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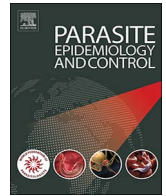
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## Seroprevalence and awareness of porcine cysticercosis across different pig production systems in south-central Cambodia

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### ABSTRACT

**Background:** Taeniasis/cysticercosis, caused by the pork tapeworm *Taenia solium*, represents an important public health and economic burden in endemic countries. However, there is a paucity of data on infection among pigs in many parts of Southeast Asia, particularly Cambodia. We aimed to estimate seroprevalence of porcine cysticercosis, and investigate husbandry practices and knowledge of the disease among livestock workers, across different pig sector units in south-central Cambodia.

**Methods:** A cross sectional survey was conducted among pig smallholders, commercial farms, slaughterhouses and traders/middlemen from south-central Cambodia, selected through multi-stage sampling in proportion to local pig populations sizes. Questionnaires were administered to 163 pig workers to obtain data pig production, trading and slaughtering practices. Sera from 620 pigs were tested for *Taenia* antigens using a commercial ELISA-based test. Associations between seroprevalence and pig husbandry practices were assessed using generalised linear mixed models, adjusting for random-effects at herd-level.

**Results:** Of 620 pigs sampled, 29 (4.7%) tested positive for *Taenia* antigens. Seropositivity was associated with type of pig sector unit ( $P = 0.008$ ), with the highest seroprevalence among pigs sampled from traders/middlemen (16.7%; 95% CI: 4.4%–37.8%), smallholders (7.6%; 95% CI: 3.8%–14.1%) and slaughterhouses (4.1%; 95% CI: 2.0%–7.5%), while none of the pigs sampled from small/medium or large commercial farms tested positive. Although the vast majority of pigs were penned, practices that might facilitate human-to-pig transmission, such as use of household waste and surface water sources to feed pigs, were prevalent among smallholders. However these were not found to be significantly associated with infection. Of 163 interviewed pig workers, 115 (70.5%) were aware of porcine cysticercosis, and 78 (47.8%) also knew it could affect humans. Twenty-six (16.0%) reported having noticed lesions typical of cysticercosis in their pigs.

**Conclusions:** Despite most pigs being kept confined in pens rather than raised in free-roaming systems, porcine cysticercosis appears to be endemic in south-central Cambodia and is associated

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with smallholder production. Further investigation is needed to identify which *Taenia* species are causing infections among pigs, and how seroprevalence and zoonotic risk may vary across the country, to understand the risks to public health and assess where interventions might be needed.

## 1. Background

Taeniasis/cysticercosis is a neglected parasitic disease caused by the adult or larval stage of cestodes in the genus *Taenia*. In Southeast Asia, *T. solium*, *T. saginata* and *T. asiatica* causes taeniasis (intestinal tapeworm infection) in humans while *T. solium*, *T. asiatica* and *T. hydatigena* causes cysticercosis in pigs (Conlan et al., 2011). Despite having been declared a potentially eradicable disease in 1992 (Schantz et al., 1993), it remains highly prevalent and a major public health and economic concern in many developing countries in Asia, Africa, and South America (Sarti et al., 1992). An important example of a One Health disease, taeniasis/cysticercosis transmission is dependent on human and animal hosts as well as environmental contamination and socioeconomic factors such as livestock husbandry practices, socioeconomic status, housing conditions, hygiene and eating habits.

Humans are the natural definitive host of *T. solium*, becoming infected through eating raw or undercooked pork contaminated with cysticerci (the larval stage), which then mature into adult tapeworms in the small intestine, causing the condition known as taeniasis. Pigs, the intermediate hosts, become infected through ingestion of the eggs or proglottids produced by adult worms and excreted in the faeces of infected humans. Within the pigs, these stages hatch and migrate to the muscle tissue forming cysticerci, causing porcine cysticercosis (García et al., 2002). Humans can also develop cysticercosis via fecal-oral transmission of *T. solium* eggs or proglottids, for example by ingesting contaminated food or water (Pal et al., 2000), and the cysts can develop in the brain or spinal cord causing neurocysticercosis (White, 2000). Pigs are also the intermediate hosts for two other *Taenia* species: *T. asiatica* (the Asian tapeworm) and *T. hydatigena* (the canine and feline tapeworm), which are also endemic in parts of Asia (Conlan et al., 2012). *T. asiatica* causes taeniasis in humans (the definitive host), but is not thought to cause human cysticercosis (Aung and Spelman, 2016). Meanwhile *T. hydatigena*, for which dogs and cats are the definitive hosts, is not thought to infect humans (Conlan et al., 2012).

Cysticercosis is an important disease in terms of both its public health and economic burden, with an estimated 2.5 million people infected with the *T. solium* tapeworm and 20 million with cysticerci (Bern et al., 1999), and causing an estimated 2.78 million DALYs (Disability Adjusted Life Years) worldwide (Pawlowski et al., 2005; Torgerson et al., 2015). Economic losses result not only from the costs of diagnosis, treatment, and loss of productivity related to human disease (Widdowson et al., 2000; Willingham et al., 2010; Carabin et al., 2005; Roberts et al., 1994), but also from the costs to livestock production. Porcine cysticercosis can decrease the value of pigs and pork meat and often results in total condemnation of carcasses upon meat inspection (Willingham et al., 2010; Ito et al., 2003). Cysticercosis does not usually produce clinical signs in live pigs, but cysts can sometimes be observed on the tongue or eyelids. Other manifestations in pigs include diarrhoea, myositis, emaciation, myocardial failure, abnormal skin sensitivity, seizures and neurological disorders (Urquhart, 1998; Trevisan et al., 2016).

Recommendations for *T. solium* control include improving the use and maintenance of latrines, improvement of pig management practices, and antiparasitic treatment of human taeniasis cases to reduce environmental contamination with, and human and pig exposure to, *T. solium* eggs in human waste. Interventions targeting pig-to-human transmission pathway include treatment of pigs, meat inspection, and thorough cooking of pork (Carabin and Traoré, 2014). A vaccine against porcine cysticercosis has demonstrated effectiveness in preventing the disease in pigs in field trials (Lightowers, 2013). Data on the seroprevalence of, and risk factors for, human and porcine cysticercosis are crucial to assess where such interventions are needed, and how they might be best targeted.

In Cambodia, as in most of the poorer countries in Southeast Asia, epidemiological data on cysticercosis is severely limited (Dorny et al., 2004). However, a seroprevalence of 10% has previously been estimated among humans (Singh et al., 2002), while an abattoir survey found that 10.9% of pig carcasses showed signs of cysticercosis (Sovyra, 2005), suggesting that the burden in this country could be substantial. Furthermore, the supply of pigs to urban areas in Cambodia still relies largely on rural smallholders (Hinrichs and Borin, 2012), where conditions can be conducive for transmission (Schantz, 2002; García-García et al., 1999; Sarti et al., 1997). Increased demand for pork consumption in recent decades in Cambodia, as in many other countries in the region, means that the pig production landscape is changing rapidly, although the implications for cysticercosis risk are unclear.

To help address the paucity of data on cysticercosis in the region, we conducted a cross-sectional survey in south-central Cambodia to estimate the seroprevalence of porcine cysticercosis across different pig production systems, slaughterhouses and traders/middlemen, identify potential pig- and herd-level risk factors associated with infection in pigs, and assess levels of awareness of the disease among pig sector workers.

## 2. Methods

### 2.1. Study area

The study was conducted in three provinces of south-central Cambodia, specifically: Phnom Penh (the capital), Kandal and Kampong Speu. The south-central region was chosen due to its varied pig production landscape, allowing for comparisons between

different types of pig sector units, and also based on logistical feasibility given proximity to the capital city. Across the three provinces, a total of six study districts (Dangkao district of Phnom Penh, Ponhea Leu, Ta Khmao and Khsach Kandal districts of Kandal province and Samraong Tong and Kong Pisei district of Kampong Speu province) were randomly selected with probability proportional to pig population sizes, based on local census data (Fig. 1).

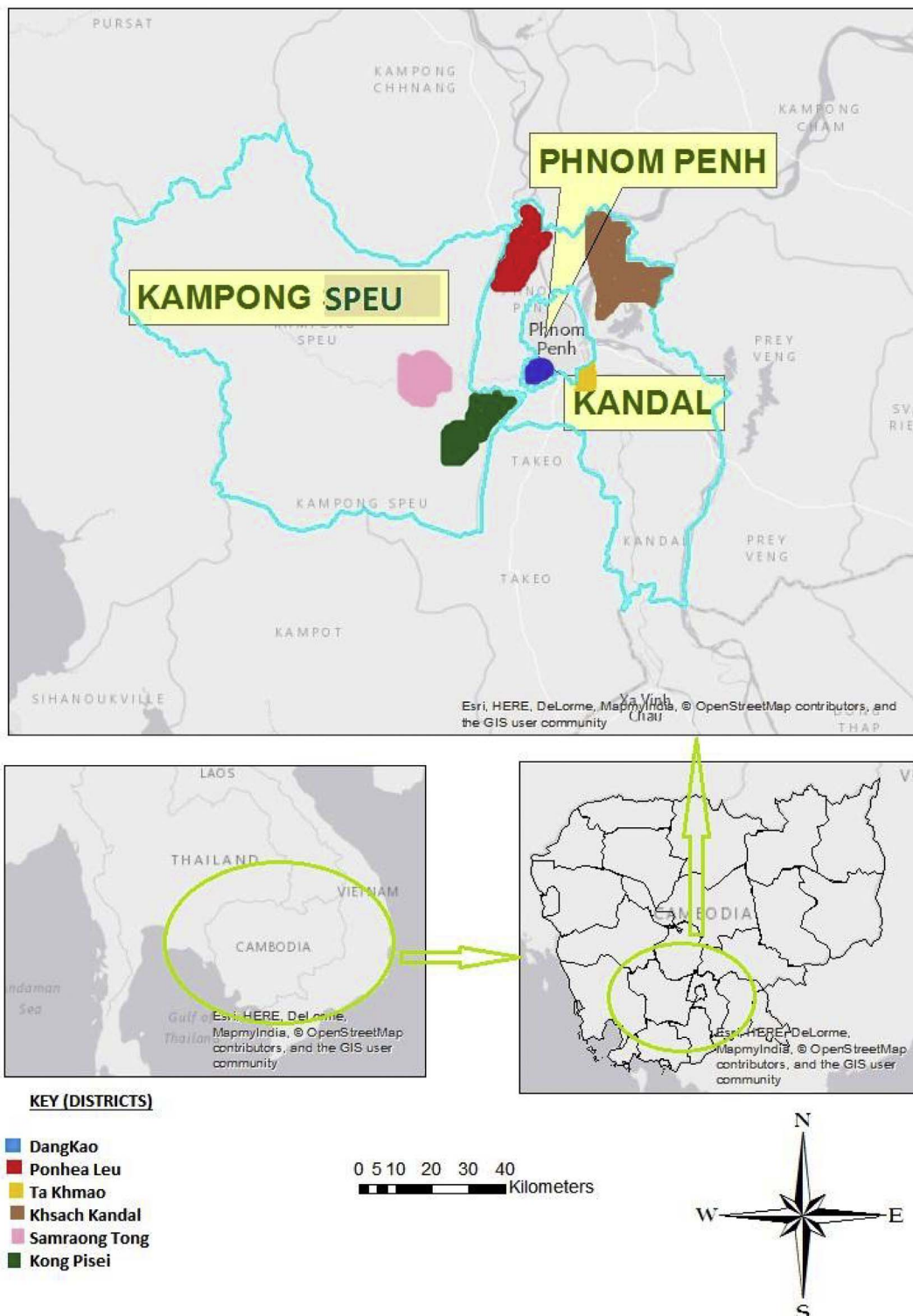


Fig. 1. Map of study area within Cambodia.

## 2.2. Study design

A cross sectional study was conducted to determine the seroprevalence of porcine cysticercosis, obtain data on pig- and herd-level risk factors for the disease, and assess the practices of pig producers, slaughterhouses and traders/middlemen. The study was integrated into a project which primarily aimed to investigate influenza virus epidemiology and diversity among pigs in Cambodia (the “PigFluCam” project); thus, the choice of study area and sampling frame were geared towards a swine influenza survey. Nevertheless, questions relating specifically to cysticercosis were also incorporated into the survey, in anticipation that pig serum samples would later be tested for this disease.

The five categories of pig sector units recruited into the PigFluCam project were classified based on a previous value chain analysis of Cambodia's pig sector ([Hinrichs and Borin, 2012](#)). These categories included three main types of producers (smallholders, small/medium farms, and large farms), along with slaughterhouses and traders/middlemen. The type of producer was determined based on herd size, with smallholders defined as households with 1–10 backyard pigs, small/medium farms with 11–200 pigs, and large farms with > 200 pigs. A total of 172 pig sector units were recruited, of which 163 (95%) and 97 (57%) participated in the interview survey and pig serosurvey, respectively (see [Table 1](#) for breakdown by category). Interviews could not be conducted in nine of the units where pigs were sampled due to human resource and time constraints in the field. Meanwhile, pigs could not be sampled at all interviewed study units for reasons including smallholders having sold or slaughtered their pigs, participants refusing to allow sampling of their pigs, and participants being absent at the point of follow-up.

Sample sizes were based on what was anticipated to be necessary in order to sample a sufficient number of pigs within each category (see below), while also considering the availability of units within the study area (e.g., large farms and slaughterhouses are much fewer in number compared to smallholders). Selection of study units was as follows. Two communes within each of the six study districts were selected by probability-proportional-to pig population sizes, based on local census data. Smallholders and small/medium farms were then selected by door-to-door convenience sampling upon visiting the selected communes until the target numbers of pigs in these categories had been sampled. Large commercial farms and slaughterhouses were selected based on those identified to be operating within the study districts, through consultation with local partners and livestock authorities. Due to their high mobility, traders/middlemen were selected through a pragmatic approach, by identifying any that were present at the farms and slaughterhouses when visiting these units.

## 2.3. Questionnaire administration

Structured questionnaires were developed to collect data on pig husbandry, trading and slaughtering practices, and awareness of cysticercosis. The questionnaires were translated into the local language (Khmer), and independently back-translated to English to ensure accuracy of translation. The purpose and procedure of the study was explained to participants and informed consent was obtained before the questionnaire was orally administered. Upon recruitment (between November 2014 and March 2015), the questionnaires were administered in Khmer to a single worker at each of the study units wherever possible. The interviewee at each study unit was either the owner/manager or someone else primarily responsible for pig husbandry/trading/slaughtering practices at the operation. Information collected included number and types of pigs kept, animal husbandry practices, feeding system adopted, source of drinking water for the pigs, availability and access of pigs to latrine facilities, trading practices, history of cysticercosis on the farm, awareness of the disease, and disease reporting and control practices.

## 2.4. Pig sampling

Approximately two months after recruitment of pig producers, slaughterhouse workers and traders/middlemen, the study participants were revisited to sample their pigs. We aimed to sample approximately 800 pigs in total, with 100–200 pigs from each of the five production/trading unit. The target pig sample sizes were guided by the PigFluCam project aims, based on what was deemed necessary to estimate swine influenza seroprevalence with sufficient precision. (Assuming a true influenza seroprevalence of ~15% in pigs ([Schantz et al., 1993](#)), and allowing for a moderate design effect of 1.5 due to clustering at herd (household/farm/

**Table 1**  
Numbers of pig sector units and pigs sampled in the interview survey and pig serosurvey.

Category of pig sector unit	Total no. of units	Interview survey	Pig survey		Total no. of pigs sampled in each category
		No. (%) of units interviewed	No. (%) of units where pigs were sampled	Median (range) of no. of pigs sampled per unit	
Smallholders	117	115 (98.3)	59 (50.4)	2 (1–6)	171
Small/medium commercial farms	26	23 (88.5)	20 (76.9)	7 (1–14)	132
Large commercial farms	6	5 (83.3)	4 (66.6)	29 (15–43)	58
Slaughterhouses	8	6 (75.0)	7 (87.5)	31 (14–40)	217
Traders/middlemen	15	14 (93.3)	7 (46.7)	5 (1–8)	42
Total	172	163 (94.8)	97 (56.4)	4 (1–43)	620

slaughterhouse)-level, we would be able to estimate seroprevalence within each category with precision of between  $\pm 6.1\%$  and  $\pm 8.6\%$  at the 95% confidence interval.)

Due to logistical challenges and drop-out of some participants between recruitment and pig sampling, the target pig sample sizes could not be reached for all categories, with a total of 620 pig sera eventually being tested for cysticercosis (see [Results](#)).

## 2.5. Sample collection and processing

Informed consent was obtained from the owners/managers of all participating units before pigs were sampled. Pigs were sampled from each herd at random; however, for safety reasons we did not sample young piglets (i.e., less than a month old) or pregnant/recently farrowed sows. The age, sex, breed (local, cross, or exotic) and type (piglet, weaning, fattening, or sow/boar for breeding) of pig were recorded before blood was collected. The blood samples were obtained from the anterior vena cava using sterile needle and syringe, then transferred into sterile EDTA vacutainer tubes and transported in a 4 °C cool box to the National Veterinary Research Institute (NaVRI) in Phnom Penh, where the laboratory work was conducted. The samples were spun down at 1500 rpm within 24 h of collection, and the plasma extracted by syringe and transferred to a new 1.5 ml tube and stored at  $-20\text{ }^{\circ}\text{C}$  until they could be processed.

A commercially available cysticercosis antigen enzyme linked immunosorbent assay (Ag-ELISA) was used for serological analysis (apDia, REF 650501, Belgium). The assay does not differentiate between infections of different *Taenia* species in pigs (*T. solium*, *T. hydatigena*, *T. asiatica*) and only indicates the presence of viable cysticerci ([Deckers and Dorny, 2010](#)). According to the manufacturers, the assay was shown to have an estimated 100% sensitivity (among 31 infected pig sera) and 99.6% specificity (among 300 negative pig sera), but the sensitivity of the assay decreases when the number of viable cysts is low (apDia, REF 650501, Belgium). Duplicates of the pre-treated controls and samples were processed following the manufacturer's protocol. The absorbance values/optic densities (OD) were determined at 450 nm within 15 min after stopping the reaction with 0.5 M  $\text{H}_2\text{SO}_4$ . Mean OD of the negative controls was used to calculate the cut-off by multiplying its value by 3.5, and the antigen index (Ag Index) of each sample was calculated by dividing the OD value of the sample (OD<sub>sample</sub>) by the cut-off value. An Ag Index  $\geq 1.3$  was considered a positive reaction, while an Ag Index  $\leq 0.8$  was considered negative. Samples with an Ag Index between 0.8 and 1.3 were considered indeterminate and were retested.

## 2.6. Data analysis

The data were entered and managed in a FileMaker Pro v14.0 relational database, and exported into R version 3.2.1 ([R Core Team, 2015](#)) for analyses. Descriptive analysis of variables relating to pig husbandry practices and cysticercosis knowledge and awareness of participants were conducted, and Fisher's exact test was used to test for associations with category of pig sector unit. Pigs with a positive Ag-ELISA test result were deemed as positive for porcine cysticercosis. Individual units (i.e., backyard farms, small/medium commercial farms, large commercial farms, tradesmen/middlemen, slaughterhouses), were deemed as positive if at least one of the pigs sampled was seropositive.

The pig survey data represent data from a complex survey design, with clustering of pigs by herd, where a herd is here defined as all pigs held by the same producer, slaughterhouse, or trader/middlemen at the time of sampling. To avoid underestimation of standard errors, analysis of the seroprevalence data accounted for this clustering wherever possible. For seroprevalence in categories where at least one pig was positive, 95% confidence intervals were calculated using Wald type-intervals adjusting for survey design, using the R package "survey" version 3.30–3 ([Lumley, 2004; Lumley, 2014](#)). Since this cannot be done for groups with an observed seroprevalence of zero, upper 95% confidence limits for zero numerators were calculated using Hanley's "rule of three" i.e.,  $3/n$  ([Hanley and Lippman-Hand, 1983](#)).

To test for associations between pig-level cysticercosis infection and herd- and pig-level covariates, generalised linear mixed-effect models (GLMMs) were used, adjusting for random effects at herd-level. However, the number of porcine cysticercosis infections in the dataset was quite low (see [Results](#)), which presents two challenges. First, maximum likelihood methods for parameter estimation are known to be biased for rare events in small datasets ([Firth, 1993](#)). Second, quasi-complete separation was an issue for many covariates, whereby no seropositive pigs were observed in at least one level of an independent categorical variable. Under such separation, parameter estimates for the covariate and its standard errors are infinite and maximum likelihood approaches will not converge ([Albert and Anderson, 1984; Abrahantes and Aerts, 2012](#)). To overcome these challenges in our clustered data, we applied a two-stage Bayesian approach for the GLMMs proposed by [Abrahantes and Aerts \(2012\)](#). In the first stage, Firth's penalized logistic regression was applied using the R package "logistf" ([Heinze et al., 2013](#)), without considering the clustered nature of the data, to inform the prior distributions for the second stage. In the second stage, a GLMM was fitted in a Bayesian framework using the R package "blme" ([Chung et al., 2013](#)), with herd as a random effect, and a t-distribution prior for the fixed effect covariate. The mean and scale for this prior distribution were based respectively on the parameter estimate and twice the variance (giving a weakly informative prior), from the Firth penalized logistic regression in the first stage.

The cut-off value for consideration of risk factors for inclusion in subsequent multivariable analysis was set at  $P < 0.1$ , although as only one variable met this criterion, multivariable analyses were not carried out.

### 3. Results

#### 3.1. Farm characteristics

Interviews among pig producers were conducted across 115 smallholders, 23 small/medium commercial farms, and five large commercial farms. The mean herd sizes were 4.3 pigs (range: 1 to 10 pigs), 27.7 (range: 11 to 131 pigs), and 587.0 (range: 493 to 800 pigs) for each of these three categories, respectively. A summary of the frequency of dichotomous variables relating to pig confinement, food and water sources, and latrine availability and access is given in Table 2. Almost all farms (139; 97.2%) reported keeping pigs in pens, either in individual (69; 48.3%) and/or shared (106; 74.1%) confinement. Ten farms (7.0%) kept pigs tethered, and only two (1.4%) reported keeping free range pigs. These parameters were not mutually exclusive, with a mixture of confinement types on 40 farms (24.5%).

Most (79.7%) of the farms reported having latrines on site, although only thirteen (9.1%) reported that their pigs could access these. Most farmers fed their pigs with rice grain (134; 93.7%), commercial pig feed (127; 88.8%), and/or household waste (101; 70.6%); with ten farms (7.0%) using homemade concentrates. Three (2.1%) reported their pigs grazed in confined areas while none reported that their pigs grazed openly. Water for the pigs was commonly sourced from wells (84; 58.7%) and surface water bodies such as ponds and streams (64; 44.8%). Dogs and cats (definitive hosts for *T. hydatigena*) were reported to be present on 113 (79.0%) and 53 (37.0%) of farms, respectively. There were statistically significant differences across the farm sizes in frequency of using household waste and rice grain to feed pigs (Fisher's exact test  $P < 0.001$ ), which tended to be more common in the smallholders and small/medium farms, and use of well water ( $P = 0.04$ ), which was more common on the large farms (Table 2).

#### 3.2. Traders/middlemen characteristics

The fourteen pig traders/middlemen that were interviewed reported trading between one to 35 pigs (mean: 5.7 pigs) daily. In the past month, most of these traders/middlemen had purchased pigs from smallholders (13; 92.8%) and/or from small/medium farms (9; 69.2%), with some also having sourced pigs from large farms (4; 30.8%) or other traders/middlemen (3; 21.4%). All except one trader/middleman reported slaughtering the pigs themselves, either in their own backyard or at a slaughterhouse, while two traders/middlemen reported selling pigs on to slaughterhouses.

#### 3.3. Slaughterhouse characteristics

The six interviewed slaughterhouses varied considerably in size, with two small “informal” sites where 1–3 pigs, sourced mainly from smallholders, were slaughtered per day, and three medium sized operations which slaughtered pigs originating from different farm sizes at a rate of ~15–20 heads per day. The largest abattoir slaughtered exotic breeds that were imported from large farms in

**Table 2**  
Frequency of dichotomous variables across the interviewed farms.

	No. (%) of farms				P-value (Fisher's exact test)
	All farms	Smallholders	Small/medium farms	Large commercial farms	
<b>N</b>	143	115	23	5	
<b>Types of pig confinement on farm</b>					
Individual	69 (48.3)	52 (45.2)	15 (65.2)	2 (40.0)	0.192
Shared	102 (71.3)	76 (66.1)	22 (95.7)	4 (80.0)	0.007
Tethered	10 (7.0)	7 (6.1)	3 (13.0)	0 (0.0)	0.568
Free range	2 (1.4)	1 (0.9)	1 (4.3)	0 (0.0)	0.359
<b>Types of pig feed</b>					
Commercial feed	127 (88.8)	99 (86.1)	23 (100.0)	5 (100.0)	0.16
Scavenge/graze in confined area	3 (2.1)	2 (1.7)	1 (4.3)	0 (0.0)	0.47
Household waste	101 (70.6)	89 (77.4)	12 (52.2)	0 (0.0)	< 0.001
Homemade concentrate	10 (7.0)	7 (6.1)	3 (13.0)	0 (0.0)	0.45
Rice grain	134 (93.7)	111 (96.5)	22 (95.7)	1 (20)	< 0.001
<b>Water sources</b>					
Surface water	64 (44.8)	55 (47.8)	9 (39.1)	0 (0.0)	0.09
Piped water	3 (2.1)	3 (2.6)	0 (0.0)	0 (0.0)	1.0
Well water	84 (58.7)	62 (53.9)	17 (73.9)	5 (100.0)	0.04
<b>Latrines</b>					
Latrine indoors	46 (32.2)	33 (28.7)	12 (52.2)	1 (20.0)	0.09
Latrine outdoors	70 (49.0)	60 (52.2)	7 (30.4)	3 (60.0)	0.14
No latrine	29 (20.3)	22 (19.1)	5 (21.7)	2 (40.0)	0.41
Pigs can access latrine	13 (9.1)	10 (8.7)	3 (13.0)	0 (0.0)	0.66
Dogs on farm	113 (79.0)	87 (75.7)	22 (95.7)	4 (80.0)	0.07
Cats on farm	53 (37.0)	39 (33.9)	12 (52.2)	2 (40.0)	0.27

neighbouring Thailand and Vietnam, at a rate of ~400 heads per day. All sites disposed of excreta and unwanted offal into nearby surface water bodies.

### 3.4. Knowledge of taeniasis/cysticercosis across pig sector workers

Participants were shown photos of signs of porcine cysticercosis (small nodules on the tongue, and eyelids, or in slaughtered carcass), and asked if they could name the disease. Of 163 participants, 30 (18.4%) responded correctly with the Khmer term for cysticercosis, “*chang angkor*” (which literally translates as “broken rice”, due to the appearance of the cysts). Ability to name the disease varied significantly across types of pig sector unit, tending to be highest among traders/middlemen (57.1%) and lowest among smallholders (12.2%) (Fisher's exact test,  $P < 0.001$ ). A further 85 participants (52.1%) said they were aware of the disease “*chang angkor*”, when told the name, while 78 (47.9%) thought it could affect humans, with no significant differences in these variables across types of pig sector unit (Table 3).

Participants were also asked if they have previously noticed signs of cysticercosis in their pigs. Fourteen (8.6%) had observed nodules on the eyelid, nine (5.5%) had noticed nodules on the tongue, and nine (5.5%) noticed cysts in pig carcasses. Twenty-six (16.0%) reported having observed at least one of these three signs. When asked to whom did they report these symptoms, five (15.4%) of the 26 that had observed the cysts reported the findings to Village Animal Health Workers and one (3.8%) to their veterinarian. Twenty-two (84.6%) reported that these symptoms affected the value of the pig, and eighteen (69.2%) reported to have treated or received treatment for pigs that showed signs of cysticercosis.

### 3.5. Pig survey

Blood samples were collected from a total of 620 pigs across 97 (56.4%) of the 172 study units (Table 1). Of the 620 samples, 171 (27.5%) were from 59 smallholder farms, 132 (21.3%) from 20 small/medium commercial farms, 58 (9.4%) from four large commercial farms, 217 (35.0%) from seven slaughterhouses, and 42 (6.8%) from seven traders/middlemen.

The mean age of pigs sampled was 4.1 months (range: 2 to 17 months), with almost equal numbers of male (308; 49.7%) and female (312; 50.3%) pigs. Most pigs (485; 78.2%) were crossbred, 91 (14.7%) were exotic breeds, while 44 (7.1%) were local breeds. The majority (542; 87.4%) were finishers or kept for fattening, 61 (9.8%) were weaners, twelve (1.9%) were sows kept for breeding, five (0.8%) were piglets.

Out of 620 pigs, 29 (4.7%) tested positive for antigens to *Taenia* spp. (*T. solium*, *T. asiatica* or *T. hydatigena*). The highest seroprevalence was observed in pigs sampled from traders/middlemen (16.7%; 95% CI [adjusting for survey design]: 4.4%–37.8%), followed by those from smallholders (7.6%; 95% CI: 3.4%–14.1%) and slaughterhouses (4.1%; 95% CI: 2.0%–7.4%) (Table 4). None of the pigs sampled from small/medium or large commercial farms tested positive for cysticercosis. Seropositive pigs were found in 9 (15.2%), 5 (71.4%) and 3 (42.9%) of the sampled smallholders, slaughterhouses, and traders/middlemen, respectively. Within these seropositive units, a median of 1 (range 1–3) seropositive pigs were detected. The intraclass correlation coefficients (ICC) within each category suggested a low but significant degree of clustering of seropositive pigs within smallholder units (ICC: 0.20; 95%CI: 0.03–0.34). ICC estimates for slaughterhouses and traders/middlemen were low with 95% CIs not significantly above 0 (Table 4). The design effect of clustering of seropositive pigs within units was calculated as 1.5, 0.5 and 1.2 for smallholders, slaughterhouses, and traders/middlemen, respectively.

In bivariate analysis, using a 2-step Bayesian GLMM approach allowing for herd-level random effects (see Methods), category of pig sector unit was a significant predictor of infection ( $P = 0.008$ ). Specifically, odds ratios (OR) estimated from the fixed-effect coefficients from this model showed that pigs from small/medium farms (OR = 0.0; 95%CI = 0.0–0.3) and large-commercial farms

**Table 3**  
Knowledge of porcine cysticercosis across pig producers, traders/middlemen, and slaughterhouses.

	Number (%) of participants						P-value (Fisher's Exact test)
	Total	Smallholders	Small/medium commercial farms	Large farms	Traders/middlemen	Slaughterhouses	
<b>N</b>	163	115	23	5	14	6	
<b>Disease awareness</b>							
Can name the disease	30 (18.4)	14 (12.2)	4 (17.4)	1 (20.0)	8 (57.1)	3 (50.0)	<b>&lt; 0.001</b>
Aware of the disease	115 (70.6)	76 (66.1)	20 (87.0)	2 (40.0)	12 (85.7)	5 (83.3)	0.07
Aware it can affect humans	78 (47.9)	53 (46.1)	14 (60.9)	0 (0.0)	7 (50.0)	4 (66.7)	0.13
<b>Previously observed symptoms in pigs</b>							
Eyelid nodules	14 (8.6)	8 (7.0)	1 (4.3)	1 (20.0)	3 (21.4)	1 (16.7)	0.75
Tongue nodules	9 (5.5)	8 (7.0)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0.92
Cysts in carcass	9 (5.5)	9 (7.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.68
Any of above symptoms	26 (16.0)	19 (16.5)	2 (8.7)	1 (20.0)	3 (21.4)	1 (16.7)	0.75

Bold shows statistical significance at  $P < 0.05$ .



**Table 4**  
Seroprevalence and clustering of porcine cysticercosis among pigs across different categories of pig sector unit in south-central Cambodia.

Category of pig sector unit	No. of units (clusters)	No. of pigs sampled	No. of pigs seropositive	Seroprevalence, % (95%CI) <sup>a</sup>	ICC <sup>b</sup> (95% CI)	Design effect
Smallholders	59	171	13	7.6 (3.4,14.1)	0.20 (0.03,0.34)	1.5
Small/medium commercial farms	20	132	0	0.0 (0.0,2.2)	–	–
Large commercial farms	4	58	0	0.0 (0.0,5.0)	–	–
Slaughterhouses	7	217	9	4.1 (2.0,7.5)	– 0.01 (– 0.02,0.07)	0.5
Traders/middlemen	7	42	7	16.7 (4.4,37.8)	0.06 (– 0.13,0.47)	1.2

<sup>a</sup> For non-zero numerators, 95% CIs for seroprevalence are adjusted for clustered survey design. For zero numerators (i.e. small/medium farms and large farms), upper 95% CIs are estimated using Hanley's rule of three.

<sup>b</sup> ICC, intraclass correlation coefficient.

(OR = 0.1; 95%CI = 0.0–0.9) were significantly less likely to be seropositive than pigs from smallholders. None of the other variables measured at pig-level or herd-level were significantly associated with infection in pigs (Table 5), thus multivariable analysis was not carried out.

#### 4. Discussion

There is paucity of information on cysticercosis in Southeast Asia, particularly regarding how infection among pigs and awareness of the disease among pig workers varies across the diverse pig production systems in this region. In this study, 4.7% (95% CI: 2.9%–7.4%) of pigs tested positive for cysticercus infection in south-central Cambodia. This is lower than seroprevalence estimates

**Table 5**  
Bivariate analysis of pig-level and herd-level factors with cysticercosis seropositivity in pigs. Results were generated from 2-stage Bayesian generalised linear mixed models adjusting for random effects at herd-level.

		$\beta$ coefficient	Std. error	Odds ratio	95% CI	P
Age	≤ 4 months	Reference				
	> 4 months	0.25	0.77	1.3	(0.3,5.8)	0.75
Sex	Female	Reference				
	Male	0.56	0.45	1.7	(0.7,4.2)	0.22
Type	Fattening/finishing	Reference				0.62
	Piglet	– 0.15	1.88	0.9	(0.0,34.1)	
	Sow	– 1.17	1.67	0.3	(0.0,8.2)	
	Weaning	– 1.35	1.12	0.3	(0.0,2.3)	
Breed	Cross-breed	Reference				0.63
	Exotic	– 0.73	1.31	0.5	(0.0,6.3)	
	Local	0.48	0.94	1.6	(0.3,10.3)	
Category of pig sector unit	Smallholder	Reference				<b>0.008</b>
	Small/medium farm	– 3.82	1.37	0.0	(0.0,0.3)	
	Large farm	– 2.92	1.44	0.1	(0.0,0.9)	
	Trader/middleman	0.84	0.70	2.3	(0.6,9.2)	
	Slaughterhouse	– 0.46	0.69	0.6	(0.2,2.5)	
Main confinement type <sup>a,b</sup>	Shared	Reference				0.93
	Individual	– 0.41	1.37	0.7	(0.0,9.8)	
	Tethered/free range	– 1.33	2.26	0.3	(0.0,22.2)	
Food sources <sup>a</sup>	Household waste	– 0.08	1.77	0.9	(0.0,29.4)	0.96
	Commercial feed	– 2.00	2.29	0.1	(0.0,12.1)	0.38
	Rice grain	– 0.77	2.76	0.5	(0.0,103.6)	0.78
	Homemade concentrate	– 1.05	3.80	0.4	(0.0,601.3)	0.78
Water sources <sup>a</sup>	Surface water	0.73	1.68	2.1	(0.1,56.2)	0.66
	Well water	– 1.69	1.64	0.2	(0.0,4.6)	0.31
Latrines <sup>a</sup>	Indoor latrine	– 0.25	1.84	0.8	(0.0,28.9)	0.89
	Outdoor latrine	– 0.89	1.74	0.4	(0.0,12.4)	0.61
	No latrine	0.05	2.00	1.1	(0.0,52.5)	0.98
	Pigs can access latrine	– 1.94	3.74	0.1	(0.0,217.8)	0.60
	Other animals on farm					
Province	Dogs	– 1.95	1.68	0.1	(0.0,3.8)	0.25
	Cats	– 1.02	1.82	0.4	(0.0,12.8)	0.58
	Phnom Penh	Reference				0.18
	Kandal	– 1.80	0.77	0.2	(0.0,0.7)	
	Kampong Speu	– 1.21	0.72	0.3	(0.1,1.2)	

Bold shows statistical significance at  $P < 0.05$ .

<sup>a</sup> Data from producers only (i.e. smallholders, small/medium and larger commercial farms).

<sup>b</sup> Main confinement type was determined based on the type of confinement in which the majority of pigs in the unit were kept.

from nearby countries of Laos (68.5%) (Conlan et al., 2012), and Myanmar (15.9%) (Khaing et al., 2015), as well as estimates from countries in sub-Saharan Africa (Komba et al., 2013; Pondja et al., 2010; Eshitera et al., 2012) and Latin America (Jayashi et al., 2012). This might be because, in our study area, the vast majority of pigs are fully confined in pens, even among smallholders, compared to more extensive (i.e., free roaming and scavenging) pig husbandry practices which may predominate in the other study settings. Indeed, it is important to emphasise that our results are unlikely to be representative of Cambodia as a whole; one would expect porcine cysticercosis seroprevalence to be considerably higher in the more remote northeastern provinces, for example, where pigs are often kept tethered or free-roaming in scavenging-based systems (Hinrichs and Borin, 2012). Another explanation for the lower seroprevalence observed in our study compared to some other settings may be due to differences in diagnostic methods, since some of the aforementioned studies (Khaing et al., 2015; Jayashi et al., 2012) tested pig sera for circulating IgG antibodies, rather than antigens.

Nevertheless, the results from our study still indicate endemicity of porcine cysticercosis which may pose a public health risk from consumption of pork produced in south central Cambodia, particularly from pigs raised by smallholders, which still account for the majority of pork production in the country. Seroprevalence of porcine cysticercosis among smallholders in our study was estimated at 7.6% (95% CI: 3.8%–14.6%). Furthermore, overall knowledge and awareness of the disease was generally lowest among smallholders compared to other pig sector workers, suggesting that any interventions targeting smallholders would also need to involve health education activities. Indeed, across all pig sector workers interviewed, less than half recognised porcine cysticercosis as a disease which can affect humans; thus knowledge and awareness of the disease may need to be increased across the pig sector.

Our results suggest significantly lower seroprevalence in small/medium and large commercial farms, where no evidence of porcine cysticercosis was found. This likely reflects improved biosecurity practices at commercial farms (Hinrichs and Borin, 2012), although some risk in the latter cannot be ruled out given the limited numbers of pigs and farms that could be sampled in these categories. Our seroprevalence estimates in pigs of traders/middlemen were lacking precision due to low sample size in this category, although the relatively high seroprevalence among those that were sampled (16.7%; 95% CI: 7.1%–34.5%) is consistent with the fact that the traders/middlemen typically source their pigs from smallholders. Furthermore, the majority of tradesmen/middlemen in this study reported slaughtering pigs in their backyards. This has important implications for zoonotic risk, since such informal slaughtering practices lack any official meat inspection procedures and therefore increase the chance of contaminated pork entering the food chain. Even at official slaughterhouses in Cambodia, the frequency and effectiveness with which meat inspection regulations are enforced is questionable (Hinrichs and Borin, 2012).

The seroprevalence we observed in slaughterhouses (4.1%; 95% CI: 2.0–7.5%) is lower than that estimated by Sovyra (2005) in a previous survey in Cambodia, in which 10.9% (95% CI: 8.1–17.2%) of pig carcasses showed signs of cysticercosis by meat inspection. Since the latter method has lower sensitivity (estimated at ~40%) (Dorny et al., 2004) than the serological assay we used, the study by Sovyra (2005) is, if anything, likely to have underestimated the true seroprevalence at that time. Thus, the differences between our estimates may reflect a real decrease in seroprevalence of porcine cysticercosis in Cambodia over the past decade. However, the variation in study areas, as well as diagnostic techniques, between the two studies mean that our results are not entirely comparable. Interestingly, one of the larger abattoirs sampled in our study, where seropositive pigs were also identified, is known to mostly slaughter pigs that have been raised and imported from large scale producers in neighbouring Thailand and Vietnam, suggesting that imported pigs could also present a risk for *Taenia* spp. transmission in Cambodia.

In terms of clustering, the results suggest low but significant clustering of seropositive pigs within smallholder units (ICC: 0.20; 95%CI: 0.03–0.34), while no significant clustering was detected within slaughterhouses or traders/middlemen. This likely reflects how pigs within the same smallholder will be exposed to the same (or similar) conditions and herd-level risk factors for infection, while pigs at slaughterhouses are sourced from a number of different herds/production units. The estimated ICCs and design effects (Table 4) will be useful for the design of future studies, and suggest that our assumption of a design effect of 1.5 for sample size calculations was reasonable.

Across our study smallholders and farms, several herd-level factors were prevalent which could facilitate human-to-pig transmission of cysticercosis, such as use of household waste as pig feed, lack of latrines, and use of surface water bodies as a water source. However, we did not find any significant associations between specific pig husbandry practices and porcine cysticercosis infection across the farms. This in contrast to studies in other countries where, for instance, lack of latrine facilities have been identified a significant risk factor (Rodriguez-Canul et al., 1999; Carrique-Mas et al., 2001; Kagira et al., 2010; Krecek et al., 2012). This might again relate to the confinement of pigs in our study area compared to free-range system in some other settings. High mobility of pigs due to frequent trading between production units in south-central Cambodia also mean that the observed porcine infections could have been acquired at another site, and thus would not be associated with the conditions or practices at the site where they were sampled. It is also important to acknowledge that, due to limited numbers of pigs that could be sampled in some categories, our study may simply have lacked sufficient power to detect such associations, as evident from the large standard errors for several of our fixed-effect coefficients in our GLMM analysis.

Indeed, the complex and clustered design of animal sampling is not always accounted for in other porcine cysticercosis surveys, which can result in underestimation of standard errors and bias in tests for associations, for example due to random effects at herd level or other sampling levels. Here, we used multi-level models to adjust for herd-level random effects in our analysis. However, as noted in the methods, a challenge we encountered in doing this was quasi-complete separation of the binary outcome for many covariates, due to relatively low seroprevalence of infection. To overcome this, we applied a two-stage Bayesian GLMM approach proposed by Abrahantes and Aerts (2012), although further methodological research is needed to determine the best approaches for analysing clustered, rare event data to reduce bias and deal with data separation issues.

Another important limitation of our study is that the Ag-ELISA test cannot differentiate between infection by *T. solium*, *T. asiatica*,

and *T. hydatigena*, all of which are found in pigs in South-East Asia (Dorny et al., 2004); thus the species that infect the positive pigs in this study could not be determined. This limits the interpretation of the results in terms of zoonotic risk, given that *T. hydatigena* is not known to infect humans. It also highlights the need for validated diagnostic tests which can differentiate between *Taenia* spp. infection in live pigs. Also, the assay may not have detected circulating antigen in pigs with low viable cysticerci and/or pigs who had been previously treated before blood was collected for this study.

In conclusion, our study suggests that porcine cysticercosis is endemic among pigs in south-central Cambodia, with infection associated with smallholder production, even though the majority of pigs in the region are raised in confined rather than free-roaming systems. Further investigation is warranted to identify the relative contribution of different *Taenia* species to the observed infection rates among pigs, along with parasitological surveys among humans, to elucidate the public health risks associated with porcine cysticercosis in Cambodia, and assess if, and where, control interventions are most needed.

### List of abbreviations

ELISA	enzyme-linked immunosorbent assay
GLMM	Generalised linear mixed model
ICC	intraclass correlation coefficient

### Ethics approval and consent to participate

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research. Ethics approvals were also obtained from the Institutional Review Board and the Animal Welfare and Ethics Review Board of the London School of Hygiene and Tropical Medicine (refs: 8302 and 2014/8N), the National Ethics Committee for Health Research, Ministry of Health, Cambodia (ref: 0274 NECHR), and the Royal Veterinary College's Ethics and Welfare Committee (ref: M2014 0029). Informed consent was obtained for conducting interviews and sampling of pigs from all participants in this study.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

Conceived and designed the study: JWR and AM, with additional contributions to study design, protocols and/or questionnaires from AA, KB, CT, SS, DH, VD, MC, GJS, RC, AV. Coordination and implementation of field surveys and specimen collection: CT, KB, VD, DH, SS, JWR. Specimen testing: AA. Data management: AA, VD, JWR. Data analysis: AA, JWR. Wrote the paper: JWR, AA, AM. All authors read and approved the final version of the manuscript.

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The opinions and assertions contained herein are those of the author(s) and are not to be construed as official or reflecting the views of the Department of the Navy, Department of Defense, or the U.S. Government. Co-author Andrew Vaughn CAPT, MC, USN is a military service member (or employee of the U.S. Government). This work was prepared as part of his official duties. Title 17 U.S.C. §105 provides that ‘Copyright protection under this title is not available for any work of the United States Government.’ Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

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