



Serodiagnosis of Porcine Cysticercosis and Risk Assessments in Pigs Slaughtered in Ywar Thar Gyi Slaughter within Yangon Region, Myanmar

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ABSTRACT

Since 2015, porcine cysticercosis has been under-reported in Myanmar, even though the country was known to be endemic. This study aimed to figure out the seroprevalence of porcine cysticercosis and risk factors relating to pigs' infection by administering questionnaires to the farm owners. We bled a total of 330 pigs to collect the sera sample before slaughter at the Ywar Thar Gyi slaughter, the major abattoir in Yangon, Myanmar. The monoclonal antibody-based B158/B60 Ag-ELISA technique was used since it has shown high diagnostic values. The presence of *Taenia* spp. cyst was also assessed by meat inspection of six different muscles. We also investigated the distribution of cysts in slaughtered pigs. Of 330 blood samples, 14.24% of pigs were tested as seropositive. However, 12.12% of pigs were examined for the presence of cysticerci by the meat inspection method. The comparison between seroprevalence and prevalence by meat inspection was made using a 2x2 matrix table. Regarding cyst distribution, 95.0% (38/40) observed cysticerci on the ventral surface of the tongue muscle, and we found the remaining two positive samples inside the heart muscle. Results have shown that husbandry practice (OR=5.1; 95% CI=2.9-8.5) and deworming practice (OR=4.1; 95% CI=2.5-7.5) had a significant association ($P<0.05$) with the presence of infection in slaughter pigs. This study indicates the presence of cysticercosis in slaughtered pigs, and the results could assist the need for further studies for the intervention of effective control strategies.

Key words: Meat Inspection, Porcine Cysticercosis, Questionnaires, Seroprevalence, *Taenia* Spp.

INTRODUCTION

The pig tape worm *Taenia* spp., a zoonotic parasite, which resides in the tissues and the high prevalence has been reported especially in low-income countries (Trevisan et al. 2018). The larval stage of *Taenia* spp. can cause neurocysticercosis (NCC) which may lead to late onset acquired epilepsy, madness, blindness and death in areas where the disease is endemic (Brutto 2012). Humans serve as definitive hosts and swine are intermediate host which carries metacestode, a larval stage of *Taenia* spp. (Gomez-Puerta et al. 2019). The genus *Taenia* revealed great impact on public health significance since condemnation of infected meat would account great economic loss (Braae et al. 2019). By ingesting infected human feces containing *Taenia* spp.

eggs, pigs become infected, and the eggs have been developed inside the muscle and brain then subsequently lead to cysticerci (Jankharia et al. 2005). Recently 50 million people have been reported of *Taenia* spp. infection and recorded more than 50,000 of human cysticercosis annually (Hoberg 2006). Pigs are usually subclinically infected and could diagnose only at necropsy by meat inspection (Nunes et al. 2000) and condemnation of whole carcasses becomes great economic losses (Ito et al. 2004). Clinical symptoms involve myositis, myocardial failure, seizures and neurological disorders in infected pigs (Trevisan et al. 2016).

To diagnose this infection, meat inspection technique is the basic method even this method revealed low sensitivity percentage (Dorny et al. 2004), indicating current need to improve diagnosis techniques with high

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diagnostic values. Considering breaking the life cycle of *Taenia* spp., the meat inspection plays critical by condemnation of infected carcasses, which in turn reduce the risks (Phiri et al. 2003). Therefore, meat inspection has been integral in order to control transmission of *Taenia* spp. infection in many developed nations (Zammarchi et al. 2013). As described by Gauci et al. (2019), definitive diagnosis can be made by detection of cysticerci at the necropsy table. Nowadays, ELISA methods of using monoclonal antibodies have been used to detect circulating antigens that parasite elicits during metacystode stage (Sciutto et al. 1998). However, this sandwich ELISA technique might not be able to determine species level (Brandt et al. 1992). With regards to Ag-ELISA assays, the diagnostic efficacy of Ag-ELISA B158/B60 was determined to be 82.7 and 86.3% of sensitivity and specificity, respectively (Kabululu et al. 2020). Apart from that, molecular detection has been used since this method offer high sensitivity and specificity than conventional microscopic techniques (Ito et al. 2016).

Even though Myanmar has shown to be least developed country and endemic region of *Taenia* spp. infection within Southeast Asian countries (Aung and Spelman 2016), very limited information on human cysticercosis and neurocysticercosis have been available in Myanmar. Briefly, 23.2% seroprevalence of neurocysticercosis has been reported in refugees from Myanmar who live in the United States (O'Neal et al. 2012) and 5.5% in Thailand-Myanmar Border (McCleery et al. 2015). Apart from that, three cases of intraocular cysticercosis infection have been reported within year 2001 to 2010 in Yangon Eye Hospital (data not published). On regards of porcine cysticercosis, 15.9% seroprevalence of slaughtered pigs in Nay Pyi Taw area has been reported, firstly (Khaing et al. 2015). On that study, personal hygiene of labour, husbandry system and feeding habits of pigs have been analyzed as potential

risks for porcine cysticercosis. Secondly, overall prevalence of 27.81% in slaughtered pigs has been reported in Ywar Thar Gyi slaughter in which age and gender were shown to have significant association with the presence of cysticerci by meat inspection in 2017 (data not published).

In Myanmar, most of the pig husbandry practice were free ranging or semi-intensive without proper sanitation system. Since combined diagnostic tools might be helpful to interpret current status of porcine cysticercosis in definite region, the aims of this study were to investigate seroprevalence of porcine cysticercosis by Ag-ELISA, to determine the prevalence of porcine cysticercosis by meat inspection technique and to determine potential risks of getting infection in Ywar Thar Gyi slaughter pigs. By attempting this, the control strategies of porcine cysticercosis can be further pursued effectively.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Animal Care and Biosafety Committee (014/20140), Myanmar Veterinarians, Yangon, Myanmar.

Study Area

A cross-sectional study was conducted in Ywar Thar Gyi slaughter, the major abattoir in Myanmar, to determine the seroprevalence of *Taenia* spp. cysticercosis in slaughtered pigs within August to December' 2021. The Ywar Thar Gyi slaughter is located between latitude 16.84°N and longitude 96.17°E (Fig. 1). The slaughtered pigs were received from 6quarters of Yangon Region, 6quarters of Irrawaddy Region and 3quarters of Bago Region. Meat inspection was performed at the necropsy unit, Ywar Thar Gyi slaughter and serological examination was performed at Parasitology Laboratory, Insein, Yangon.

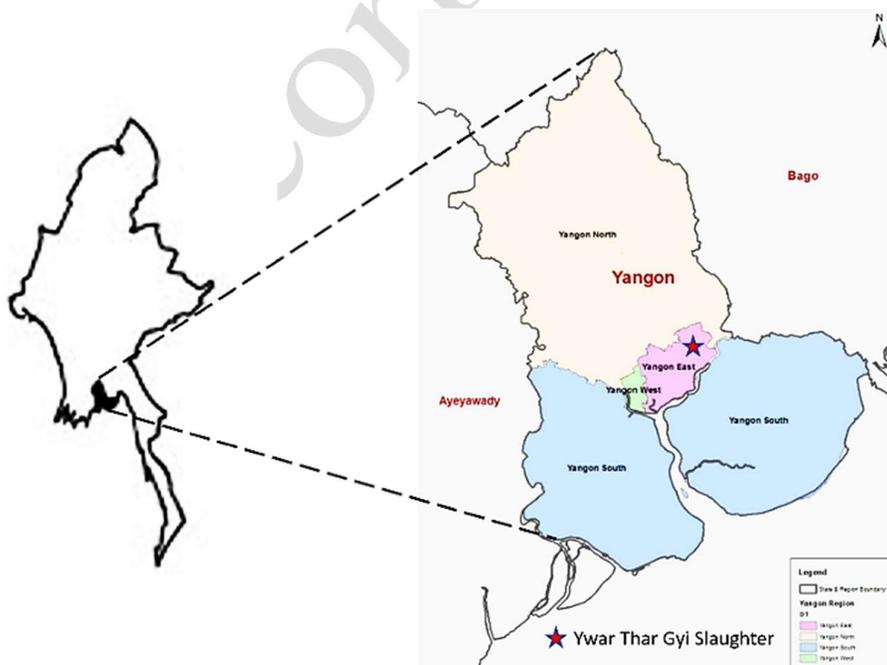


Fig. 1: Location map of Ywar Thar Gyi Slaughter, Yangon.

Questionnaires Survey

At the time of sample collection, the questionnaire was designed to get to know human characteristics and pig characteristics. With regards to the potentially relevant risks, two categories were implied for statistical analysis with the presence of infection.

Sample Size Determination

In Ywar Thar Gyi slaughter, the number of slaughtered pigs per month was approximately 25,000 pigs during study period. Sample size was calculated using the formula previously described by Thrusfield (1995). In the present study, a total of 330 pigs were inspected with an expected prevalence 30 at 95% confidence level.

Antigen-ELISA Assay

To determine seroprevalence, the sampled pigs were bled using sterile needle and syringe before slaughter. Briefly, at least 1mL of blood sample from each pig was obtained from anterior vena cava and transferred to 1.5mL Eppendorf. The Eppendorf containing blood sample was kept in 4°C cool box and transported to Parasitology Laboratory, Insein, Yangon, where the laboratory work was conducted. After that, the sera samples were separated by centrifugation at 3000xg at 4°C for 5min (Helgeland et al. 2000) and stored at -20°C until analysis. Thereafter, the circulating antigens of *Taenia* spp. cysticerci from sera sample were analyzed using antigen ELISA kit. In this study, B158/B60 monoclonal based sandwich enzyme-linked immunosorbent assay (Ag-ELISA) was performed as described previously by Dorny et al. (2004). Briefly, the plates were treated with 100mL of monoclonal antibody (MoAb) diluted to 10mg/mL in 0.1M Na Borate, 0.07M NaCl buffer, pH8.2, and then incubated overnight at 4°C. Thereafter, the plates were washed at least three times for 5min with 0.15M NaCl containing 0.05% Tween 20. Then, blocking was done by using PBS-T containing 1% bovine serum albumin and 0.05% Tween 20 and left at 25°C for 1hr before washing. Diluted serum samples 1:2v/v in horse serum were added and incubated for 30min at 37°C followed by washing with the homologous biotinylated MoAb HP10 diluted in PBS-T at optimum concentration for 30min at 37°C. After incubation and washing, biotinylated peroxidase streptavidin conjugate was added (Thermo Fisher Scientific Inc., Waltham, USA), diluted in PBS-T to optimum concentration and incubated for 30min at 37°C. In addition to this, the plates were washed again and 100mL of tetramethyl benzidine substrate (TMB) was added and incubated for 30min at 4°C. The color reaction was stopped using 100mL of 0.2M H₂SO₄. Lastly, the absorbance values were measured at 450nm in an ELISA reader (Human GmbH, Humareader, Model 2106). With regards to negative control sera, no cysticercus cellulosa cyst at the time of meat inspection and seronegative sample was used. After performing Ag-ELISA, the optical densities were compared with negative control sera.

Meat Inspection

Meat inspection was performed as described previously Boa et al. (2002). A total of 330 randomly selected pigs were involved. For purpose of meat

inspection, six different muscles (brain, heart, tongue, diaphragmatic, masseter and shoulder muscle) of each pig were inspected using direct knife-eye method. Therefore, total of 1980 muscle were dissected and inspected for the presence of *Taenia* spp. cyst. Briefly, a deep long incision was made parallel to masseter muscles and examined the presence of any cysts. When pericardium was opened, a deep incision was made to the heart muscle to expose any metacestodes. The remaining muscle were palpated and examined visually after deep incision with <0.5cm. The viable and non-viable cysticerci were classified as described by Kabululu et al. (2020). Briefly, the translucent fluid-filled vesicles were viable cysticerci, whereas caseous or calcified vesicles were non-viable. In the current study, the pig with one viable cysticerci was considered as positive. Thereafter, the positive samples were fixed in 70% alcohol and thorough examination was carried at Parasitology Laboratory, Insein, Yangon.

Statistical Analysis

The data were entered into Microsoft Excel Sheet and comparison between meat inspection and serological examination was done using 2x2 matrix table. Pearson Chi-square test was performed with 95% confidence interval using SPSS version 20 to understand the associated risks.

RESULTS

Questionnaires Survey

The descriptive data according to questionnaires survey was described in Table 1. Briefly, household data and pig data were collected before slaughter from the farm owners who interested to participate in current study.

Comparison of Seroprevalence and Meat Inspection

Out of 330 blood samples, 14.24% of pigs were tested as seropositive in the present study. However, 12.12% of pigs were examined for the presence of cysticerci by meat inspection method. The comparison between seroprevalence and prevalence by meat inspection was made by 2x2 table (Table 2). By means of meat inspection, 95.0% were observed cysticerci on the ventral surface of tongue muscle and the remaining two positive samples were found inside the heart muscle (Table 3). To extend our knowledge, the prevalence percentage of porcine cysticercosis within Southeast Asian Countries according to previous studies were collected and shown in Table 4.

Risk Factors Associated with Seroprevalence of Porcine Cysticercosis

Univariate analysis of hypothesized risk factors was performed accordingly. On behalf of human factors, personal hygiene (OR=2.9; 95% CI=1.7-5.1), deworming practice (OR=3.6; 95% CI=2.4-5.3), owner's knowledge about food-borne diseases (OR=4.5; 95% CI=2.4-6.8) were significantly associated with presence of infection (P<0.05) whereas eating habit of raw/undercooked meat had no association. In accordance with pigs, the factors of breed, age, gender and source of origin were not associated (P>0.05), however husbandry practice (OR=5.1; 95% CI=2.9-8.5) and deworming practice

Table 1: Descriptive data of 330 samples collected as per questionnaire

Categories	Samples falling in category	
	Number	%
Human characteristics		
Personal hygiene		
Good	103	31.21
Poor	227	68.79
Deworming practice		
Yes	241	73.04
No	89	26.96
Eating habit of raw/undercooked meat		
Yes	98	29.69
No	232	70.31
Knowledge about food-borne diseases		
Yes	56	24.34
No	174	75.66
Pig characteristics		
Breed		
Local	137	41.51
Crossbred	193	58.49
Gender		
Male	218	66.06
Female	112	33.94
Age (months)		
<6	113	34.25
≥6	217	65.75
Source of origin		
Yangon	127	38.48
Irrawady	94	28.48
Bago	109	33.04
Husbandry practice		
Near to human house	212	64.25
Separated	118	35.75
Deworming practice		
Yes	97	29.4
No	233	70.6

Table 2: Comparison of seroprevalence and prevalence from meat inspection (n=330)

Method	Positive/Negative	No.	%
Ag-ELISA	Positive	47	14.24
	Negative	283	85.76
Meat inspection	Positive	40	12.12
	Negative	290	87.88

Table 3: Distribution of cysts

Inspected muscle	Number of positive sample (%)
Brain	0
Tongue	38/40 (95.0)
Heart	2/40 (5.0)
Shoulder	0
Masseter	0
Diaphragmatic	0

(OR=4.1; 95% CI=2.5-7.5) were significantly associated. The distribution and odds ratio of analyzed risk factors relating to porcine cysticercosis are shown in Table 5.

DISCUSSION

Cysticercosis contributes a serious health problem in community where the pigs have been raised without sanitation practice in developing countries (Lekule and Kyvsgaard 2000) and eventually lead to great economic losses by condemnation of infected carcass (Dahourou et al. 2018). Within year 2014-2015, the seroprevalence of

porcine cysticercosis was studied in the central area of Myanmar using Ab-ELISA technique (Khaing et al. 2015). Currently, the diagnostic values of different assays were interpreted in different ways. Apart from that, the prevalence of porcine cysticercosis becomes critical to consider human cysticercosis in country level when effective control measures have been relied on the limited data availability. So, this study aimed to identify the current status of porcine cysticercosis at major abattoir in Myanmar after seven years. In the present study, a total of 330 pigs were involved. A serodiagnosis and meat inspection were performed in order to determine the presence of *Taenia* spp. cyst before slaughter. For this purpose, Ag-ELISA of B158/B60 was used since this test shown to have high diagnostic values and could be useful in surveillance studies with large sample sizes (Kabululu et al. 2020).

In the present study, the overall prevalence of porcine cysticercosis was 12.12% (40/330) by means of meat inspection. Out of 40 positive samples, 38 samples were observed cysticerci on the ventral surface of tongue muscle whereas the remaining two were found inside the heart muscle. As meat inspection of slaughter pigs largely depends on the parasite predilection sites, the parasite was especially found in diaphragm, heart and tongue (Devleesschauwer et al. 2013). In accordance with meat inspection, the tongue examination has been reported as critical to identify *Taenia* spp. infected pigs. However, the efficacy could differ from the degree of infection and location site of incisions (Boa et al. 2002). Apart from that, the sensitivity of meat inspection showed up to 70% in heavy infected pigs and the lower sensitivity has been reported in light infection cases (Pray et al. 2017). Therefore, tongue could be the most predilection site for *Taenia* spp. in which tongue was found as most condemned organ relating to porcine cysticercosis (Goussanou et al. 2013). In line with above mentioned studies, 95.0% (38/40) of positive sample was found from examination of tongue in current study. In contrast with this, the shoulder and masseter muscle have shown as the parasite predilection site (Gauci et al. 2019). Therefore, the standard meat inspection procedure should be guided for *Taenia* spp. endemic area.

Serodiagnosis with high diagnostic values plays crucial role to understand different characteristics within parasitic infections (Ahn et al. 2019). In current study, 14.24% (47/330) was found to be seropositive. As previously, the Ag-ELISA assay has been described as reliable test compared to Ab-ELISA assay in which low diagnostic values have been reported by post-mortem confirmation (Sciutto et al. 1998). In current study, the seroprevalence of porcine cysticercosis is similar to previous study in which 15.93% (58/364) has been reported in Nay Pyi Taw area, Myanmar (Khaing et al. 2015) which revealed urgent need of support from veterinary and medical health authorities. Since cross reaction with the presence of *T. hydatigena* has been reported (Bustos et al. 2019), further studies with another intermediate host should be performed. Apart from that, presence of *Taenia* spp. infected dogs within study area could favor to occur relatively high prevalence of cysticercosis (Jansen et al. 2021). In our study, Ag-ELISA serodiagnosis showed relatively more prevalence than

Table 4: Prevalence of porcine cysticercosis within Southeast Asian Countries

Countries	No. of samples tested	Prevalence		Diagnostic methods	References
		No.	%		
Thailand	188	37	19.7	Serodiagnosis	Chaisiri et al. (2019)
Vietnam	408	4	0.9	Serodiagnosis	Ng-Nguyen et al. (2018)
Myanmar	364	58	15.9	Serodiagnosis	Khaing et al. (2015)
Indonesia	35	27	77.1	Meat inspection	Assa et al. (2018)
Cambodia	242	27	11.2	Serodiagnosis	Söderberg et al. (2021)
Laos	590	330	55.9	Meat inspection	Conlan et al. (2012)
Myanmar	330	47	14.24	Serodiagnosis	Current study

Table 5: Distribution and odds ratio of analyzed risk factors relating to seroprevalence of porcine cysticercosis

Risk Factors	χ^2	Odds ratio (95%CI)	P-value
Human characteristics			
Personal hygiene			
Good			
Poor	16.065	2.925 (1.736-5.084)	0.002***
Deworming practice			
Yes			
No	5.462	3.643 (2.353-5.348)	0.001***
Knowledge about food-borne diseases			
Yes			
No	15.763	4.482 (2.385-6.832)	0.000***
Pig characteristics			
Husbandry practice			
Near to human house			
Separated	39.972	5.056 (2.987-8.563)	0.000***
Deworming practice			
Yes			
No	21.072	4.075 (2.458-7.513)	0.001***

meat inspection since meat inspection technique would favor missed cases such as immature cysticerci could escape during inspection (Kabululu et al. 2020), some false positive Ag-ELISA results should not be ruled out, vice versa.

In the study area, most of the pig farms were backyard and lack of proper sanitation practice, for example do not use of latrine for defecation since lack of basic knowledge for personal hygiene. Therefore, lack of personal hygiene in study area was associated with the presence of infection in pigs from which pigs could access infected human feces elsewhere (Komba et al. 2013). In present study, deworming practice was shown to have association with the presence of infection. By administering questionnaires to farm owners, most of them were not practiced for regular usage of anthelmintics. The open defecation in study area may also favor to get infection as described by Jorga et al. (2020). In Myanmar, the people live in rural area who mainly practiced small scale backyard farming with 10-30 pigs, were lacking knowledge about food-borne parasitic diseases. According to our study, only 56 out of 230 owners reported that they had known at least one food-borne parasite disease, even though none of them couldn't be able to name cysticercosis. The lack of health awareness has been shown major intrinsic factors in

cysticercosis (Khaing et al. 2015), the intensive husbandry practice and health educational program for farm owners should be developed in Myanmar.

With regards to potential risk factors for pigs with the presence of infection, the breed, age, gender and source of origin were the factors which are not associated with the presence of infection. These results are in accordance with previous studies of Gweba et al. (2010) in which age, gender and breed were not associated with porcine cysticercosis. However, factors of age, gender and breed have been mentioned as significant risk factors in previous studies (Pondja et al. 2010). In the present study, the sample pigs were come from the three regions, Irrawaddy, Yangon and Bago in which all of these were involved in delta regions and had similar husbandry practice, so there would be no association between source of origin and presence of infection.

In the study area, the pigs were raised nearby human house which could lead to favor free roaming. So, the husbandry practice of pigs without separating with the human house was more likely to get infection in pigs (Komba et al. 2013). In accordance with previous report by Khaing et al. (2015), anthelmintics usage was significantly associated with the presence of infection in the current study. Until now, none of the treatment has been reported for total removal of parasite, regular deworming practice could be efficiently effective in pig husbandry practice within Myanmar. Since human neurocysticercosis has been reported in Myanmar woman (O'Neal et al. 2012), public awareness should be taken with regards to relatively high prevalence in current study. As Myanmar is endemic area for this parasite, this information might help to provide important data for disease intervention and control measures for this zoonotic helminth.

Conclusion

All the obtained results provide the current status of porcine cysticercosis in Myanmar. From the present study, it was concluded that the seroprevalence and meat inspection prevalence of *Taenia* spp. cyst in slaughtered pigs were 14.24 and 12.12%, respectively. The tongue was shown as parasite predilection site. The potential risk factors were statistically analyzed. The further studies in different region should be performed to interpret the infection in country level as baseline data from which effective control strategies should be further pursued.

Declaration of Competing Interests

The authors declare that they have no competing interests.

Authors' Contribution

BKS was responsible for the study design and conceptualization. BKS, KMM, KMT and TWN were responsible for sample collection, sample identification and data analysis, while AN supervised the serological assay interpretation. BKS carried out drafting, reviewed and revised the manuscript. All authors approved the final version of manuscript.

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