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Parasite Epidemiology and Control

# The first seroepidemiological study on *Toxoplasma gondii* in backyard pigs in Myanmar

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#### ABSTRACT

A cross-sectional study of *Toxoplasma gondii* infection in pigs was carried out in backyard farms in three townships, within Nay Pyi Taw area from June 2014 to August 2014. Blood samples were randomly collected from 256 pigs in 129 farms. Using commercial Latex Agglutination Test kits, specific antibodies to *T. gondii* were analyzed. Based on LAT results, among 256 serum samples examined, 47 samples (18.4%) were found positive to *T. gondii*. The numbers of samples showing specific antibody titres from 47 positive pig sera were 20 at 1:64, 2 samples at 1:128, 9 samples at 1:256, 3 samples at 1:512 and 13 samples at 1:1024. Among the hypothesized risk factors, roaming of cats around the farm was found associated to *T. gondii* seropositivity in pigs (OR = 3.13; 95% CI = 1.33–7.34). This study provides information on seroepidemiology study of *T. gondii* in backyard pigs for the first time in Myanmar. This information will be useful in developing strategies for the control of *T. gondii* infection in pigs.

# 1. Introduction

*Toxoplasma gondii* infection is caused by an intracellular protozoan parasite that can infect in humans and many animals. While it circulates in different intermediate hosts, the feline is a definitive host (Montoya and Liesenfeld, 2004). The infection is spread through the ingestion of sporulated oocysts from contaminated soil or water directly or through contaminated food, consumption of raw or undercooked meat from infected animals, and vertical transmission from mother to foetus ((Dubey, 2008)). This infection is a highly significant parasitic disease, and recognized as an important foodborne zoonosis. The infection was ranked as the fourth worldwide foodborne parasite infections by the WHO and the FAO (Torgerson et al., 2015).

Because of its omnivorous nature, pigs can acquire *T. gondii* by ingesting sporulated oocysts in food and water, by ingesting infected meat offal or infected rodent carcasses. Consumption of raw or undercooked pork from *T. gondii* infected pigs is a major source of human toxoplasmosis in some countries (Kijlstra et al., 2008; Dubey, 2008; Dubey et al., 2012).

In general, although raw pork consumption is not common in Myanmar, however, nowadays there is potential for unsafe processing and preparation for pork leading to undercooked pork consumption. Furthermore, due to its simple and low cost, the backyard farming

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system (intensive or semi-intensive) with poor biosecurity remains common among the farmers in most villages. Pigs are kept indoors in intensive farming system. Pigs in semi-intensive farming system have access to outdoor pens during the day and are only confined to pens at night. Due to poor biosecurity, it may lead to decrease food safety because zoonotic diseases including parasites could be reintroduced. Although seropositivity does not always accurately represent true prevalence, serological investigations are useful as epidemiological indicator for assessing the risk that results from eating pork (Gardner et al., 2010). The prevalence of *T. gondii* infection among pigs is unknown in Myanmar. The aims of the present study were therefore to conduct the serological survey of *T. gondii* infection in pigs in backyard farms and to identify its associated factors within Nay Pyi Taw area.

#### 2. Materials and methods

## 2.1. Study area

A cross-sectional study on serological survey of *T. gondii* infection in pigs was carried out in Lewe, Pyinmana and Tatkon Townships within Nay Pyi Taw area, located between latitude 19° 45′N and longitude 96° 06′E, which is situated in central area of Myanmar. This study was conducted between June and August of 2014.

#### 2.2. Sample size

According to the data from Livestock Breeding and Veterinary Department (LBVD), the total pig population in the three townships in 2013 was approximately 180,000. The sample size was calculated as described by Martin et al. (1987) using estimated prevalence (20%) based on the seropositive rate of *T. gondii* infection in goats reported in Myanmar (Bawm et al., 2016). The estimated sample sizes for each township were calculated as 122, 56 and 78 from Lewe, Pyinmana and Tatkon Townships, respectively.

#### 2.3. Sample collection

A total of 129 herds, including 94 from Lewe, 23 from Pyinmana and 12 from Tatkon Townships were randomly selected for blood sampling. Sample collection was focused mainly in suburban and rural areas within townships, where the large number of pig farms was located. In 125 herds, the average herd size was 3 to 7 pigs, in four herds, the average herd size was 8 to 15 pigs per herd. One or two serum samples and four serum samples were collected from the herd size of 3 to 7 pigs and 8 to 15 pigs, respectively. Totally, 256 serum samples were collected. While taking blood samples, pigs under the age of four months were not sampled because maternal antibody can be present till four months of age. From the jugular vein of each pig, 5 ml of blood samples were collected using disposable syringes and needles, then put in vacuum tube and kept in an ice box. Samples were then carried to the laboratory and centrifuged at 769g for 10 mins to obtain the sera. Serum samples were stored at -20 °C until examined by the Latex Agglutination test (LAT).

#### 2.4. Data collection

During sample collection in the farms, questionnaire interview to the farm owners was conducted by using the prepared questionnaire sheet, which included necessary information focusing on potential risk factors for *T. gondii* infection such as history and health status of pig, breeding and feeding management system including breed, sex, age, intensive or semi-intensive, veterinary care, type of feed, water source etc. and roaming of cats and rodents around the farm. In intensive farming system, pigs were kept mostly on concrete bedding. Pigs were fed concentrates and received veterinary care. In semi-intensive farming system, pigs were kept on straw bedding and fed kitchen wastes (meat offal, swill and kitchen leftovers etc.) mixed with concentrates. Before feeding, some farmers boiled kitchen wastes to sterilize it, but most of farmers in the study area did not. Furthermore, none of the farms in the study area were in good sanitary conditions (lack of cleanliness in the pens, as well as a lack of proper disposal of sewage and animal feed wastes).

#### 2.5. Serological test

In order to detect *T. gondii* antibodies, all the collected sera were tested using the commercially available modified Toxo-reagent Eiken latex agglutination test (LAT) kits (Eiken Chemical Company LTD., Taito, 110–8408, Tokyo, Japan). Serology test was performed as described in previous report (Bawm et al., 2016). The cut-off titre was 1:64 according to the manufacturer's instructions. According to limitation of *T. gondii*-antigen-coated latex beads of LAT, detection of *T. gondii* antibody titre was conducted till 1:1024 titre for the positive samples.

#### 2.6. Statistical analysis

Based on LAT results and information from questionnaire interview, these data were entered and validated in Microsoft Excel 2010 and imported into SPSS 20, then, the hypothesized risk factors were examined by Pearson Chi-square test. Among the collected data of questionnaire, the factors including age, sex, farming system, type of feed, and roaming of cat were used for statistical analysis. Other factors such as breed, water source, veterinary care, and presence of rodent, were found to be consistent across farms. Association between seropositivity of *T. gondii* infection and hypothesized risk factors was examined by comparing the difference in exposures to

risk factors in seropositive and seronegative groups, then by analyzing with Chi square tests. Variables for which the difference between seropositive and seronegative showed P value <0.05 were regarded as significantly associated with the status of disease.

## 3. Results

In this study, the overall prevalence was 18.4%, 47 of 256 samples were positive for *T. gondii* antibodies. The highest prevalence was noted in Tatkon and the lowest in Pyinmana (Table 1). Among 129 farms, 21 farms (16.2%) were found as positive. The number of positive samples of different antibody titres in each township is shown in Table 2.

Roaming of cats around the pig farm was evaluated as having significant association with *T. gondii* infection both in individual and farm levels (P < 0.05) (Tables 3 and 4) in this study, the only hypothesized factor. There were about 3 times more odds of being *T. gondii* seropositive in pigs having cat around the farm than pigs not having cat around the farm.

#### 4. Discussion

This study reports the first information on *T. gondii* infection in pigs in Myanmar. Since this survey was conducted in 2014, the situation in the country may have changed. Overall prevalence of *T. gondii* in pigs in Nay Pyi Taw area was 18.4% in this study. According to Fayer (1981), the worldwide seroprevalence of *T. gondii* in pigs was estimated to be 29.0%. The prevalence of *T. gondii* infection in pigs worldwide varies widely with as low as 2.7% in the USA (Hill et al., 2010) to as high as 65.0% in tropical climates of Oaxaca State, Mexico (Alvarado-Esquivel et al., 2012) tested by ELISA (enzyme-linked immunosorbent assay). Reports from some Asian countries showed as 5.2%–17.5% in Nepal (Devleesschauwer et al., 2013) and 13.8% in China (Zhang et al., 2020) using ELISA, and 27.2% in Vietnam (Huong and Dubey, 2007) tested by MAT (modified agglutination test). In addition, using LAT method, seroprevalence of *T. gondii* in pigs was higher in Myanmar than in other Asian countries, including Indonesia (2.3%) (Tuda et al., 2017), Japan (5.2%) (Matsuo et al., 2014), and the Philippines (13.4%) (Ybañez et al., 2019). However, it was lower than those in South Africa (33.9%) (Tagwireyi et al., 2019) and Nigeria (46.2%) (Ishaku et al., 2018) tested by LAT.

Within the study areas, Lewe, Pyinmana and Tatkon Townships, the seroprevalence were observed as 18.85%, 10.7% and 23.07%, respectively. Seroprevalence variations within the different areas observed in this study were likely due to different farm management systems and a varying number of infected cats in each township. Since it is one of the neglected diseases, *T. gondii* infection in cats was neither treated nor controlled in Myanmar. *T. gondii* infection in cats in Myanmar has been reported as 13% by microscopic examination in the Yezin, Nay Pyi Taw area (Htet, 2012), 41.3% by serological study and molecular detection of *T. gondii* from faecal oocyst within Yangon area was also reported (Bawm et al., 2020). Therefore, the number of infected cats might be variable in different townships and might influence the seroprevalence of *T. gondii*. In this study, roaming of cats around the farm was found to be significantly associated (P < 0.05) with seroprevalence in pigs.

Since ingesting oocysts from infected cat was one of the ways of acquiring *T. gondii* infection in pigs, roaming of cats on farms has been shown to be a major risk factor for seropositivity of *T. gondii* infection in sows (Lehmann et al., 2003). Similar results were also reported from Brazil (de Sousa et al., 2014) and Spain (García-Bocanegra et al., 2010). Additionally, the serological study in goats in Myanmar has also shown that similar results with this study (Bawm et al., 2016). In the study area, infected cats can spread oocysts into the environment, water, and food, posing a direct transmission risk to pigs or rodents. Infected rodents can be eaten by pigs as well as cats, and rodents in general can reinforce the presence of cats in farms. The questionnaire interview revealed that rodents were present in all pig farms, even though farmers used traps to control rodents, which may favour *T. gondii* positivity in pigs (Kijlstra et al., 2008). This could be the rationale why roaming of cats around the pig farm was found to be associated risk factor in the present study.

Although no significant association with farming system existed in this study, the seroprevalence of *T. gondii* in intensive farming was numerically higher than semi-intensive farming. All semi-intensive farms had ground floor and were with wood or bamboo sheet. Moreover, all examined pig farms were in poor sanitary condition with poor housing system. Therefore, cats and rodents can easily enter the farms and therefore all pigs would have same chance of contact with *T. gondii* oocysts shed by infected cats in all farms owing to the same poor sanitary condition, although farming system was different. Weigel et al. (1995) also noted that the seroprevalence of pigs kept in confined and non-confined facilities did not vary significantly.

In this study, no significant association with *T. gondii* infection was found for type of feed, age (3–9 months) and sex of pigs. All farmers used kitchen waste as basal diet to feed their pigs and usually do not boil the pig feed before feeding and all the study pig farms were not in good hygienic status. Therefore, it might be owing to have same chances for entering of cats and rodents to the farms and pigs' feed.

Controlling the infection of *T. gondii* in livestock was a major factor in reducing human infection reservoirs (Klun et al., 2006). Therefore, expanding public awareness of *T. gondii* infection as an important zoonotic disease is very important. In conclusion, the

Table 1	
No. of Toxoplasma gondii seropositive pigs in each township.	

Township	No. examined	No. seropositive (%)
Lewe	122	23(18.85)
Pyinmana	56	6 (10.7)
Tatkon	78	18(23.07)
Total	256	47(18.4)

#### Table 2

Distribution of	of specific	antibody t	itres of	positive	pig sera	in each	township.

Township	No. of positive sera at different titres						
	1:64	1:128	1:256	1:512	1:1024		
Lewe	9	1	2	2	9	23	
Pyinmana	2	-	2	1	1	6	
Tatkon	9	1	5	_	3	18	
Total	20	2	9	3	13	47	

#### Table 3

Seroprevalence and risk factors for T. gondii seropositivity in pigs in Nay Pyi Taw area, Myanmar.

No.	Factor	Total	Positive No. (%)	OR	$\chi^2$	P-value	95%CI
1.	Age						
	$\leq 6$ months	145	26 (17.90%)				
	>6 months	111	21 (18.90%)	1.10	0.04	0.84	0.42-2.82
2.	Sex						
	Male	162	32(19.75%)	1.29	0.57	0.45	0.66-2.54
	Female	94	15 (15.96%)				
3.	Farming system						
	Intensive	117	27 (23.08%)	1.78	3.19	0.074	0.94-3.38
	Semi-intensive	139	20 (14.39%)				
4.	Type of feed						
	Mixed with concentrate	69	14 (20.29%)	0.84	0.23	0.62	0.41-1.69
	Kitchen waste	187	33 (17.65%)				
5.	Roaming of cat						
	Yes	186	40 (21.51%)	3.13	7.46	0.006*	1.33-7.34
	No	70	7 (10.00%)				

CI, confidence interval; OR, odds ratio.

\* Significant statistical findings (P < 0.05).

# Table 4

Seroprevalence and risk factors for T. gondii seropositivity in p	pigs at farm J	level.
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No.	Factor	Total	Positive No. (%)	OR	$\chi^2$	P-value	95%CI
1	Farming system						
	Intensive	25	7 (28.0%)	2.5	3.12	0.07	0.88-7.06
	Semi-intensive	104	14 (13.4%)				
2.	Type of feed						
	Mixed with concentrate	115	19 (16.5%)	1.18	0.04	0.83	0.24-5.74
	Kitchen waste	14	2 (14.2%)				
3.	Roaming of cat						
	Yes	95	19 (19.8%)	4.00	3.66	0.05*	0.87-18.18
	No	34	2 (5.9%)				

CI, confidence interval; OR, odds ratio.

\* Significant statistical findings (P < 0.05).

findings of the study provide useful insights into current situation of *T. gondii* infection in pigs that could be used in Myanmar's pig farms for the prevention and control of infection with *T. gondii*. However, for consumer safety, further investigation into the prevalence of *T. gondii* infection in other intermediate hosts, in particular meat animals from other regions of Myanmar, is required.

# Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Written informed consent was taken from owners of the pig farms involved in this study prior to collection of samples. Collection of serum samples from pigs was approved by the University of Veterinary Science.

#### **Declaration of Competing Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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