *T. gondii* at that age and in male mandrills seems to corroborate with their social behavior, characterized by fighting for male dominance (Setchell et al., 2006), resulting in severe injuries with bleeding or death, exposing males to infected blood.

This report also indicates a level of environmental contamination. The existence of a sylvatic cycle implicating wild canids for *N. caninum* or felids for *T. gondii* is suggested by these results on animals living in the forest. The fact that *T. gondii* DNA has been detected in fruit and vegetables (Lasso et al., 2012) and interaction with other wild species in the forest, makes the hypothesis of environmental contamination plausible. Estimation of antibodies gives a good indication of the exposure of animals to *N. caninum* and *T. gondii*. For *N. caninum*, although a confirmatory test such as Western blot or the immunofluorescence antibody test (IFAT) was not carried out, a competitive ELISA does not need specific secondary antibodies. However, the principle of competition indicates that the test can be used in different species (McCann et al., 2008). The retrospective examination of medical files did not allow us to relate clinical signs to *T. gondii* or *N.caninum* in these primates due to the nonspecific symptoms observed. However, the fact that antibodies remained high, as shown by the titer 1/4000, suggests that Old World primates may harbor the parasite either in a cystic form (bradyzoite) or as reactivation of an old infection (due to incoming tachyzoites). Reinfection (by trophozoites from a new infection) can occur quite often in a contaminated environment. The subsequent immune reaction may help contain the infection, resulting in mild or transient clinical signs (Drapper et al., 1971), which is substantiated by the fact that in chimpanzees experimental infection showed that a naive animal remains ill for a few days with a rise in the antibody titer for 6 months, while the other animals that had previous contact with *T. gondii* remain well. This suggests that old world primates may control their infection quickly, either by their immune system or other mechanisms that require clarification.

No studies have been conducted on this aspect on the other primates examined in this study. This suggests that the means of spreading the disease in primates require further study to determine their potential role in dispersion of these parasites.

Conclusion

This study suggests that old world primates are possible reservoirs of *T. gondii,* with variability depending on the species. Although antibodies against *N. caninum* were found, no parasite material was detected.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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