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Full Length Research Paper

Seroprevalence of two abortive parasites: *Toxoplasma gondii* and *Neospora caninum* in domestic animals in Franceville, Gabon

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Management of livestock maybe an important factor in limiting the spread of abortive parasites. Anti-*Toxoplasma gondii* measured with the modified agglutination test (MAT) and *Neospora caninum* with an Enzyme-Linked Immunosorbent Assay (ELISA) were screened in 212 domestic animals. Sheep had the highest seroprevalence (82.14%), followed by cattle (26%), pigs (20%), ducks (19.05%), and chickens (17.78%). Two breeding systems were distinguished: (1) the rural method in which animals breed in the wild, no food is given, and there is no drinking water point and no shelter; (2) the modern type, characterized by providing food, water, shelter, fences, and hygiene. When comparing the different species according to the breeding system, we found that for chickens, the rural style of breeding was associated with the highest seroprevalence of *T. gondii* compared with the modern breeding method (40% versus 4%; *p*=0.0004). Similarly, pigs bred according to the rural method had a higher seroprevalence compared with the modern breeding method (48% versus 0%; *p*=0.0001). *N.caninum* antibodies were present in 32.14% of sheep and 50% of goats. This study suggests that seroprevalence to *N. caninum and T. gondii* may vary according to the breeding method.

Keywords: *Toxoplasma gondii*, *Neospora caninum*, seroprevalence, animals, Gabon.

INTRODUCTION

Toxoplasma gondii is a zoonotic protozoa infecting humans and a wide range of animals. It is prevalent in Gabon (Beauvais et al., 1978; Duong et al., 1992) and the main source of contamination is mostly telluric at an early age (Bisvigou et al., 2009). It is known that *T. gondii* can cause spontaneous abortion in humans and animals, congenital malformations, and death due to encephalitis in immune compromised patients (e.g., cancer patients, transplant recipients, AIDS patients, etc.). Recently, various outbreaks (Vaudaux et al. 2010; Carme et al., 2009) and serious clinical symptoms in immunocompetent individuals (Carme et al., 2002) have been reported. This suggests that virulence may vary depending on the strain or the existence of new strains. The distribution and circulation of such strains in the wild among humans and animals is not well known.

In Gabon, *T. gondii* in particular is highly prevalent in humans. However, few studies have looked at the overall prevalence in animals in Africa (Hammond et al., 2014), and nothing is known about *Neospora caninum*, which was discovered in 1984 (Bjerkas et al., 1984) and confirmed in 1988 (Dubey et al., 1988). It has a life cycle

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involving many animal species and canids as definitive hosts. Similarly, many clinical symptoms have been described in animals, such as spontaneous abortion and neonatal mortality in cattle, sheep, and goats. As shown here, *T. gondii* and *N. caninum* can cause significant economic loss and public health problems. *T. gondii* may become a serious threat to the increasing number of immunodeficient individuals for whom acute or reactivated infection with *T. gondii* can be fatal, even though there is no proof of *N. caninum* causing such problems in humans. Studies of experimental infection of non-human primates have shown that there is a development of clinical symptoms in these primates (Barr et al., 1994). The close relation (e.g., cross-reactive antigens) between the two species suggests that *N. caninum* is potentially pathogenic to the same animals. In the absence of data on *N. caninum*, a clear assertion cannot be made on its clinical importance, particularly in Gabon. This country has a traditional way of raising animals based on free roaming, resulting in a low production of protein; therefore, meat, particularly poultry, is imported, amounting to 26 billion CFA a year (Lawson, 2012). Consequently, local production needs to be developed and improved. One way to improve this situation is to avoid the infection of animals by these abortive parasites (*T. gondii*, *N. caninum*) by simple methods of livestock management. This is substantiated by the fact that the environment may also affect the distribution of this parasite (Eymann et al., 2006) and that antibodies to this infection can be elevated in rural animals (Hornok et al., 2008). Moreover, it has been shown that domestic dogs had a lower rate of infection than stray dogs (Hosseininejad and Hosseini, 2011). Also, free-roaming dogs in Nigeria (Ayinmode et al., 2016) had more antibodies than caged dogs. Therefore, this study was undertaken to establish preliminary data on the possible distribution of both *T. gondii* and *N. caninum* in domestic animals.

MATERIAL AND METHODS

Animal sample collection

Domestic animals from farms or free-roaming animals around Franceville were bled with the owner's consent: blood was taken from the jugular vein or the saphenous vein for large animals and from the axillary vein for birds. Depending on animal size, 1–5 mL of blood was collected. Sera or plasma was obtained by centrifugation of total blood for 3000rpm/min for 15 min. The plasma was stored at −20°C until use.

Detection of anti-*T.gondii* antibodies

Antibodies against *T. gondii* in different samples were detected using a modified agglutination test (MAT); ToxoScreen DA (Biomerieux, Lyon, France) according to the manufacturer's instructions. Briefly, sera or plasma was diluted in phosphate buffered saline (PBS); samples and controls were incubated overnight (18h) at room temperature in duplicate at 1/40 and1/4000 dilution in 96-wellplates coated with formalin-treated *T. gondii* trophozoites and 0.2 M/L of 2-mercaptoethanol. Plaques were quantified 18 h later. A positive agglutination test was characterized by a mat covering about half of the well base, whereas a negative sample was characterized by sedimentation of trophozoites in the bottom of the well base.

Detection of *N. caninum* antibodies

N. caninum antibodies were detected by an ELISA following the ID screen *N. caninum* procedure (ID Vet Co., Belgium), which detects antibodies from multiple species. Briefly, the plates were precoated with *N. caninum* antigen and incubated with serum or plasma from the control and the sample diluted to1/10. The plates were then incubated for 45 min at room temperature, washed, multiple species conjugate was added, and they were incubated for a further 30 min followed by three washes. Subsequently, substrate was added and the plates were incubated for 15 min longer. The results were read at 450 nm in a spectrophotometer using the following formula: OD sample − OD negative control/ OD positive control − OD negative control × 100. A sample with a ratio greater than 50 was considered positive.

Statistical analysis

Analysis was performed to determine different prevalence levels with a 95% confidence interval using Graph Pad software. Fischer's exact test was used to compare the variation in breeding modes between the groups. A *p*-value less than or equal to 0.05 was considered significant.

RESULTS

Study sample

In all, 212 animals were bled: chickens, sheep, ducks, pigs, goats, cattle, and geese (Table 1). These animals were either free-roaming with food and water not controlled or on farms with clear zootechnical procedures in place, with food and water provided by the owner.

Prevalence of *T. gondii* antibodies

A total of 212 domestic animals were screened for anti-*T. gondii* antibodies. The overall prevalence was 28.3%. Sheep had the highest seroprevalence (82.14%), followed

Table 1. Seroprevalence of *T. gondii* and *N. caninum* according to species.

Species	Number (n)	Prevalence *T. gondii* CI 95%	Prevalence *N. caninum* CI 95%	Coinfection (%) CI 95%
Gallus gallus	45	17.78 (6.6-29)	-	-
Duck	21	19.05 (2.3-35.8)	-	-
Geese	4	0	-	-
Pig	60	20.00 (9.9-30.1)	-	-
Goats	4	0	66.66 (20.5-112.9)	-
Sheep	28	82.14 (68-96.3)	32.14 (14.8-49.4)	32.14 (14.8-49.4)
Cattle	50	26.00 (13.8-38.2)	-	-
Total	212	28.30 (22.2-34.4)	-	-

by cattle (26%), pigs (20%), ducks (19.05%), and chickens (17.7%), and none of the four goats or four geese tested was seropositive (Table 1). We then compared animals roaming free around the owners' compounds (no specific food, drinking point, or shelter) with animals raised with modern farming methods (provision of food, water, shelter, fencing, and hygiene). Free-roaming chickens (40% versus 4%; $p = 0.0004$) and pigs (48% versus 0%; $p = 0.0001$) had far higher seroprevalence rates than their farmed counterparts (Fig. 1).

Prevalence of *N. caninum* antibodies

The sample comprised the following species: sheep, goats, pigs, and cattle. The results showed that *N. caninum* antibodies were highly prevalent in sheep (32.14%), while two of the four goats examined were positive (50%). However, none of the pigs ($n = 60$) or cattle ($n = 50$) was positive for *N. caninum* antibodies (Table 1).

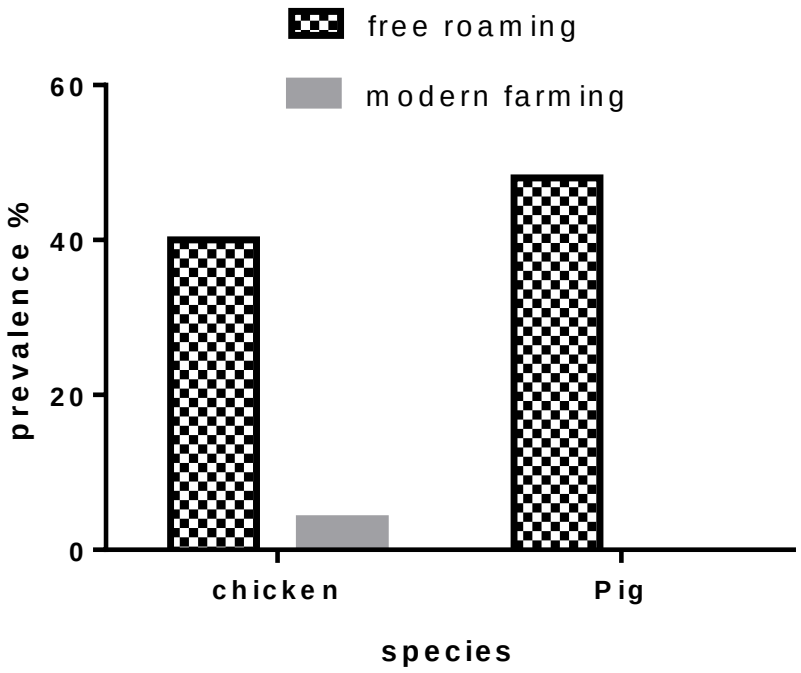
Prevalence of *T. gondii*/*N. caninum* coinfection

To analyze the potential coinfection between *T. gondii* and *N. caninum*, antibodies against both were sought at the same time in the same sample. The results showed that 32.14% of sheep had antibodies against both *T. gondii* and *N. caninum.* No other coinfection was found in the other species examined (goats, pigs, cattle).

DISCUSSION

We showed the existence of *T. gondii* and *N. caninum* among domestic animals in the city of Franceville. The prevalence of *T. gondii* in sheep and goats in Franceville contrasts with the 31% prevalence rate seen in Uganda for goats and sheep (Bisson et al., 2000). This prevalence varies from 10% to 50% in Zimbabwe depending on rainfall (Hove et al., 2005); seroprevalence rates in sheep from South Africa vary from 4.3% to 5.6% (Abu Samra et al., 2007). These variations between African countries (Kamani et al., 2010; Van DerPuije et al., 2000; Sawadogo et al., 2005) are related to the climate, with a trend of lower seroprevalence rates in arid areas (Abu Samra et al., 2007). In the present study, *T. gondii* was not detected in geese, although it has been detected in this species elsewhere (Yang et al., 2011; Na Young et al., 2012). The prevalence in ducks was high (33.3%), because this species and chickens are considered good indicators of soil contamination by *T. gondii* oocysts. The prevalence seen in ducks and chickens in this study may indicate the high contamination in the environment. Free-roaming pigs had high prevalence rates compared with their farmed counterparts, and a similar observation was made with chickens, indicating that animals exposed to the environment are more contaminated. In this study, cattle had a high prevalence of *T. gondii* (26%) compared with findings from Tanzania (Luuk et al., 2009). This may be due to the management method used but also to the fact that cattle forage at a high level from contaminated ground and it seems they are more resistant to *T. gondii* infection (Dubey and Jones, 2008). Only a few reports on infection of African domestic animals have been published, and data on this type of zoonosis seroprevalence will help define a control strategy. Antibodies in this case are used more to determine the seroprevalence of these infections. Although there is a conflicting result in terms of sensibility (MAT methods) and specificity (IFAT method), it is

Fig. 1 Distribution of *T. gondii* according to the breeding method. The prevalence of *T. gondii* in the animals tested was determined and this was plotted against the breeding method recorded in free-roaming and modern farming groups for two representative species (chickens and pigs).



difficult to see oocyst shedding in feces (Dubey et al.,1995), whereas the presence of antibodies means that animals have been in contact with the parasite but not necessarily infected. For example, elevated antibodies may indicate protective immunity or a new contamination. Also, the presence of antibodies may indicate a repetitive infection.

There are very few data on *N. caninum* in domestic animals in Africa, particularly in Gabon. This is the first report on the existence of this parasite in Gabon. Its prevalence among domestic animals is high in sheep and goats. It was not found in the other species examined, but the technique used may have been limited in detecting antibodies in these species. This parasite, which has been discovered only recently, may be an obstacle in the development of intensive farming in the country, given that both *T. gondii* and *N. caninum* may induce considerable economic loss with reduced meat production due to frequent abortion. Measures must be taken to limit the dispersion of these pathogens. Coinfection was seen in sheep only, but not in goats or cattle. Whether this distribution suggests resistance of some animal species to *N. caninum* or is due to the size of the sample studied needs further investigation.

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Animal rights all applicable international and national guidelines for the care and use of animals was followed.

CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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