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Full Length Research Paper

Seroprevalence and virulence of *Toxoplasma gondii* in human and animal populations in a village in southeast Gabon

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Abstract

The seroprevalence of *Toxoplasma gondii* in animals and humans sharing the same biotope was examined, to determine the means of controlling this parasite in the tropical ecosystem. Humans (*n*=198) and animals (*n*=369) were tested simultaneously for the presence of anti-*T. gondii* antibodies. Human samples were tested using an enzyme-linked fluorescent assay (ELFA), while animal samples were analysed using the agglutination test. A bioassay using Swiss mice for testing strain virulence was also performed. Two people had IgM, the IgG isotype were found in 73.23% of humans, 92.85% of cats, 58.82% of dogs, 46.42% of chickens, 87.05% of goats and sheep, 6.66% of house rodents, 10.20% of wild rodents and 38.8% for bush meat consumed by villagers. A subsequent bioassay showed that one strain derived from chickens and one from small ruminants induced ascites in mice, while nine strains isolated from chickens and nine from small ruminants were asymptomatic despite the presence of anti-*T. gondii* antibodies in 95% of infected mice. These results suggest that the strains circulating in this environment might be complex. The high seroprevalence observed is associated with domestic cats and the spread of this parasite is due to the mode of management of domestic animals.

Key words: Biotope, *T. gondii*, circulation, IgG, virulence, Gabon.

INTRODUCTION

Toxoplasma gondii is a cosmopolitan parasite which infects humans and animals worldwide. The infection is acquired by ingestion of undercooked or raw meat containing viable tissue cyst or by ingestion of food and water that is contaminated with oocysts shed by cats. The majority of infected humans remain asymptomatic. However, the reaction of latent infection occurs in immunocompromised patients, causing encephalitis or cerebral toxoplasmosis (Wong et al., 1984; Israelski et al., 1993).

In Gabon the prevalence of HIV increased from 1.6% in 2000 to 5.9% in 2006 (Gabon, 2012; www.broad casting hiv) This suggests a higher risk of developing clinical symptoms in case of AIDS in toxoplasmosis-infected individuals. Transplacental transmission is possible; therefore, the foetus and neonate are at risk of developing congenital toxoplasmosis. The virulence of the *T. gondii* strain is in general analysed by bioassay using mice (Pena et al., 2008). Three lineages of *T. gondii* with genetic differences have been described

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according to their virulence in mice: type I is considered the most virulent in mice, while type II and type III are less virulent (Pena et al., 2008). In some countries, the clinical expression of *T. gondii* seems to differ from what is seen in Europe and North America, where types II and III are very common. In Brazil for example, it has been shown that ocular toxoplasmosis is very common and the complexity of the strain isolate contributes to the clinical outcome of toxoplasmosis (Asis Khan et al., 2006; Lenildo de Moura et al., 2006). In Africa, most studies have used chickens as indicators of oocyst spread of different strains of *T. gondii*(Dubey et al., 2005; Velmurungan et al., 2008). Thus, while some studies in North Africa (e.g., Egypt) show a predominance of asymptomatic type II and type III in chickens but only type III in ducks (Dubey et al., 2003), other studies in West and East Africa show that a type II avirulent strain for mice was present in chickens (Dubey et al., 2008a). Furthermore, studies in Uganda on genotype and mouse virulence have shown that a high level of multiple infections and polymorphic strains (Lindstrom et al., 2008) can occur in addition to the three dominant strains (I, II and III). The appearance of severe toxoplasmosis cases in immunocompetent individuals exhibiting an atypical genotype in tropical areas (Carme et al., 2002) raises the question of the possible existence of a sylvatic cycle and other types of *T. gondii* that differ from the classical domestic type in addition to a cycle that includes cats as the reservoir. It has been suggested that wild meat handling and consumption of undercooked meat, or untreated river water can be a source of contamination by this parasite. The *T. gondii* reservoir in this case is wild felids. The existence of neotropical atypical strains inducing severe clinical signs observed in infected immunocompetent adults (Carme et al., 2006) has therefore been suggested. However, this assertion was not confirmed by several studies in tropical areas.

In central Africa, Gabon is a typical tropical country in which most toxoplasmosis reports involve pregnant woman (Nabias et al., 1998; Billiault et al., 1987), with few in the general population (Duong et al.,1992a; Duong et al., 1992b) and no reports on the prevalence of *T. gondii* in animals or the virulence of the parasite. Yet meat consumption, particularly wild meat, is a custom in this area. Knowledge of epidemiological factors that influence transmission and clinical expression of *T. gondii* will allow the implementation of preventive or curative measures to limit the incidence of the severe clinical outcomes of toxoplasmosis.

Therefore, the relationship between humans, domestic animals, wildlife and circulating *T. gondii* strains needs to be investigated. Although several methods for preventing infection by *T. gondii* are known (CDC, 2003), they cannot be applied to all circumstances. It is therefore important to identify the most appropriate factor involved in a specific environment in order to take proper measures among those already known. Therefore, this study presents one of the first reports on *T. gondii* in animals and humans in Equatorial Africa, particularly Gabon.

MATERIALS AND METHODS

Study site

Dienga is a village that is a CIRMF field station for the study of tropical disease parasites and viruses. It is situated in the southeast of Gabon, in the Ogooué Lolo province. The village is occupied by 186 families with 1500 inhabitants. This study covers in part the medical surveillance necessary for the individuals participating in different research programs. Families live mainly from agriculture, hunting and fishing. Traditional customs remain in this ecosystem, characterised by eating food cooked at more than 100°C, free-roaming livestock, stray dogs and cats, manual work for agriculture and fishing, hunting bush meat for human consumption, and household wastes usually deposited approximately 50 m behind the kitchen. These wastes are an important source of feeding for domestic animals. The objective of this study was explained to the villagers in the local language, as were the material and methods used. Informed consent was obtained at different levels of authorities: the Ministry of Health, the village chief, the individual, animal owners, and parents for children.

Human blood sample collection

Random sampling was used for blood collected from individuals (*n*=198) after explaining the study in the local language and obtaining their informed consent. Five milliliters of venous blood was drawn from each person in a tube without anticoagulant. After centrifugation at 352 *g* for10 min, the serum was divided into 100-µl aliquots and kept at −20°C until use.

Blood collection from domestic animals

Blood from domestic animals: small ruminants (*n*=139), cats (*n*=14), dogs (*n*=51) and chickens (*n*=84) were collected with the consent of the owner and in his presence after a clear explanation. Dogs' blood was collected from the cephalic vein in a 5-ml syringe. Cats were bled under anaesthesia using Ketamine (Ketamine ^{R}) 1g/10ml and drops of Xylazine (Rompum^{R}). The leg was shaved at the bleeding point and 5 ml of blood was drawn. Goats and sheep were bled from the jugular vein followed by their identification using a ring. All animals were marked for identification after bleeding. Chicken blood was collected using a 1-ml syringe from the wing vein followed by identification marking with scotch tape. Animals were all free- roaming,

caught randomly throughout the village. The full blood was transferred to a tube and left for clotting to obtain their sera.

Blood collection from wild animals

Blood from rodents: Domestic rodents (*n*=15) and wild rodents (*n*=48) were captured using either local traps or Sherman traps placed randomly in different houses throughout the village and in the forest around the village. Rodents were bled under anesthesia mixed with Xylazine (drop) and Imalgene. Blood was taken from the retro-orbital sinus of the eye, yielding between 0.3 and 0.4 ml of blood. After clotting, the clot was separated with serum by centrifugation at 352 *g* for 10 min and kept at −20°C until use. Domestic rodents were those captured in and around the house. Wild rodents were those captured in the bush within a 2- to 3-km radius around the village.
Blood from bush meat (*n*=18): No specific action was taken to incite hunting of bush meat. Also, no specific strategy was taken to interfere with the villagers' traditional lifestyle. However, given that bush meat consumption was a local custom, the animals used were killed by hunters for their own consumption according to their own needs. By negotiating with the villagers, the research team member was able to collect either blood or exudate from the meat. These samples were stored frozen until use.

***T. gondii*-specific antibody detection in animals**

Anti-*T. gondii* antibodies in domestic or wild animals were detected using the Direct Agglutination test (Toxo-Screen DA, Biomerieux^{R}SA, Lyon, France). The sample was diluted at 1/40 and 1/4000 according to the protocol described by the manufacturer, then incubated for 5–18 h. A positive sample was characterised by agglutination of *Toxoplasma* in a mat covering half of the well base, while a negative sample was characterised by sedimentation of *Toxoplasma* in a button.

Measurement of specific IgG and IgM of *T. gondii* in humans

IgG were measured from 100µl of plasma or serum using the enzyme-linked fluorescent assay (ELFA) operated on an immunoanalyser (Vidas) according to the manufacturer's instructions (Biomerieux^{R}). Samples with a titer higher than or equal to 8 IU/ml were considered positive. For IgM, 10µl of plasma or serum was used in an ELFA technique, which used an immunocomplex of *T. gondii* antigen coupled to a monoclonal antibody. The reagents were used according to the manufacturer's instructions (Biomerieux^{R}). Samples were considered positive when the value of the titre was greater than or equal to 0.65 IU/ml.

Biological assay for virulence of *T. gondii* strain

Some sheep (*n*=5), goats (*n*=4) and chickens (*n*=11) found IgG-positive for anti-*T. gondii* were killed to study the virulence of the circulating strains of *T. gondii*. Their heart (30–40g) or brain was collected. These organs were sliced in digestion buffer which included 0.4% Trypsin and 40µg/ml of gentamycin in 0.9% NaCl solution using a blender (2 min), followed by incubation at 37°C in a water bath for 3 h under rotation and then filtered through gauze and washed three times at 978 *g* for 10 min each. The pellet was resuspended in 1–2ml or 6–12 ml depending on the organ and animal species. We inoculated each pair of Swiss mice intraperitoneally with 0.5ml of this suspension. The mice were followed up for 1 month depending on their physical appearance and bled to collect their serum (detection of IgG). Brains and ascites were collected for isolation of potential *T.gondii* parasites (Saki and Khademvatan, 2014; Fernandes et al., 2017).

Statistical analysis

Statistical analysis was performed using the Minitab program (Minitab Inc., State College, PA, USA), and R software (R Development Core Team, 2013) for Pearson's chi-squared test with Yates' continuity correction. The differences between two groups of samples were analysed statistically using the chi-square test. For multiple comparisons, a contingency table was used and a Yates correction applied when the number of samples per case was less than five. A *p*-value ≤0.05 was considered significant.

Ethics approval

This study was approved by Gabon's Ministry of Health and the Gabon National Ethics committee; additional authorisation was obtained from the animals' owners.

RESULTS

Study population

Different populations of humans and animals sharing the same biotope were analysed. These populations contributed a total of 567 samples from different species including 135 females and 85 males varying in age from 0 to 80 years, sheep, goats, chickens, dogs, cats, domestic rats, wild rats and different species of animals consumed by the villagers.
This population of animals was composed of small ruminants: goats, sheep (*n*=139), chickens (*Gallus*

domesticus, *n*=84), dogs (*Canis*,*n*=51), cats(*n*=14), rats (*n*=63) and wild meat (*n*=18).

Prevalence of *T.gondii* in the human population from Dienga

The distribution of *T.gondii* in the human population was determined using the detection of specific IgG and IgM. Only two individuals out of 220 had specific IgM, while 145 individuals had specific IgG (73.2%; 95% CI: 66.4–79.2). There was no significant difference between males 70.5% (95% CI: 55.3–79.4) and females 76.1% (95% CI: 67.4–82.5) (X^2=2.67; dl=1; *p*=0.101). The prevalence between 0 and 5 years of age was 41.17% (7/17) and became more pronounced between 6 and 10 years of age (13/19 individuals, 68.9%). The relationship between age and *T. gondii* (Figure 1) within this village showed that the prevalence increased from 0–10 years to 11–20 years of age (*p*=0.012), then dropped between 21 and 30 years of age (*p*=0.362). In the 31- to 40-year-old, 41- to 50-year-old, and over-50-year-old age groups, a significant increase was seen in comparison with the prevalence in the 0- to 10-year-old age group (*p*=0.007, *p*=0.041, *p*=0.027, respectively). From 11 to 50 years of age, the fluctuation seen in different age groups was not significant (*p*>0.05).

Seroprevalence of *T. gondii* in animals

The contact between *T. gondii* and different species of domestic and wild animals was assessed using the direct agglutination test. Table 1 shows the distribution of *T. gondii* amongst domestic animals with the highest prevalence for cats, 92.8% (95% CI: 66.1–99.8), followed by small ruminants (sheep and goats), 87.05% (95% CI: 80.3–92.1). The general prevalence in both wild and domestic rodents was 9.5%. For wild animals or bush meat, 18 animals of different species were tested for contact with *T. gondii*: only seven were positive (38.8%, 95% CI: 17.2–64.2).

Comparative analysis of seroprevalence in different populations sharing the same biotope

It appears that the prevalence of *T. gondii* among the seven groups was significantly different (X^2=141. DF=7; *p*<0.001), with cats having the highest prevalence (92%) and rats having the lowest (9.52%) (Figure 2). No significant differences existed between cats, small ruminants and humans (*p*>0.05), whereas the difference between humans, chickens and dogs was significant (*p*<0.01 and *p*=0.003, respectively). Similarly, the difference between small ruminants, chickens and dogs was also significant. (*p*<0.001 and *p*<0.001, respectively). The potential relationship between cats and two other groups (dogs and bush meat) was examined by comparing the anti-*T. gondii* prevalence between these groups. These prevalence rates were significantly different (*p*=0.023 and *p*=0.02 for dogs and bush meat, respectively). Furthermore, the prevalence rates between bush meat and any other domestic species including humans were significant (*p*<0.05). The same observation was made between rats and other species (*p*<0.05). Finally, it appears that cats had elevated prevalence (Figure 2) followed by small ruminants and sequentially by humans, dogs, chickens, bush meat, wild rats and domestic rats. Examination of the cat environment to identify the risk of being infected revealed (Figure 3) a strong correlation between seroprevalence in individuals with contact with cats compared to those without contact with cats (X^2=66.8, df=1, *p*<0.003).

Study of virulence in mice

Swiss mice were inoculated with different trypsin-digested organs from animals found positive during the survey in Dienga; however, the number of parasites in each inoculums was not known. Of the 22 isolates inoculated to mice, variations were seen depending on the inoculums or the animal from which the inoculate was derived (Table 2). Inoculums obtained from bush meat did not generate any response. However, some mice developed ascites: 4.7% (95% CI: 1.3–11.7**)**. Many other mice 73.7% (95% CI: 62.7–82.9) were only serologically positive for *T. gondii* IgG, with no physical symptoms over 1 month of follow-up.

DISCUSSION

T.gondii is a ubiquitous parasite. Its medical importance is increasing in Gabon due to the increase in the population at risk: pregnant women and HIV-positive individuals. *T. gondii* also has economic importance because it may provoke abortion in sheep and goats. In this country, bush meat consumption is a traditional way of providing protein. Timber exploitation threatens the ecosystem, restricting animals' natural habitat, and human–animal contact is on the rise. Therefore, re-emerging or emerging zoonosis is increasing (Duong et al., 1992b; Prasad, 2010). One way to overcome this danger is to understand different aspects of transmission of the disease from one species to another. Studies investigating humans and animals sharing the same biotope simultaneously are scarce. Yet this can help understand the circulation of *T. gondii* strains in animals and humans and the development of a control strategy.

In Gabon, no studies have been conducted on domestic or wild animal species simultaneously. This study is the first attempt toward meeting this objective. A typical Gabonese village that has retained its indigenous way of life was taken for this first study and all species of animals living with humans and the humans themselves

Table 1. Seroprevalence distribution of *T. Gondii* in the study population.

Species	Number	Prevalence (%)
Small ruminants	139	121 (87.05)
Cats	14	13 (92.85)
Dogs	51	30 (58.82)
Chicken (*Gallus domesticus*)	84	39 (46.42)
Ratus ratus (wild)	48	5 (10.2)
Ratus domesticus	15	1 (6.66)
Bush meat	18	7 (38.8)

Table 2. Virulence of *T. gondii* strain in mice.

				Clinical	signs	*T. gondii*IgG+
Origin of isolate	Mice (n)	Isolate (n)	Death	Symptoms	No symptoms	*n* (%)
Ruminant	60	10	0	2	58	
Chicken	22	11	0	2	20	73.7[a]
Bush meat	2	1	0	0	2	
Total	84	22	0	4 (4.7%)[b]	80	95.2[c]

[a]: The percentage of total mice(*n*=84) that received isolate without presenting any symptoms. [b]: Percentage of mice inoculated with one isolate among the total used(*n*=84) with symptoms
[c] :Percentage of mice among those which received inoculate (*n*=84) with IgG+ only without any symptoms; *n*= total number of mice that received isolate (*n*=84)

were analysed for the prevalence of *T. gondii* antibodies. It was found that the prevalence in humans at Dienga remains high, in agreement with a previous report on a larger population (Bisvigou et al., 2009), suggesting the stability of the transmission process over 1year in the village. This does not differ from the prevalence reported earlier (Beauvais et al., 1978) in the general population. The result contrasts with other African countries such as Niger with 18–18.2% (Develoux et al., 1988; Julvez et al., 1996) and Mali 21% (Maiga et al., 2001) but in agreement with others such as Ivory Coast (Adoubryn et al., 2004).

It is likely that the equatorial environment is favourable to *T. gondii* proliferation more than dry areas. This observation shows the importance of the ecosystem in the spread of *T. gondii.* Cats had higher prevalence in Dienga than stray cats in Italy (Papini et al., 2006). Despite their important role, few studies on cats have been conducted in Africa, particularly Gabon. This is surprising given that these animals are considered as the definitive host. Interestingly, both cats and humans had very high prevalence, confirming the observation by others indicating that the seroprevalence in cats correlates with that in humans (Meireles et al., 2004). Dogs are also considered a good indicator of the current circulation status of *T. gondii* in absence of cats. In Dienga, the prevalence in dogs is high (58%) but slightly lower than dogs in Sri Lanka: 67.4% (Dubey et al., 2008b). This suggests an intense circulation of *T. gondii* amongst species. Chickens have been used to characterise different strains of *T. gondii* throughout the world. They are considered indicators of the current spread of oocysts in the soil. At Dienga, 46.42% were positive for *T. gondii*. Similarly**,** small ruminants (sheep and goats) have a high prevalence (87%) compared to sheep from South Africa, with a prevalence varying from 3.4% to 7.9% (Abu Samra et al., 2007), Morocco: 27.6% (Sawadogo et al., 2005) and other dry countries of Africa: 0–25.6% (Deconinck et al., 1996). The prevalence in rodents seems to follow the general trend in the world, while in an urban area of the UK the prevalence in mice is 51% (Gai Murphy et al., 2008), in Niamey this prevalence is 1.96% (Mercier et al., 2013), in Thailand only 4.2% of Muridae (Sathaporn et al.,

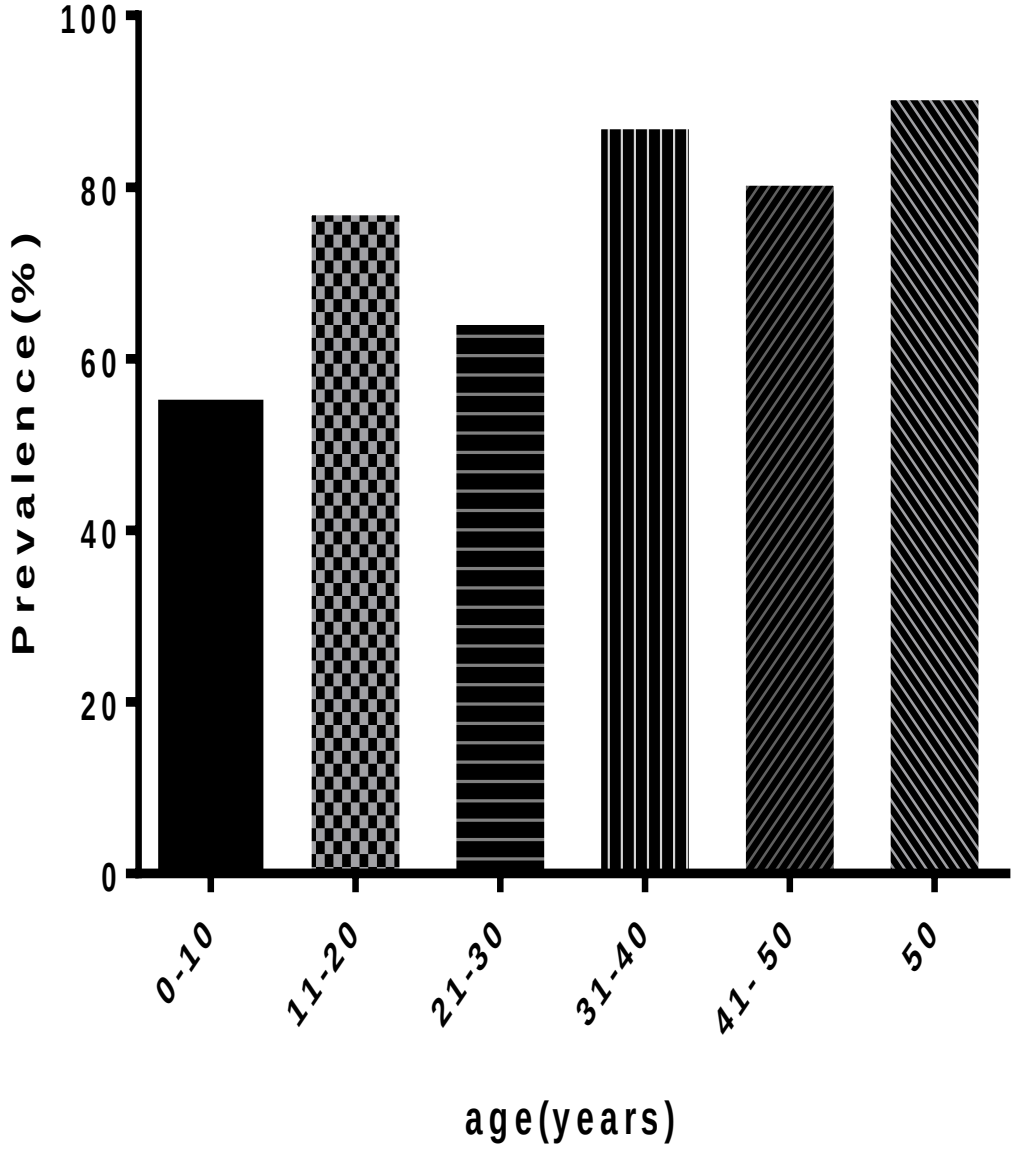

Figure 1. Seroprevalence (%) of *T. gondii* according to age(years). Specific anti-*T. gondii* IgG was measured using ELFA. The percentage of positive individuals per 10-year age group was plotted against the total number of individuals in the group.

2010) and 0.8% in the West Indies (Dubey et al., 2006). Therefore, the prevalence observed in mice is not a bias on the sample. Furthermore, it has been shown that this prevalence varies with species and environment (Mercier et al., 2013).

With these data on human and domestic animals, its seems clear that the environment plays an important role in contamination of different species by *T. gondii* oocysts. If cats can be considered reservoirs shedding oocysts in their faeces, other animals feeding in this environment can be considered mechanical dispersers of *T. gondii*, which is substantiated by experiments on dogs (Lindsay et al., 1997). These experiments show that dogs can shed a sporulated oocyst 2 days after its ingestion, and the oocyst can stay on the skin of a dog for several days. Secondly, small ruminants (sheep, goats) are not carnivores; therefore, wild meat consumption cannot explain the high prevalence in these

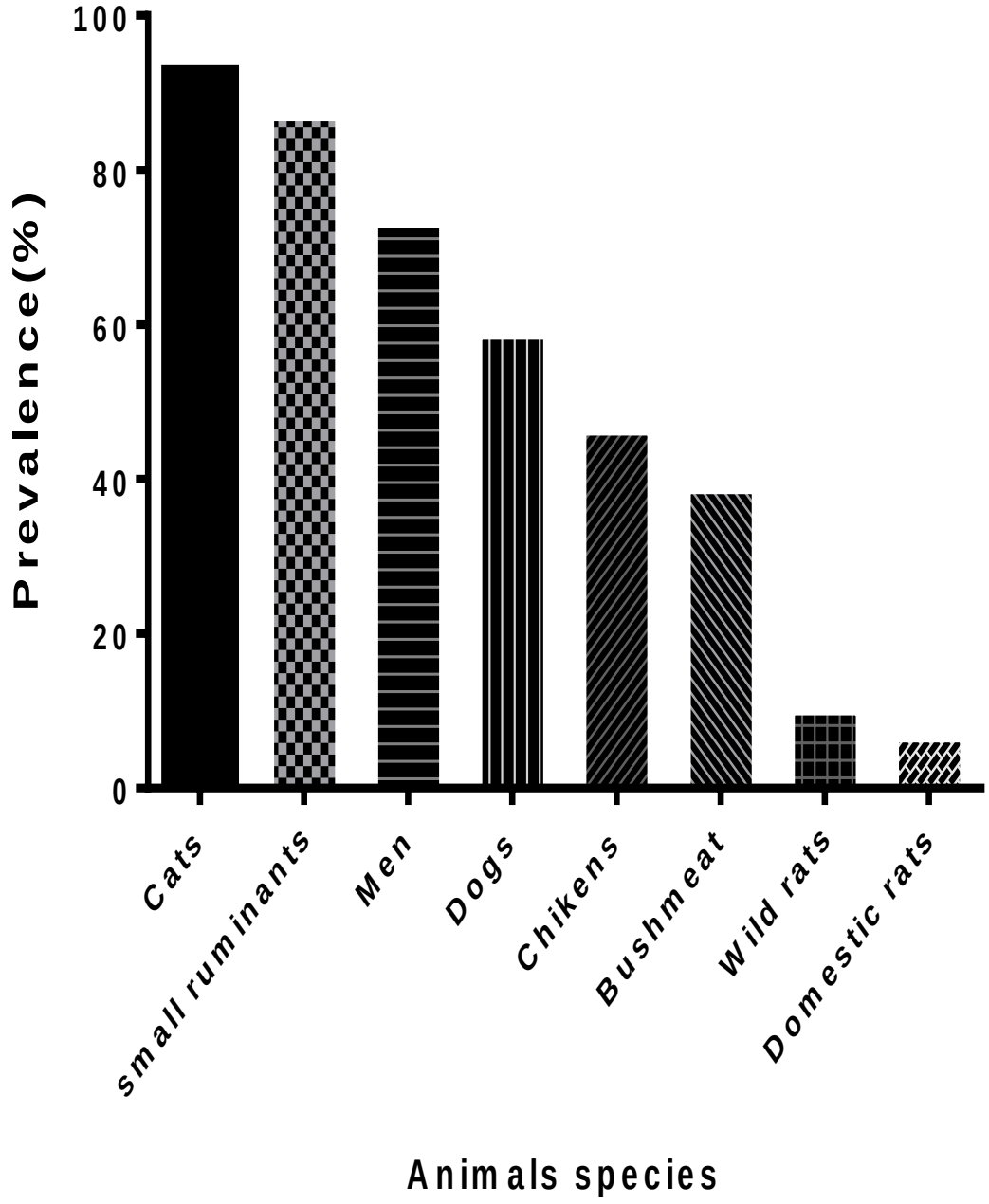

Figure 2. Seroprevalence (%) of *T. gondii* according to Animal species, small ruminantsand humans. Anti-*T. gondii* IgG were measured using ELFA for human samples or ToxoScreen for animals. The percentage of positive individuals was plotted against the total number of individuals in this species. The figure presents the resulting histograms.

animals. Interestingly, the prevalence of *T. gondii* in wild meat was low compared to any of the domestic animals studied, although the number of animals per wild species tested was low. Other studies in wild animals in Africa found 13% of 157 animals from 12 species (Riemann et al., 1975). Outside Africa, prevalence varies between 4% and 5.9% in gazelles and *Oryx* out of 608 animals tested (Osama et al., 1994; Tuntasuva et al., 2001); others found 15.4% amongst captive felids in Thailand (Khongsak et al., 2006). Furthermore, the present study shows that there is a gradient of prevalence with the epicenter in domestic cats. This suggests that the greatest contamination in humans is from their immediate environment. Overall, the rubbish stock behind the kitchen that served as a source for feeding domestic animals must be burned. Another possible

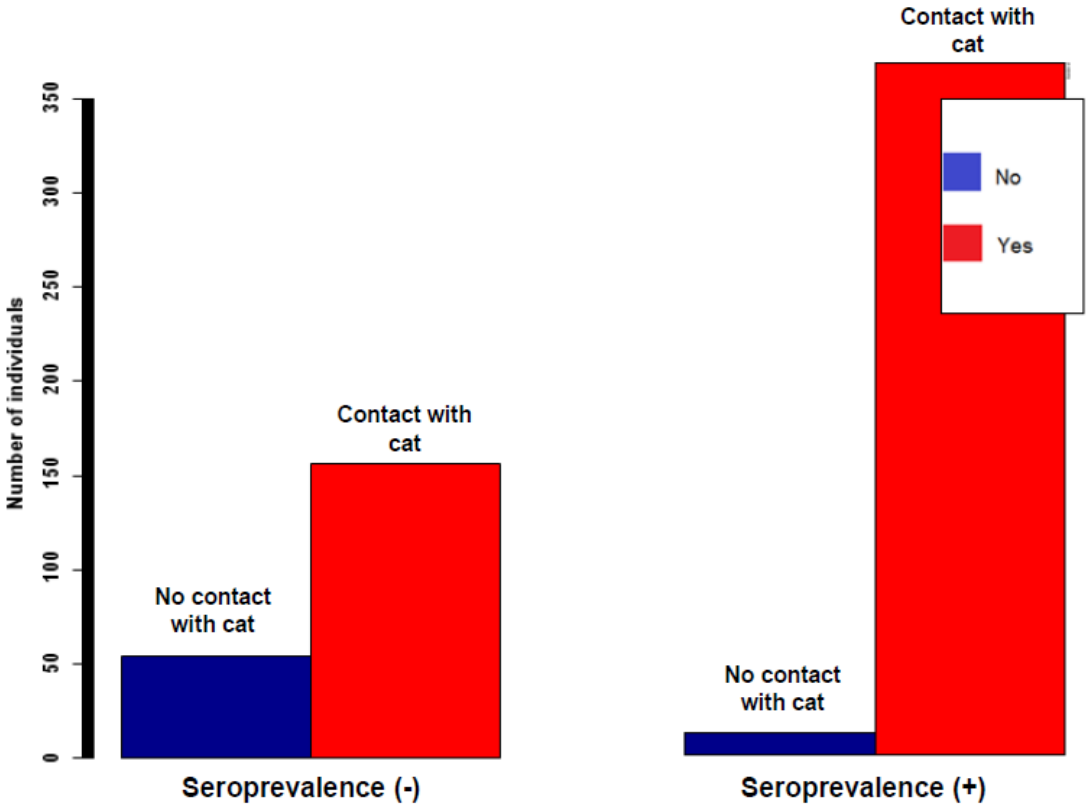

Figure 3. Seroprevalence of *T.gondii* according to contact with cats. Antibodies measured by both ELFA (for humans) and Toxoscreen (for animals) were plotted against the number of individuals without contact with cats (NO=bleu) and those in contact with cats (YES=red). Individuals found positive with antibodies are on theright side [seroprevalence (+)], while those without antibodies [seroprevalence (–)] are on theleft side of the graph.

strategy might be to limit the circulation of domestic animals ranging free in the local tradition. Since it has been shown that the prevalence of *T. gondii* may vary with the type of nutrition, acting on the cat's diet may also be a solution. Cats eating raw meat are likely to be more infected than those fed cooked meat (Ewa et al., 2003;Papini et al., 2006). Another possibility is treating cats and their nests with drugs. Food is traditionally well cooked and therefore an action based on consumption of rare steak is unnecessary.

The virulence of strains circulating in the village of Dienga was suggested by the virulence of inoculum in Swiss mice. Some developed ascites, others had no symptoms. The number of parasites in each inoculum was not known, so the variation between the strains could be due to the differences in inoculum size. This suggests the heterogeneity of strains circulating in the village, at least in animals. Further studies on the genetic characterisation of the strains circulating amongst species have shown the presence of type I-related strain design such as Africa 1 and 3 (Mercier et al., 2010) and in many cases type III isolates. Due to the complexity of the structure of these strains, the relationship with their clinical expression in humans and other animal species might also be complex, given that it has been shown that even within a haplogroup, expression of some virulent factors may vary according to the degree of virulence (LiMin et al., 2014).

CONCLUSIONS

These results suggest that the *T. Gondii* parasite circulating in the village is complex and derived from cats rather than bush meat, while other domestic animals are important vehicles for *T. gondii* circulation. The risks to the human population can be reduced by fencing livestock, burning rubbish, feeding cats cooked meat and drug treatment.

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Conflict of interest

We declare no conflict of interest

Authors' contributions

JPA conceived the study, study design, data analysis and writing the manuscript. NB coordinated specimen collection, laboratory work and analysis of data. MP participated in collection of specimens, data analysis and writing of the manuscript. BU participated in study design, specimen collection and clinical analysis. All authors read and approved the final manuscript.

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