



A serological survey of *Toxoplasma gondii* infection in sheep and goat from Benin, West-Africa

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Abstract *Toxoplasma gondii* is a parasitic protozoan, the etiological agent of toxoplasmosis, a worldwide zoonosis responsible for abortion and congenital malformation in animal and human. The present study reports, for the first time, the occurrence of *T. gondii* infection among sheep and goats from Benin. A total of 368 small ruminants: 215 serum samples from sheep raised in Sahelian area of North Benin and 153 serum samples from goats raised in a family farm from South-Benin, were collected and screened for anti-*T. gondii* IgG antibodies by the ELISA-indirect method. The results show the presence of anti-*T. gondii* IgG in 53% (83/153) of goats and 1.4% of sheep (3/215). Age, sex and breed did not seem to affect the frequency of this infection. Among goats, *T. gondii* infection was higher in animals reared in the coastal zone (Cotonou municipality) than those raised on the island (Allada municipality) [odds ratio (OR) = 4, 95% confidence interval (CI) =

1.07–15.002, $p = 0.032$, (χ^2) test]. Humidity would be the determining factor in the disparity of recorded infection rates among sheep and goat. The high prevalence of caprine toxoplasmosis observed in southern Benin shows strong environmental contamination. Sensitization campaigns should therefore be undertaken by the public health authorities to inform the inhabitants of this area about risks and preventive measures of this zoonose.

Keywords Zoonose · *Toxoplasma* · Sheep · Seroprevalence · Goat · Benin

Introduction

Toxoplasmosis is a major worldwide zoonosis. It is caused by the protozoan *Toxoplasma gondii* which can parasitize all warm-blooded animals (Dubey 2016). Cat is the definitive host of the parasite in which sexual reproduction occurs, while other animal species (poultry and mammals) serve as intermediate hosts (Dubey et al. 1970). In humans, the consumption of undercooked meat infected with *T. gondii* is an important risk factor for human toxoplasmosis (Cook et al. 2000; Tenter et al. 2000). Cases of human toxoplasmosis acquired after consumption of ovine meat infected with *T. gondii* have been reported in Europe (Condoleo et al. 2017), United States (Guo et al. 2016), China (Zhou et al. 2011) and other countries (Kijlstra and Jongert 2008). Infection by *T. gondii* in sheep and goats may cause foetal death, mummification, abortion, stillbirth and neonatal death and thus can be responsible for heavy economic losses (Innes et al. 2009; Dubey 2016). In Africa, seroprevalence of *T. gondii* in food animals has previously been summarized (Tonouhewa et al. 2017). The results revealed that pigs, sheep and goats are the most affected

The original version of this article was revised: the name and affiliation of the author 'Anjum Sherasiya' was incorrect and it has been corrected now.

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species, posing a major risk to consumers with prevalence ranging from 26.1% (17.0–37.0%) to 22.9% (12.3–36.0%) for sheep and goats respectively. *T. gondii* infections are not restricted to domestic animals, but wild game, especially wild boars, can be infected with a risk of infection for game meat consumers. According to Rostami et al. (2017), 23% of the world's wild-boar population will be infected with *T. gondii*.

In Benin, Small ruminant farming is practiced by rural populations in all agro-ecological areas and constitutes a saving easily mobilized for the owners. The most common sheep breeds are Djallonké and Sahelian, while for goats the dwarf goat is the main breed encountered (Djenontin et al. 2017). In 2013, the small ruminant herd was estimated at 2,576,000 (Countrystat 2018). The majority of sheep flock was concentrated in the north of the country, including the municipalities of Malanville, Karimama and Kandi located in the far north where the Sahelian breeds are mainly found (Djenontin et al. 2017). Otherwise, goat herds are typically found in family farms in all regions, but the majority of this population was concentrated in the south of the country. Sheep and goats contribute significantly to food security in Benin. As follows, national meat production is estimated at 61,646 tonnes in 2014, and small ruminants contribute 7.45% and as the rank third highest contributor after cattle and poultry (Countrystat 2018). In Benin, there is no data about *T. gondii* infection in different animals destined for human consumption and the prevalence of infection in sheep and goats is not yet available to our knowledge; however, the consumption of undercooked sheep or goats' meat infected with *T. gondii* is an important risk factor for human toxoplasmosis. The present study was therefore conducted to find out the prevalence of *T. gondii* infections in sheep and goat raised in Northern and Southern Benin and intended for human consumption.

Materials and methods

Ethic statement

This study has been approved by the Committee for Animal Experiments of the Department of Animal Production and Health of Polytechnic School of University of Abomey-Calavi of Benin. The methods used in this protocol involving animals were in accordance with the ethical standards of this institution. Before blood sampling, all owners of the identified goat and sheep herds were approached to collect their permission and informed consent after full information on the protocol used. Only the animals belonging to the owners who gave their agreements were taken part for this study.

a. Study site

The study was undertaken in two different parts of Benin. The northern region is located in the Department of Alibori where a steppe climate prevails (Fig. 1). At any time of the year it is very hot, the rainfall is low with an average of 750 mm/year and the ambient temperature oscillates around 35 °C. For the climate, the Köppen-Geiger classification is BSh (Peel et al. 2007). This region is favourable for sheep and cattle breeding which evolved in a transhumant breeding system. For the study, all sheep sampled came from three municipalities in this region: Malanville, Karimama, and Kandi (Fig. 1). The second region studied is in the southern part of the country. Topical humid climate characterized by a great rainy season from March to November is dominant in the region. It rains almost all year, with an average rainfall of 1244 mm/year, while the average temperature is 27 °C. These conditions are thus favourable for survival of *T. gondii* oocysts in the environment throughout the year. The Köppen-Geiger classification for the climate is Aw (Peel et al. 2007). All goats were sampled in three municipalities: Cotonou, Abomey-Calavi and Allada from three agro-climatic zones of this region (Fig. 1).

b. Sampling

A seroepidemiological survey was conducted between February 2017 and July 2017 in Northern and Southern

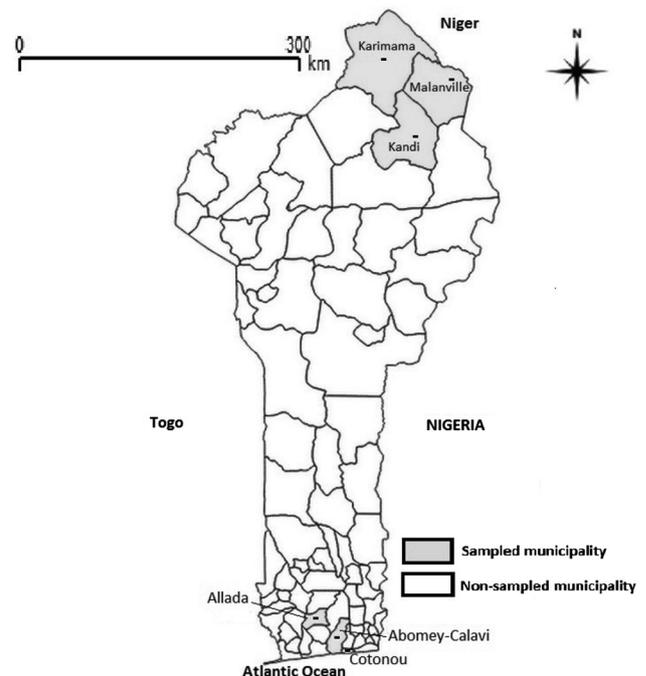


Fig. 1 Map of study sites

Benin to discover the prevalence of Toxoplasmosis among sheep and goats. Blood samples collection was performed using a simple random type sampling. For this, a minimum sample size of 97 was calculated to estimate prevalence with at least 10% accuracy, an expected prevalence of 50% and a 95% confidence level according to Thrusfield (2010) formula:

$$n = \frac{Z^2 \times P(1 - p)}{d^2}$$

In which n = number of sampled properties; Z = value of the normal distribution for confidence level of 95%; p = expected prevalence of 50% (no previous study in Benin), d = absolute error of 10%.

Initially, 153 goats were sampled randomly in family farms at 31 households in three municipalities of southern Benin. These were: Abomey-calavi, Allada and Cotonou. The study included animals raised in family farms that graze near homes, meaning that most of the time they share the same environment as humans and cats. In addition to these samples, 215 Sahelian sheep reared in extensive systems in three municipalities of Northern Benin were included. All animals sampled are raised in Benin and intended for human consumption in the southern region of the country. From each randomly selected animal, approximately 5 ml of blood was collected from the jugular vein. The blood was collected in vacutainer tube and then sent to the laboratory in refrigerated coolers at a temperature between 8 and 12 °C. After centrifugation, the sera from each tube were transferred into two Eppendorf tubes marked with the identifier of each animal and stored in freezers at a temperature of 20 °C until the serological tests. In addition, each sample was followed by a quiz giving information on the geographical location of the sampling area, age, gender, breed of the animal, presence of cats in the households. These data were used to identify the risk factors influencing sheep and goat infection by *Toxoplasma gondii*.

c. Serological examination

The detection of anti-*Toxoplasma gondii* antibodies among goat and sheep sera has been performed using the ELISA INDIRECT kit from IDvet, targeting P30 surface antigen of *T. gondii* (ID Screen Toxoplasmosis Indirect Multi-Species, ID Vet Innovative Diagnostic, Montpellier, France). It is a serological diagnostic test used to detect the presence of *T. gondii* specific immunoglobulin G resulting from infection with this parasite according the manufacturer's instruction. This kit can be used for specific antibodies to *T. gondii* in pig, sheep, goat and other mammals, including cat and dog. The test involves contacting the antigens and serum samples to be tested in a 96-well

microplate. The wells are sensitized with the *T. gondii* specific antigen P30. *Toxoplasma*-specific antibodies, if present form an antigen-antibody complex. After washing, a peroxidase-labeled anti-multi-species conjugate (HRP) is dispensed into the wells. It binds to antibodies, forming an antigen-antibody-conjugate-HRP complex. After removal of the excess conjugate by washing, the reaction is revealed by a revealing solution (TMB). The resulting coloration is related to the amount of specific antibodies present in the test sample: in the presence of antibodies in the sample, a blue color appears which becomes yellow after blocking. In the absence of antibodies in the sample, no coloration appears. The reading is performed at 450 nm with a microplate Absorbance spectrophotometer (Biorad, marnes la coquette, France). According to manufacturer, the test is validated if: the average value of optical density of the positive controls (DOCP) is greater than 0.350 (DOCP > 0.350); the ratio of the average of the positive controls (DOCP) to the average of the negative controls (DOCN) is greater than 3.5, (DOCP/DOCN > 3.5). Sera with S/P%: less than or equal to 40% are considered negative, between 40 and 50% are considered doubtful, greater than or equal to 50% and less than 200% are considered positive and 200% or more are considered as derived from an animal with an acute clinical expression.

d. Statistical analysis

Prevalence's are expressed in percentages. Data was statistically analyzed using Chi square test or exact Fischer test to compare seroprevalence values between modalities of different variables. Independent variables with a significant difference between modalities were selected for multiple logistic regression analysis to identify independent risk factors for seroprevalence, computing odds ratios (OR) and their 95% CI. Data analyses were performed by EPI-INFO 7 (Epi-Info 7, CDC, USA) for windows while differences between proportion were considered statistically significant at the 5 per cent level ($p < 0.05$).

Result

The occurrence of *T.gondii* infections in sheep and goats raised in traditional extensive farming from two different region of Benin was evaluated for the first time in this study. A total of 153 goats were sampled from 31 households in southern Benin. In this breeding system, animal are reared wandering around the households, where they usually benefit from agricultural by-products and kitchen waste. Under this management system, animals are usually exposed to *T. gondii* oocysts if they were present in the environment around the household. For 153 goat sera

analyzed by ELISA method, 82 were positive for antibodies against *T. gondii*, representing an overall prevalence of 53.6% (CI 95 = 45.7–61.5%). Animals older than 1½ (38/61) had 2.6 higher chance of being infected than animals aged 6 months (8/21), but the χ^2 test performed did not show a significant difference between the observed values ($p > 0.05$). Similarly, we did not observe a significant difference between the infection rate of males and females ($p > 0.05$), although seroprevalence in females was higher than males. On the other hand, the seroprevalence has strongly evolved according to the agroclimatic region. Indeed, we obtained a very high seroprevalence (92.6%) in animals raised in the coastal region (Cotonou municipality) compared to those raised in inland regions (30% in Allada). Animals raised in Coastal region (Cotonou) were, therefore, four times more likely to be infected with *T. gondii* oocysts than those Allada (inland region) with a significant difference at the 5% threshold ($p < 0.05$). The overall results are summarized in Table 1.

Table 2 shows the infection rates observed in sheep from the Sahelian areas of Northern Benin. We observed a very low prevalence of *T. gondii* infection in animals from this region (3/215), i.e. a seroprevalence of 1.4% (CI 95 = 0.2–3%). Three positive sera were from Ouda Sahelian sheep, while all West African dwarf sheep and Bali-Bali Sahelian samples were negative. We did not observe a variation in prevalence according to the genus, age and breed of animals.

Discussion

Toxoplasmosis is a zoonotic infection responsible for abortions and other reproductive disorders in sheep, goat and pregnant women. Several cases of cerebral

toxoplasmosis are frequently reported in HIV/AIDS patients at CNHU-HKM National University Hospital in Cotonou, Benin, but the sources of infection have never been studied (Zannou et al. 2004). Cats are definitive hosts and represent the key element in the epidemiology of this disease. The role of oocysts shedding by cats in the transmission of this parasite have been studied (Wallace 1973; Ruiz and Frenkel 1980; Etheredge et al. 2004). It was shown that the absence of cats was associated with an absence of human and animal antibodies to *Toxoplasma*, on islands in the Pacific Ocean (Etheredge et al. 2004). In Benin, the presence of resident and stray cats has been observed in the majority of households where they are used as measures of rodent control (Avode et al. 2005). This assumes a high contamination of the household environment by *T. gondii* oocysts. In sheep and goat, toxoplasmosis has never been diagnosed in Benin before, although cases of abortions and stillbirths are frequently reported on farms. In this study, a high seroprevalence (53.60%) was observed in goats compared to sheep ($p < 0.05$), for which we observed a very low level of infection (1.4%). In Cotonou, goat infection was particularly high compared to Abomey-Calavi and Allada ($p < 0.05$). This result highlights the large spatial distribution of *T. gondii* infections within the same species. This result may be related to environmental factors such as rainfall and temperature. Many studies have shown that moisture and heat are important factors for the sporulation and survival of *T. gondii* oocysts excreted in feces of cats (Davoust et al. 2015; Dubey 2008; Schares et al. 2016). In Benin, rainfall varies considerably by geographical area. Rainfall is particularly high on the coast and gradually declines in the country from south to north. In addition, the ambient temperature follows the same evolutionary curve, which results in a humid tropical climate that is progressively

Table 1 Seropositivity and risk factor of *T. gondii* infection in goat from South-Benin

Risk factor	Variable	No. sample	No. positive	Prevalence (%)	OR	95% CI
Species	Goat	153	82	53.60		45.70–61.50
Sex	Male	47	20	30.72	1	
	Female	106	62	69.28	1.9 ($p = 0.08$)	0.97–3.81
Age	< 6 Months	21	8	38.10	1	
	7–18 Months	71	36	50.70	1.67 ($p = 0.44$)	0.61–4.52
	> 18 Months	61	38	62.29	2.68 ($p = 0.09$)	0.96–7.45
Municipality	Allada	44	14	31.81	1	
	Abomey-calavi	68	30	44.11	1.69 ($p = 0.23$)	0.76–3.74
	Cotonou	41	38	92.68	4 ($p = 0.032$)	1.07–15.00
Presence of cat	Yes	112	58	51.78	1	
	No	41	24	58.53	1.31 ($p = 0.47$)	0.63–2.70

OR Odd ratio; CI confidence interval

Significant = ($p < 0.05$)

Table 2 Seropositivity and Risk factor of *T. gondii* infection in sheep from North-Benin

Risk factor	Variable	No. sample	No. positive	Prevalence (%)	OR	95% CI
Specie	Sheep	215	3	1.40		0.2–3
Sex	Female	160	1	0.62	1	
	Male	55	2	3.63	6 ($p = 0.32$)	0.30–356.13
Age	< 6 Months	6	0	0	0	–
	7–18 Months	34	0	0	0	–
	> 18 Months	175	3	1.71	– 1	–
Breed	West African dwarf sheep	73	0	0	0	–
	Bali-Bali Sahelian sheep	72	0	0	0	–
	Ouda Sahelian sheep	70	3	4.28	– 1	–

OR Odd ratio; CI confidence Interval

Significant = ($p < 0.05$)

evolving towards a dry tropical climate as one progresses from the south (1244 mm/year) to the north of the country (750 mm/year). This dynamic justifies the high prevalence observed in the zone 8 located on the wetter coast, where the rainfall and ambient temperature are higher than the two other agro-ecological zones located inland. The climatic conditions are thus more favourable for the maturation and survival of *T. gondii* oocysts on the ground in the Southern region of the country than in the other northern regions, where the ground is drier and less watered. This also justifies the very low seropositivity recorded in sheep coming from the Sahelian zone of northern Benin, which is less watered and where it is extremely hot most of the year. In Benin, goats are raised mostly in family farms. In this system, animals share the same environment as humans and live near the huts where they eat leftovers and kitchen waste, as well as cohabiting with cats used for rat extermination. This proximity favours the level of infection observed in this animal species. On the other hand, sheep are generally raised in herds, which graze most of the time in the savannah far from household where the contamination of the environment by the oocysts of *T. gondii* is lower compared to the proximity of household. This situation could also justify very low prevalence obtained in sheep. In our study the prevalence obtained in goats (52.3%) is higher than that obtained in Senegal in 52 goats (9.6%) (Davoust et al. 2015) and in 323 goats (15.48%) in Central Ethiopia (Gebremedhin et al. 2014). Swai and Kaaya (2012) also obtained lower prevalence levels in 337 goats (19%) in Tanzania while Van der Puije et al. (2000) found 29% prevalence of toxoplasmosis in 526 Ghana goats. Moreover, higher infection level have been reported in goats from some African countries. In Ethiopia, prevalence in goats was 74.6% in 641, and 69% in 312 goats in Zimbabwe (Hove et al. 2005; Teshale et al. 2007).

In sheep, the seroprevalence obtained in the present study is far lower compared to those obtained from studies

in other parts of Africa. Most studies on the prevalence of toxoplasmosis in sheep showed a variable infection level of 0% in 45 sheep in North Nigeria and 71% in 5806 sheep in Libya (Al-Mabruk et al. 2013). In the Western Cape state (South Africa), Hammond-Aryee et al. (2015) recorded an 8% infection rate on 282 merino's sheep, while the prevalence was twice as high (16%) in 43 sheep from Senegal (Davoust et al. 2015). In West Africa, Van der puije et al. (2000) recorded 33.2% seroprevalence among 732 Ghanaian sheep. However, a lower prevalence level (6.7%) was obtained among 372 sheep in Borno State, Nigeria (Kamani et al. 2010a).

In Northern Nigeria, a country that shares the same climatic conditions as Benin, toxoplasmosis has been reported in goat, sheep, cats and humans. A study using Polymerase Chain Reaction (PCR) to detect *T. gondii* infection in aborted fetuses from 45 sheep and 31 goats respectively were negative (0%) (Kamani et al. 2010b). This result is consistent with the low seroprevalence obtained in sheep from Northern Benin for this study (1.43%), as this region shares the same climate as Northern Nigeria.

Given the proximity of goats reared in family farms with households, this species could be used as a sentinel for environmental risk assessment in Benin. Goats are one of the main animal species used for meat production in Benin and around the world. Due to high prevalence obtained in this species in Southern Benin, special attention should be given to cooking meat. At-risk populations such as pregnant women and people living with HIV / AIDS should avoid methods of preparation that do not favor meat-based cooking: for example, braised and grilled meats on barbecues that is widely consumed in Benin. The high prevalence of infection in animals in Southern Benin is a wake-up call for public health authorities, who should pay more attention to this neglected zoonotic disease in the tropics.

In pregnant women seroprevalence is much higher in southern Benin. Infection levels between 70 and 36.1% were observed in 1997 and 2018 (Akpovi et al. 1998; Zannou et al. 2004; Ogouyèmi-Hounto et al. 2014) respectively. This trend is decreasing over time, suggesting an increase in the proportion of pregnant women who are not immunized against infection and therefore at risk for congenital toxoplasmosis (Ogouyèmi-Hounto et al. 2014). It is important to advise them to avoid eating undercooked meat, raw milk, unsafe water, unwashed vegetable contaminated with cat feces, and contact with cat feces and soil. In Benin, the population is accustomed to eating well-cooked meat, but cooking methods that do not favor thorough cooking of meat such as barbecues exist and should be avoided.

Conclusion and recommendation

T. gondii is present and circulates in small ruminants which are available for human consumption in Southern Benin. The infection is much higher in goats in family farms in Southern Benin than in sheep from Northern Benin. This result highlights the high contamination of the environment by *T. gondii* oocysts excreted by cats and the high exposure of populations to the risk of human infection in Southern Benin. Sensitization campaigns should therefore be undertaken by the public health authorities to inform the Beninese population about risks and preventive measures. As toxoplasmosis may be a public health problem in Benin, it would be particularly useful to obtain information on the genetic diversity of *T. gondii* and to isolate strains of this parasite affecting humans and animals in this country.

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Author's contributions ABNT and YA conducted the survey and wrote the manuscript. AS helped in interpreting the results and worked on the manuscript. PS and JMA performed statistical analyses and designed the figures. GLA aided to perform the serological analyses and interpretation of results. MNA, IY and SF participated in the planning and supervision of the work.

Compliance with ethical standards

Conflict of interest The authors state that they have no competing interests.

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