



TOXOPLASMA GONDII INFECTIONS IN PIGS FROM SOUTH BENIN AND ASSESSMENT OF BREEDERS' KNOWLEDGE ABOUT TOXOPLASMOSIS

A. B. N. TONOUHEWA¹, Y. AKPO^{1,3}, P. SESSOU¹, C. SALANON¹,
G. L. APLOGAN⁴, M. N. ASSOGBA², I. A. K. YOUSSAO² & S. FAROUGOU¹

¹Communicable Disease Research Unit (URMaT), University of Abomey-Calavi, Cotonou, Benin; ²Department of Animal Health and Production, University of Abomey-Calavi, Cotonou, Benin; ³Laboratory of Ecology, Health and animal Production, Faculty of Agronomy, University of Parakou, Parakou, Benin; ⁴Laboratory for Veterinary Diagnosis and Serosurveillance of Animal Diseases, Ministry of Agriculture, Livestock and Fisheries, Benin

Summary

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Toxoplasma gondii is a ubiquitous apicomplexan parasite that causes toxoplasmosis, a cosmopolitan zoonosis responsible for abortion and congenital malformation in animals and humans. Pigs can be a reservoir for *T. gondii* and act as one of the sources for parasite transmission to humans. Currently, there are no data about *T. gondii* infection in any animal in Benin. After the report of several cases of abortions and reproductive problems in pigs in several livestock farms in the southern region of Benin, this study was carried out to investigate the occurrence of this zoonotic parasite in pigs. A total of 184 pig serum samples have been collected in two municipalities and sent to the laboratory for the detection of anti-*T. gondii* IgG antibodies by ELISA indirect method. The results showed the presence of anti-*Toxoplasma gondii* IgG in 23% (IC₉₅ = 22.56–23.44) of pigs (42/184). Age, sex, and race did not seem to affect the frequency of this infection. Moreover, reproductive failure has been reported on many farms. These were cases of stillbirths in 40% of farms followed by neonatal mortality (36%) and abortion cases which were frequently observed in 27% of farms. *T. gondii* is present in pigs from south Benin and could be responsible for reported abortions in the areas. In addition, the raw and undercook meat of pigs could be important source of *Toxoplasma gondii* infection in humans from south Benin.

Key words: abortion, Benin, ELISA, IgG, pig, *Toxoplasma*

INTRODUCTION

The protozoan parasite *Toxoplasma gondii* can infect all mammals and birds on all

continents (Lindsay & Weiss, 2008; Dubey, 2016). It is an intracellular coccidian

of apicomplexan phylum, the etiologic agent of toxoplasmosis. This disease is a major zoonosis responsible for abortion and congenital malformation in humans and animals. Among animal species, sheep, goats and pigs are the most affected. In pigs, the infection usually occurs postnatally by ingestion of oocysts from contaminated environment and ingestion of infected tissues of animals, or prenatally by transplacental transmission of the parasite (Dubey, 2009). The disease can then be severe or fatal with abortion cases and mortality especially in piglets. In humans, who can get infected by the consumption of food contaminated with oocysts excreted by cats or by undercook meat containing tissue cysts, most infections are asymptomatic, except in immunocompromised people and pregnant women (Tenter *et al.*, 2000). In Africa, most studies on animal toxoplasmosis have shown the variability in the level of infection for different animal species and areas, with high prevalence in chickens and low prevalence in cattle (Tonouhewa *et al.*, 2017). In pigs, a prevalence varying from 9% to 39% was reported (Tonouhewa *et al.*, 2017).

Benin is an African country where approximately half of the human population is infected, with an estimated prevalence of 48% for toxoplasmosis in the southern region (Ogouyèmi-Hounto *et al.*, 2014). Studies carried out between 1997 and 2014 showed a decreasing proportion of 63% and 48% of seropositive pregnant women (Akpovi *et al.*, 1998; Ogouyèmi-Hounto *et al.*, 2014). That implies a gradual increase in the proportion of women not immunised against toxoplasmosis among Beninese population, therefore exposed to congenital toxoplasmosis. Otherwise in the country, pig farming is one of the main activities in

the breeding sector and pork is highly appreciated by consumers particularly in the southern region. Unfortunately, the pork industry is confronted with many health problems, including the failure of reproductions on farms and hygienic and sanitary quality of pork destined for human consumption, now that pigs are considered as an important intermediate host of many parasites including *T. gondii*. Human toxoplasmosis acquired after pork consumption has been reported in some countries (Dubey & Jones, 2008; Wang *et al.*, 2012; Hill & Dubey, 2016). To date, there seems to be no report on the presence of *T. gondii* among any animal species in Benin, although the infection has been reported in humans.

The present study was therefore undertaken to provide current information on the presence of *T. gondii* infection in pigs raised for human consumption in south Benin, and is the first to examine *T. gondii* prevalence in food animals in the study area.

MATERIALS AND METHODS

Ethical standards

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.

Study area

This study was conducted in the Atlantic and Littoral departments of southern Benin between September 2016 and March 2017.

Survey and sampling

Following the reports on the frequency of abortions in pig farms in southern Benin, a cross-sectional survey was conducted in 100 livestock farms in the Atlantic Department located in the region. The objective was to assess current farming practices, the magnitude of reproduction failure and knowledge of breeders about toxoplasmosis, a zoonosis responsible for abortion in pigs. In farms chosen for the survey with the collaboration of the veterinary service of the Ministry of Agriculture, Breeding and Fishery (MAEP/Benin), the information about breeding practice, history of reproduction, knowledge on toxoplasmosis and the role of cats as vectors of zoonosis transmission to animals and humans has been collected from breeders.

Subsequently, a seroepidemiological survey on *T. gondii* infection in pigs was conducted between October 2016 and January 2017 in two municipalities to estimate the level of infection in pig in the area. For sampling, a minimum sample size of 97 was determined in order to estimate the prevalence with at least 10% precision at 50% expected prevalence and 95% level of confidence according to Thrusfield (2005). A total of 184 pig samples were collected using a simple random sampling technique. The sampling was made at livestock farms identified in the municipalities of Abomey-Calavi and the slaughterhouse of Cotonou where animals raised in the region are slaughtered for human consumption.

Serological examination

Pig sera was tested using commercial indirect ELISA Kits (ID Screen Toxoplasmosis Indirect Multi-Species from ID Vet Innovative Diagnostic, Montpellier, France) to determine the presence of

Toxoplasma specific IgG antibodies, resulting from infection with *T. gondii* according to the manufacturer's instruction. This kit has been used for detecting specific antibodies to *T. gondii* in pigs, sheep, and other mammals including cats and dogs. For serological examination, a 96-well plate was prepared containing the test and control specimens, before transferring them into the ELISA microplate using a multichannel pipette, in order to avoid differences in incubation times between specimens. According to the manufacturers' recommendation, sera with sample-to-positive (S/P %) ratio less than or equal to 40% were considered negative, between 40 and 50% were considered doubtful and greater than or equal to 50% – positive. The test was validated if the mean value of the positive control optical density (ODPC) was 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) was greater than 3.5 (ODPC/ODNC > 3.5). Doubtful samples were repeated for certainty.

Statistical analysis

The results were expressed in percentages. Data were statistically analysed using chi-square test or exact Fischer test to compare seroprevalence values between modalities of different variables. Data analyses were performed by Epi-Info 7 for Windows (Epi-Info 7, CDC, USA). The differences were considered statistically significant at $P < 0.05$.

RESULTS

Pig farms management

The surveyed breeders consisted of 96% men and 4% women. The enrolment rate was 27% with 13.04% at the primary le-

vels, 65.21% and 14.81% at the secondary and university levels respectively. Most of the farms had a pig population between 5 and 216 with an average of 55.2±49.70. Out of all farms, 62% practiced confined animal production system compared to 38% with outdoor system. The different reared breeds consisted of Me-tis (61%) followed by local breed (20%) and Large White, Landrace (19%). With regard to animal feeding, complete food rations were offered in 40% of farms, agro-food by-products associated with food waste – in 50%, while 10% of breeders served leftovers to their animals. The drinking water was 75% well-water or uncovered tank, and 90% of breeders used self-medication in case of pig diseases, while 10% waited for the advice of a veterinarian to administer health care.

Reproductive disorders

Table 1 shows reproduction disorders observed at the surveyed farms. The most frequent reproductive failure was stillbirth (40%) and neonatal mortality observed in 36% of farms. Otherwise, abortions cases were frequently observed in 27% of

farms. Toxoplasmosis is recognised as an abortive disease, so these reproductive disorders could be related to this zoonotic infection.

Knowledge about toxoplasmosis

Regarding the knowledge of pig breeders about toxoplasmosis (Table 1), most breeders had no information about this parasitic zoonosis. Only 6% of pig farmers have heard of toxoplasmosis at least once and knew that infection could be responsible for abortion in pregnant women. All respondents (100%) were unaware that pigs can contract toxoplasmosis. Only 5% of breeders knew that the cat is the definitive host of this parasite and can transmit *T. gondii* infection to pigs or humans. The presence of resident or wandering cats has been observed in more than half of the farms and respondents were not aware of the role played by the cat in the epidemiology of this parasitic zoonosis. Finally, on 29% of the farms measures of rodent control were not applied, although rats were recognised as *T. gondii* reservoir.

Table 1. Knowledge of breeders about toxoplasmosis

Questions	Response	Number	P
Reproduction disorders on farms in the last 6 months	Neonatal mortality	36	≤ 0.05
	Stillbirths	40	
	Abortions	27	
Have you ever read, heard or seen any information about <i>Toxoplasmosis</i> ?	No	94	≤ 0.01
	Yes	6	
Can pigs contract toxoplasmosis?	No	100	≤ 0.01
	Yes	–	
Do cats run on the farm?	No	58	≥ 0.05
	Yes	42	
Can cat transmit toxoplasmosis to pigs or humans?	No	94	≤ 0.05
	Yes	5	
Rodent control at the farm	No	29	≤ 0.01
	Yes	71	

Table 2. Seropositivity of *Toxoplasma gondii* infection in pigs from south Benin

Variable	Number of samples	Number of positive samples	Prevalence
<i>Sex</i>			
Female	106	26	24.52
Male	78	16	20.51
<i>Age, years</i>			
< 1	33	7	21.22
> 2	151	35	23.17
<i>Breed</i>			
Local	50	11	22.00
Exotic	133	31	23.00
<i>Municipality</i>			
Ab-Calavi	96	25	26.00
Cotonou	88	17	19.31
Total	184	42	23.00

T. gondii infection among pigs

In the present study, antibodies against *T. gondii* were detected in 23% of pigs (42/186). This implies a continuous exposure of this animal species to the oocyst of *T. gondii* excreted in the environment by cats in study area where stray cat presence was reported. The highest seroprevalence was observed in sows: 25% (26/106) compared to boars (21%; 16/78). *T. gondii* infection was higher in Abomey-Calavi municipality (26%; 25/95) than in Cotonou municipality (19.31%; 18/88). In addition, higher levels of infection were recorded in animals older than 1 year (23.11%) compared to younger ones (21.22%). Moreover, there was no significant difference between the prevalence levels observed according to the different variables at the 5% level of statistical significance. This result shows that the spatial distribution of the infection may be related to the circulation of stray cats, which are abundant in southern Benin.

DISCUSSION

This is the first epidemiological report of *T. gondii* infection in any animal in Benin. The results show that this zoonotic parasite was present among pigs in Benin and can be transmitted to humans, which confirms the high seroprevalence of human toxoplasmosis recorded in south Benin, attributed to the fact that food animals can be reservoirs for *T. gondii* and act as one of the sources for parasite transmission to humans.

There are different methods to evaluate pig infection by *T. gondii*. Among these diagnostic methods, serological techniques are the most commonly used (Gamble *et al.*, 2005). For the present study, the ELISA test was used. This serologic technique is not specific to all animal species, but is used among many animal species including pigs for seroepidemiological studies because of the specificity and high sensitivity (Gamble *et al.*, 2005; Dubey, 2009). Villena *et al.* (2012) and Villard *et al.* (2016) showed that there was a high agreement between the ELISA

technique, the modified agglutination test and the reference test, e.g. the bioassay in mice in detection of *Toxoplasma gondii* infections in animals.

The results of this study revealed a relatively high level of pig infection by *T. gondii* in southern Benin. Indeed, about one-fifth of pigs were infected by *T. gondii*, which highlights the presence and circulation of this parasite in the farms investigated. This result confirms the frequency of reproduction failures recorded on farms in the areas. Toxoplasmosis is generally asymptomatic in farm animals. However, in pigs, the infection is responsible for reproductive disorders such as neonatal mortality, stillbirths and abortions (Dubey, 2009). Unfortunately, most breeders (96%) had no knowledge about this disease and its zoonotic potential, while animal husbandry practices carried out during the survey are likely to favour this infection. Many factors can affect pig infection by *T. gondii*. Among these factors, the practice of outdoor breeding favours animals contact with *T. gondii* oocysts contaminating the environment, as environmental conditions are favourable for the survival of *T. gondii* oocysts in the soil. The presence of cats and the lack of rodent control in pig farms promotes the spread of the parasite (Kijlstra *et al.*, 2008; Dubey, 2009). However, during the survey, most of these factors were recorded on pig farms. Benin has a humid subtropical climate with an average annual temperature of 27.5 °C and a high humidity from March to November (Aw climate type) according to the Köppen-Geiger classification (Peel *et al.*, 2007). These environmental conditions are favourable for the survival of *T. gondii* oocysts throughout the year (Dubey, 2016).

Other studies have been carried out on the seroprevalence of toxoplasmosis in

pigs from West African countries. Ayinmode & Olaosebikan (2013) have studied the prevalence of *T. gondii* infections in pigs from Ibadan, Nigeria. The results showed a level of infection (25% among 100 pigs) comparable to that obtained in Benin for the present study (23%, 43/184). This can be explained by the geographical position and the similar climatic conditions prevailing in these two countries. In fact, Benin and Nigeria are border countries and there is a humid subtropical climate in the southern region of the two countries where the studies were carried out, and pig management system is comparable in the different West African countries. In Bobo-Dioulasso (Burkina Faso) Bamba *et al.* (2016) obtained a prevalence of 29% among 300 pigs, higher than that recorded in the present study. This disparity could be related to different populations of pigs sampled. Indeed, the authors studied the seroprevalence of *T. gondii* infections only in wandering pigs more exposed to the risk of contamination by *T. gondii* oocysts than animals raised in confinement system. However, for the present study, exotic pigs reared in confinement generally were taken into account, as well as wandering pig raised in extensive system. In Ghana and Ethiopia, however, infection levels (39% among 641 pigs and 32% among 402 pigs respectively) were significantly higher compared to those obtained for the present study (Arko-Mensah *et al.*, 2000; Gebremedhin *et al.*, 2015). Moreover, Hove *et al.* (2005) reported very low seroprevalence (9% among 97 pigs) of *T. gondii* infections in the same species in Zimbabwe, while in Madagascar similar infection levels to those obtained in this study (23% among 250 pigs) were recorded by Rakotoharinome *et al.* (2012), which shows a high

variability in the seroprevalence of infections by country and region.

Although toxoplasmosis is a zoonosis, responsible for abortions in women and reproductive disorders in pig farming, it is still a little-known disease for most breeders in Benin. In this study, only 6% of pig breeders had heard of it but most of them were unaware that animals could contract the infection, resulting in reproductive disorders in infected sows. In addition, given the magnitude of the reproductive history observed in some farms and the level of *T. gondii* infection recorded in this study, it is likely that this history was due in part to this zoonosis. Finally, toxoplasmosis is not the only disease that can cause reproductive disorders in pigs, other additional studies are needed to confirm this hypothesis and show the link between this disease and reproductive problems observed.

Human toxoplasmosis has been described in newborn, pregnant and immunocompromised individuals in south Benin (Zannou *et al.*, 2004; Avode *et al.*, 2005). This situation can therefore be related to the presence and circulation of *T. gondii* in domestic animals including pig, that maintain the parasite's life cycle in the environment and thus promote the transmission of infection to humans (Tenter *et al.*, 2000; Dubey, 2016). Therefore, pork consumption can result in the acquisition of toxoplasmosis if the pork is raw or undercooked. This is the first time that *T. gondii* infection is described in animals from Benin. So, further work is required to assess infection level among other animal species and the risk factors for human and animal infections.

CONCLUSION

The parasite *T. gondii* is present in domestic animals from Benin, especially pigs. This is the first epidemiological report of *T. gondii* prevalence in any animals from Benin. It is shown that *T. gondii* is present in food animals in this country and that their consumption may be a potential source of infection for human.

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Tonouhewa, A., Y. Akpo, P. Sessou, C. Salanon, G. Aplogan, M. Assogba, I. Youssao & S. Farougou

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Correspondence:

Aretas B. N. Tonouhewa
03BP 2253 Cotonou/Jericho, Benin,
phone : 0022966823375,
e-mail: tonouhewaaretas@gmail.com