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Serological evidence and factors associated with porcine toxoplasmosis in three villages of Fara's division in Burkina Faso

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ABSTRACT

Porcine toxoplasmosis is a worldwide zoonosis. This study was conducted to establish evidence of toxoplasmosis and its associated factors among pigs in three villages of Balés province, Burkina Faso. Serums samples were collected from 182 pigs and data was collected on farmers' sociodemographics, origin (village) of pigs, pigs' sex, age, breed and keeping systems through a household questionnaire interview. Serum samples were analyzed using indirect ELISA targeting IgG antibodies to *Toxoplasma gondii*. Results of the study showed an overall sero-prevalence of 16.5 % (95% CI: 11.1% – 21.9%). The sero-prevalence was higher in Toné (23.1%; 95% CI: 12.8% – 33.3%) and Kabourou (20.7%; 95% CI: 10.3% – 31.1%) compared to Sadon Bobo (5.1%; 95% CI: 0% – 10.7%) ($p=0.01$). It was also higher in pigs older than 12 months (23.2%; 95% CI: 14.9% – 31.5%) compared to pigs less than 12 months (8.4%; 95% CI: 02.4% – 14.4%) ($p=0.00731$). During rainy season, tethered pigs (7.1%; 95% CI: 0.40% – 13.8%) were less infected than housed pigs (20.6%; 95% CI: 11.1% – 21.9%) ($p=0.02$). Multivariate logistic regression model shows that pigs older than 12 months were more likely to get infected compared to pigs less than 12 months old (OR = 2.58; 95% CI = 1.00 - 6.62; $p=0.04$). These results provided evidence for the presence of *T. gondii* in pigs in this area.

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Keywords: Burkina Faso, pigs, *Toxoplasma gondii*, seroepidemiological studies, zoonosis.

INTRODUCTION

Toxoplasmosis is caused by *Toxoplasma gondii*. It is major zoonosis disease of medical and socio-economic importance (Sonar and Brahmabhatt, 2010).

Felidae family, especially the cat, are the only known definitive hosts of the parasite (Dubey et al., 2012) releasing millions of parasite oocysts in the environment. These oocysts after sporulation can maintain their infectivity for

several months in water and soil and are responsible for infestation of intermediate hosts including human and other animals like sheep, goats, pigs, chickens (Buxton et al., 2007). Intermediate hosts could become infected through consumption of food or water contaminated with oocysts (Dubey and Jones, 2008). Ingestion of raw or undercooked meat of infected animals is the most common way of human infection (Belloco et al., 2016; De Berardinis et al., 2017). The transmission risk could be higher with sheeps' and pigs' meat as *T. gondii* infection is common in those animals. However, infection of cattle, horses and water buffaloes with *T. gondii* is less common than infection in sheep or pigs (Hill and Dubey, 2002). There is also congenital transmission in human which lead to serious neurological or ocular disorders with cardiac and brain abnormalities such as hydrocephalus, mental retardation, seizures or fetal mortality (Jones et al., 2001; Remington et al., 2006). The disease is usually asymptomatic in immunocompetent individuals but can lead to serious complications in immunosuppressed individuals. It is common in pregnant women in Burkina Faso (Bamba et al., 2017b) and can lead to abortions. In rural communities of Burkina Faso, pig meat is mostly consumed boiled (Ngowi et al., 2017; Dahourou et al., 2018). So, consumption of pork could be at risk for *T. gondii* transmission to humans, especially when the meat is undercooked (Dubey, 2009). In Burkina Faso, like in other African countries, pigs are mainly reared in traditional way and free ranging is very common in this pigs' management system (Mopate et al., 2011; Kiendrebeogo et al., 2012; Djimenou et al., 2017). However, this type of breeding system is associated with higher risk of toxoplasmosis in pigs (Stelzer et al., 2019).

Studies on animal toxoplasmosis, mainly on pigs, are scarce in West Africa. Studies were carried out in Burkina Faso and Côte d'Ivoire (Pangré et al., 2009; Bamba et al., 2016) but have only focused in pigs' in slaughterhouses located in the cities. Otherwise no sero epidemiological study, focused on porcine toxoplasmosis, has been carried out in

rural area in Burkina Faso. Hence, this study aimed at evaluating the serological evidence of porcine toxoplasmosis and its associated factors among free roaming pigs in three villages of Burkina Faso.

MATERIALS AND METHODS

Study area

The study took place in province of the Balés, located in the Boucle du Mouhoun region. Three villages, in Fara division, were selected for this study. The villages are located at 60 km from national road one, on the regional road 11 between *Poura Carrefour* village and the border with Ghana. Populations of this area are mostly animists with an important pig population except for Kabourou where we have many Muslims, with a small pig population. The area has a tropical climate with two seasons. The rainy season from May to September with an average rainfall of 871 mm. The dry season is between October and April.

In the study area, there is no data on pig numbers and pigs' husbandry practices depend on the season. During rainy season, pigs are tethered or kept confined (housed) in pigsties; while during dry season, pigs are left scavenging for food. According to veterinary services in this area, pigs' main reported diseases are African swine fever, porcine cysticercosis and gastro intestinal parasitic infections.

Sample size and pig selection

At the beginning, blood samples collection was designed for porcine cysticercosis prevalence study in this province. During field activities, the presence of toxoplasmosis has been suspected. As samples collected were enough for a study on toxoplasmosis, serum collected was used to assess the evidence of toxoplasmosis in pigs to increase knowledge on zoonosis in the study area.

Sampling size was calculated using Epitools Epidemiological calculators with the following formula: $N = (Z^2 * P (1-P))/e^2$; where N is the sample size, Z is the value from standard normal distribution corresponding to 95% confidence level, P is the expected

prevalence and e is the precision. The precision was set at 7% and expected prevalence was estimated to 28.8 % (Bamba et al., 2017b) and pig population of 270 504 pigs in the region (Institut National de la Statistique et de la démographie, 2016). The minimum sample required for this study was 162, but 182 samples were sampled. From each animal, blood was drawn for serum harvesting. In each village, households were randomly selected from all pig-keeping households and each selected household were visited and one pig were randomly selected.

Questionnaire survey

A questionnaire was administered to each farmer whose animal was included in the study to collect information on potential associated factors to pigs' infection with *T. gondii*. Data on farmer gender and their experience with pig husbandry, pigs' sex, age and breed, pig keeping type was collected. Data was recorded on sheets and linked to the animal biodata. Same predictors of sero-positivity to porcine cysticercosis were used in the risk factor analysis of *T. gondii*.

Serological analysis

Pig sera samples were tested using commercial indirect ELISA Kits (ID Screen® Toxoplasmosis Indirect Multi-Species from ID Vet Innovative Diagnostic, Montpellier, France) which detects *Toxoplasma sp* specific IgG antibodies in pigs' sera. This ELISA test used microplates precoated by *Toxoplasma gondii* specific P30 antigen. The aim of the reaction is to build antigen-antibody-conjugate complex which make a reaction with a substrate and produce a coloration detected by the measurement of optical densities. Optical densities (OD) were read at 450 nm. The test was validated if the mean value of the positive control O.D. (ODPC) is greater than 0.350 (ODPC >0.350) and the ratio of the mean O.D. values of the positive and negative controls (ODPC and ODNC) is greater than 3.5 (ODPC/ODNC >3.5). According to the manufacturer's recommendation, sera or meat juice with S/P% less than or equal to 40% are considered negative, between 40 and 50% are

considered doubtful and greater than or equal to 50% are considered positive. Samples with S/P % greater than or equal to 200% are considered as acute infection. Doubtful samples were repeated for certainty.

Statistical analysis

Data was transferred on Microsoft (2007) Excel sheet, and statistical analysis was performed using STATA 13. Apparent sero-prevalences were calculated and expressed in percentage with 95% confidence interval (CI). Descriptive statistics were used to report demographic characteristics of respondents, as well as pig characteristics. Univariate analysis was carried out on each identified potential risk factors independently. Significant variables to univariate analysis at $P \leq 0.1$ were included in a multivariable logistic regression model to compute adjusted odds ratios (AOR) at 95% CI. Tests for significance of associations were performed at significance level of 0.05.

RESULTS

Sero-prevalence of toxoplasmosis in pigs is summarized in table 1. The overall sero-prevalence was 16.5% (95% CI: 11.1% – 21.9%) with 40% of acute toxoplasmosis. This prevalence was significantly associated with village, pig age and keeping type during rainy season. The highest prevalence was found in Toné village (23.1%; 95% CI: 12.8% – 33.3%) and the lowest at Sadon Bobo village (5.1%; 95% CI: 0 – 10.7%). There was also a difference in prevalence between village ($p=0.01$). The prevalence was higher in older pigs (more than 12 months) (23.2 %; 95% CI: 14.9% – 31.5%) compared to younger pigs (less than 12 months) (8.4%; 95% CI: 2.4% – 14.4%) ($p=0.007$). Prevalence was higher in confined pigs during rainy season (20.6%; 95% CI: 13.5% – 27.6%) compared to tethered (7.1%; 95% CI: 0.40% – 13.8%) ($p=0.02$).

The prevalence was 10.6% (95% CI: 11.3% – 22.3%) in animals kept by women while none animal kept by men was infected in the present study ($p=0.43$). Pigs that belong to farmers with longer experience in pig keeping (more than five years) were more infected then pigs that belong to farmers with short

experience in pig husbandry (Table 1). The infection was higher in females (19.8 %; 95% CI: 12.5% – 27.05%) than in males (10.6%; 95% CI: 3.2% – 18.02%) ($p=0.107$). Variation was noted on prevalence regarding pigs' breed and the prevalence was 18.8% (95% CI: 12.5% – 25.1%) and 6.1% (95% CI: 0% – 14.3%) in local and mixed pigs respectively ($p=0.07$).

Univariate logistic regression revealed that different factors were associated with *T. gondii* infection. Pigs which were more than 12 months were 3.28 times more likely to be infected by *T. gondii* than those less than 12 months (Table 2) ($p=0.01$). Pigs tethered were

also, less likely to be infected with *T. gondii* (OR = 0.29; CI95 = 0.09 – 0.89; $p=0.03$) than confined pigs during rainy season. Also, Pigs from Sadon bobo village were less likely to be infected with *T. gondii* (OR = 0.18; CI95: 0.04 – 0.65; $p=0.009$) when compared with pigs from other villages (Table 2).

After multivariable regression analysis, only pig age was associated to infection with *T. gondii*. Pigs which were more than 12 months were 2.58 times more likely to have *T. gondii* than animals less than 12 months (Table 3) ($p=0.04$).

Table 1: Descriptive characteristics of farmers, sampled pigs with associated *T. gondii* seroprevalences in Balés provinces, Burkina Faso.

Variables	Category	n	Seroprevalence and 95 % CI	p-value
Village	Kabourou	58	20.7 (10.3 – 31.1)	0.01
	Sadon Bobo	59	05.1 (0 – 10.7)	
	Toné	65	23.1 (12.8 – 33.3)	
Farmer experience	Less than 5 years	10	10 (0 - 28.6)	0.84
	Between 5 and 10 years	72	16.7 (8.1 – 25.3)	
	Above 10 years	100	17 (9.6 – 24.4)	
Farmer gender	Men	3	0	0.43
	Women	179	16.8 (11.3 – 22.3)	
Pig sex	Male	66	10.6 (3.2 – 18.02)	0.107
	Female	116	19.8 (12.5 – 27.05)	
Pig breed	Local	149	18.8 (12.5 – 25.1)	0.07
	Mixed	33	06.1 (0 – 14.3)	
Age class	[0 - 12 months]	83	08.4 (2.4 – 14.4)	0.00731
	> 12 months	99	23.2 (14.9 – 31.5)	
Pigs living area	Piggeries	126	20.6 (13.5 – 27.6)	0.02
	Tethered	56	7.1 (0.40 – 13.8)	
Total animal sampled	-	182	16.5 (11.1 – 21.9)	

n: number of sampled animals for the category; CI: Confident Interval

Table 2: Univariable logistic regression analysis of potential explanatory of *T. gondii* infections in pigs in Balés province, Burkina Faso.

Variables	Category	Crude OR and 95 % CI	p-value
Village	Kabourou	0.86 (0.36 - 02.05)	0.750
	Sadon Bobo	0.178 (0.04 - 0.65)	0.009
	Toné	1	
Farmer experience	Less than 5 years	1	
	Between 5 and 10 years	1.80 (0.20 - 15.56)	0.593
	Above 10 years	1.84 (0.21 - 15.52)	0.574
Farmer gender	Men	0.00 (0.00 – 10.9)	0.991
	Women	1	
Pig sex	Male	0.479 (0.19 - 1.18)	0.11
	Female	1	
Pig breed	Local	1	
	Mixed	0.27 (0.06 -1.23)	0.09
Age class	[0 - 12 months]	1	
	> 12 months	3.28 (1.3 - 8.11)	0.01
Pigs living area	Piggeries	1	
	Tethered	0.29 (0.09 - 0.89)	0.03

OR: Odds Ratio ; CI : Confident Interval

Table 3: Factors associated with *Toxoplasma gondii* infection in pigs in Balés Province, Burkina Faso.

Variable	Category	Adjusted OR	95% CI for OR	P-Value
Village of origin	Toné	1		
	Sadon Bobo	0.33	0.04 – 1.46	0.12
	Kabourou	0.89	0.33 – 1.93	0.62
Pig Age Class	[0-12 months]	1		
	> 12 months	2.58	1.00 - 6.62	0.04
Pig sex	Male	0.69	0.26 – 1.81	0.46
	Female	1		
Pigs' living area	Piggeries	1		
	Tethered	0.75	0.16 – 3.48	0.72

OR : Odds Ratio; CI : Confident Interval

DISCUSSION

Toxoplasmosis was described in animals and mainly in pigs over the world (Dubey, 2009) and in Africa (Tonouhewa et al., 2017). This first serological evidence of *T. gondii* on pigs, from Balés Province, reported a seroprevalence of 16.5% (CI95: 11.1 – 21.9%). In Burkina Faso, Bamba et al. (2016) and Bamba et al. (2017b) have respectively reported a prevalence of 29% and 28.8% in pork in an abattoir survey in Bobo Dioulasso. This indicate evidence of porcine toxoplasmosis in pigs in Burkina Faso and regarding this situation, pork eaters could be at risk for toxoplasmosis. Furthermore, in Burkina Faso, the prevalence of toxoplasmosis in pregnant women is 31.1% and consumption of meat including pork was reported as risk factor of human infection (Bamba et al, 2017a).

In Côte d'Ivoire, Pangré et al. (2009) found a prevalence of 8.8 % in an abattoir survey on pork. Arko-Mensah et al. (2000) in Ghana, Gebremedhin et al. (2015) in Ethiopia and Hove et al. (2005) in Zimbabwe have reported prevalence of 39%, 32.1% and 3.51% respectively. In Nigeria, Obijiaku et al. (2017) and Onyiche and Ademola (2015) have reported 4.4% and 29.14% respectively. In the others part of world, Hill et al (2010) reported a prevalence of 2.6% in United States and Alvarado-esquivel et al. (2011) found 12.7% in Mexico. In Asia, 27% has been reported in pigs by Zhou et al. (2010) in China. Lopes et al. (2013) in Portugal and Garcia-Bocanegra et al. (2010) in Spain have reported 9.8% and 19% respectively. Such seroprevalence variations might be linked to differences in climatic conditions (altitude, rainfall, soil type, temperature) and animals management systems (intensive, extensive) and also pigs access to cats scat (Dubey, 2009; Innes, 2010).

According to different explanatory variables, the infection of pigs was associated with location (village), pigs age and pigs living area. The higher seroprevalence in Toné and Kabourou villages, compared to Sadon Bobo village ($P < 0.05$) could be associated with high density of cats observed in Toné and Kabourou (personal observations). In different studies carried out in United States and The

Netherlands, cats' access to pigs and the number of cats in farms lead to high prevalences of *T. gondii* infection in pigs (Dubey, 2009). Pigs' age has been found to be a risk factor for porcine toxoplasmosis in different studies (Arko-Mensah et al., 2000; Gebremedhin et al., 2015). The higher seroprevalence of toxoplasmosis in pigs older than 12 months compared to pigs less than 12 months might be due to the longer contact time of older animals with a potentially infected environment containing *T. gondii* oocysts and/or tissue cysts (Villari et al., 2009). The study found that pigs kept in piggeries during rainy season were more infected with *T. gondii* compared to those that were tethered. During rainy season, cats get access to piggeries for food and might exposed pigs to *T. gondii* oocysts though their infected scats. In Togo, neighboring country of Burkina Faso, Degbe et al. (2018) found that a high proportion of cats around Lomé release oocysts in their scats.

Some limitations have been noted for this study. During field activities, many associated factors were not added on the survey questionnaire for the identification of risk factors. This suggests that further studies are needed at national level to identify on animal level risk factor of toxoplasmosis on animal.

Conclusion

Toxoplasmosis is an important zoonotic disease which affects many animal species and human. The present study, conducted in a division of Burkina Faso, reported a prevalence of 16.5% for porcine toxoplasmosis and has highlighted that pigs older than 12 months were more likely to be infected with *T. gondii* compared to those less than 12 months. As human toxoplasmosis transmission through infected meat and regarding the important prevalence, it is necessary for health sector stakeholders to implement sensitization actions in this area.

COMPETING INTERESTS

Authors declare that there is no competing interests.

AUTHORS' CONTRIBUTIONS

The study has been designed by LDD, OBG and AT. SMR and ARST collected data on the field and made laboratory analysis. LDD made statistical analysis and proposed the draft of the manuscript. All authors reviewed the manuscript before submission.

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