Manuscript Details

Manuscript number	PAREPI_2017_26_R1
Title	Seroprevalence and awareness of porcine cysticercosis across different pig production systems in south-central Cambodia
Article type	Full length article

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 husbandary 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 multistage 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 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.

Keywords	porcine cysticercosis; Taenia; livestock production; zoonosis; pigs; Cambodia

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Submission Files Included in this PDF

File Name [File Type]

R1_Cover letter and responses.doc [Response to Reviewers]

Porcine cysticercosis in Cambodia_R1 clean.docx [Manuscript File]

Figure1.tif [Figure]

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31st October 2017

Dear Editors,

Please find enclosed the revised version of our manuscript entitled “Seroprevalence and awareness of porcine cysticercosis across different pig production systems in south-central Cambodia”.

We are very grateful to the two reviewers for their useful comments; we have endeavored to address all of these the in the revised version. In particular, we have clarified sample sizes for the different surveys, incorporated further details and discussion on clustering of seropositive pigs, and made several other changes as detailed in out point-by-point response below.

We believe these revisions have strengthened the clarity, detail and reproducibility of the study without substantially affecting the main findings and conclusions.

Thank you again for your consideration and we look forward to hearing from you.

Yours faithfully,

James W Rudge *PhD*, *MSc*

(Corresponding author)

Comments from the editors and reviewers (author responses in blue font).

Reviewer 1

Major comments

The antigen-ELISA from apDia has been used for detection of circulating antigen in serum. Although authors mention that the test was performed according to kit manual, a brief description of the conducted method would be favored. Cutoff values (Ag-Index) should be mentioned. The authors might wish to mention that the sensitivity of the AG-ELISA strongly depends on the number of viable cysts present and might not detect low level infections.

Response: Agreed and amended – a brief description of the ELISA method is now included.

The numbers of recruited pig production/trading units is not conclusive. Table 1 shows 172 units in total, 117 smallholders, 26 small/medium commercial farms, 6 large farms. In the results section (Line 219), it is mentioned that 115 smallholders, 23 small/medium and 5 large commercial farms were included. The same applies for the traders (15 in Table 1 versus 14 in results section) and slaughterhouse (8 in Table 1 versus 7 in results sect.).

Response: We apologise if this was unclear. The confusion lies in the fact that different numbers of units were included in the interview and pig surveys. While this was mentioned in the text, we have clarified in the Methods and also revised Table 1 to clearly show the number of units per category in the interview and pig surveys.

The manuscript is not showing how many pigs were sampled per production unit and type of unit, it only shows the total number of pigs sampled and the number of units per category included in the study. It would be important to know the sample per unit or at least per category (smallholders, small/medium, etc…) if association of infection is calculated to keeping methods or other parameters.

Response: Agreed and amended. While the pig sample sizes by pig sector category were originally given in the text of the results section, they are now also shown in revised Table 1.

The authors mention that some of interviewed pig workers reported treatment of pigs after notice of signs of cysticercosis (Line 273). Treatment can be insufficient but can have considerable impact on the detection of circulation antigen in serum. Treated pigs can still harbor few viable cysts but circulating antigen will be below detection level. It would be important to mention this in the discussion section. Were the pig workers asked if sampled pigs were treated before sampling? Is there any prophylactic anthelminthic treatment in large commercial pig farms?

Response: Unfortunately the pig workers were not asked about prophylactic use of anthelminthics, but we have now acknowledged as a limitation in the discussion how this could impact on detection of circulating antigens.

A limitation of the study is detection of circulating antigen compared to antibody detection, the latter being more sensitive. The advantage of the AG-ELISA clearly is the detection of only viable

cysts, but sensitivity strongly depends on the number of these cysts. The method might miss a high percentage of low level infections, which could be detected by an EITB.

Response: Agreed – this limitation is now acknowledged in the discussion.

Line 307-309 in the discussion section: The authors observe a much lower seroprevalence in the present study than estimates form nearby countries, Africa and Latin America and conclude that this might be due to animal husbandry conditions. It should be mentioned that in most of the other surveys IgG was detected by ELISA or EITB, and not circulating antigen. Therefore the seroprevalence is expected to be much higher in the other countries applying another diagnostic method, compared to the present study!

Response: Amended. While most of the studies we reference here did in fact report using a similar Ag-ELISA approach to ours, and therefore are more comparable, two of the studies used antibody (Ig) detection, so we have now also acknowledged this as a potential explanation for the higher seroprevalence observed in some other settings.

MINOR

In the methods section the data analysis section is disproportionately high.

Response: We have tried to make this section more concise. Overall, however, we feel that a reasonable amount of detail is needed here, in order to clearly explain how we addressed some important aspects of the statistical analysis (such as clustering of the data and quasi-complete separation of the response variable in our generalised linear mixed models).

Reviewer 2

This is a well analysed and written manuscript on a study on cysticercosis nested within a swine flu study. It is good that the authors have considered a design effect for sample calculation and clustering (random effect) for analyses.

Response: Thank you.

A general comment: The authors should harmonize (choose) and use as consistently as possibly a same term throughout the manuscript

- seroprevalence (and not prevalence)

- seropositivity/seropositive (and not infected or diseased)

- pig vs. swine;

- production category vs. category vs. unit vs. system vs. mode vs. stage vs. type vs. value change vs. farm sites… Traders/middlemen vs. traders… Essentially, there were units in 3 different production systems, traders and slaughterhouses. “Production category” is strange when also used for traders and slaughterhouses.

Response: Agreed and amended. We now consistently use the terms “pig” for pig or swine; “seroprevalence/seropositive” to indicate test results at population/individual level; “pig sector unit” as a general term encompassing any study farm, slaughterhouse, or trader/middleman; and “category of pig sector unit” in reference to which of these categories describes the pig sector unit. “Production system” is now only used when referring to farms (smallholders, small/medium farms and large commercial farms) but not traders or slaughterhouses.

Abstract

Please mention the multi-stage sampling proportional to of pig populations and ELISA in methods.

Response: agreed and amended.

Were the 29 seropositive pigs homogeneously distributed in sampled units? (see below)

Response: In the interests of keeping the abstract concise, we focus on presenting seroprevalence by category of unit as per the main study objectives. Furthermore, the confidence intervals for these seroprevalence estimates adjust for clustering by unit. However, we have now included further details on clustering by unit in the main results section of the manuscript, and in new Table 4 (see below).

Conclusions: possible endemicity is not further mentioned or discussed in the manuscript.

Response: This is now explicitly mentioned in the Discussion of the manuscript.

Background

L. 54 socio-economic factors are a bit obscure without adding ‘poor’

Response: We how now added “socio-economic status” as one of the example factors in this sentence.

L. 66 please rephrase for better clarity

Response: Agreed and amended.

LL 70/71 please add ‘worldwide’ and please provide the original source of the figures on the estimated cases (as stated in the Pawlowski et al. publication): Bern, C., Garcia, H.H., Evans, C., Gonzalez, A.E., Verastegui, M., Tsang, V.C., Gilman, R.H., 1999. Magnitude of the disease burden from neurocysticercosis in a developing country. Clin. Infect. Dis. 29, 1203–1209.

Response: Agreed and amended.

I was astonished to not read anything about human neurocysticercosis in the presentation of human clinical signs and DALYs.

Response: Neurocysticercosis as a form of the human disease is now mentioned at the end of page 4.

L. 83 please state that the vaccine is for pigs.

Response: This is now specified more clearly.

Methods

Ll 114 please drop 'knowledge, attitudes and practices'. This was not a KAP study and should not suggest so! Attitudes were not assessed and knowledge was limited to knowing or having heard of the disease.

Response: Agreed and amended. We now only refer to assessing pig sector practices and awareness of the disease.

L 126 Table 1 with the 172 pig production/traders recruited. The multi-stage sampling is described, but here I would like to know the approximate proportion of eligible units in the selected communes that were registered (note that I would replace 'recruited' with 'registered' since not all units were enrolled in the study). Were all eligible units registered (and asked for informed consent?)?. If only a proportion (say 60%) were registered, how were those enrolled selected? With this additional information, Table 1 could be updated (and presented in results) with the actual units interviewed (and also showing the sampled units) per region.

Response: There were no official registries of all pig sector units operating within the study communes and districts, so the exact numbers of eligible units are unknown. For smallholders and small/medium farms, these were therefore selected based on door to door convenience sampling until target numbers of pigs in these categories had been sampled. Large commercial farms and slaughterhouses were selected based on all of those identified to be operating within the selected study districts through consultation with local partners and livestock authorities. This is now clarified in the manuscript. Table 1 is now also updated to specify numbers of units included in the interview surveys and pig surveys.

L 141 I believe the questionnaire was translated to Khmer - ? And, if yes, was a back-translation done? If the questionnaires were in Khmer, the interviews have also been done in Khmer and this should be stated.

Response: This is all correct, and is now clarified in the manuscript.

L 142 How was the interview partner in a unit selected? Were there any exclusion criteria (e.g. somebody who is not old enough or is not sufficiently knowledgeable about practices used in the unit?).

Response: 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. Thus, all interviewees should have been sufficiently knowledgeable about practices in the unit. This is now stated in the manuscript.

L 165 It is not stated how many (or the proportion of) pigs per unit were sampled. Probably this can be left out here, but in the results I've expected to see median numbers of pigs sampled in units per category.

Response: Median (and range) of number of pigs sampled per unit in each category are now given in Table 1.

L 179 Reference needed

Response: This information was from the ELISA test manufacturer's protocols, now cited.

L 192 please rephrase 'data should account for' to 'data accounted for' (because I believe this is what the authors did – and, if not, no need to mention in methods).

Response: Agreed and amended.

LL 214-216 Please rephrase something like: No multivariable analysis was done because only one variable ….

Response: Agreed and amended.

Results

As stated above, I would replace Table 1 with the actual interviews and sampling per region. I suppose that no unit without interview was sampled - ? (however, I may be wrong because in L 276 we read that 97 out of 172 were sampled. Should the latter be replaced with 163?).

Response: We have now revised Table 1 to clearly show total numbers of study units how many of these were included in the interview survey and pig serosurvey. Interviews were not conducted at a small number of units (9) where pigs were sampled due to human resource and time constraints in the field surveys. This is also clarified in the Methods section.

L 227 please give the proportion of herds with more than one confinement type

Response: The revised manuscript now states that 40 farms had more than one confinement type.

L 229 Table 2 revealed (for me) also the interesting fact that two commercial units did not have latrines…

Response: We suspect this is because they had some other form of sanitation infrastructure for human waste. In light of this, along with the small number of large commercial farms, we have decided not to highlight this finding in narrative. Of greater relevance to the study is that pigs on the large farms were unable to access latrines, unlike on some of the smallholders and smaller farms.

L 243 'or' should be replaced with 'and/or'

Response: Agreed and amended.

L 251 please rephrase 'from a range…'

Response: Agreed and amended.

Paragraph on slaughterhouses: I would have wanted to read here something on meat inspection – and likely also if there was any information from the slaughterhouses regarding past frequencies of condemned carcasses due to cysticercosis.

Response: The surveys did not specifically ask about meat inspection, so this is not mentioned in our results section. However, in the discussion we do mention the following: *"…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 [24]"*. This is based on a previous study of pig value chains in Cambodia by one of our co-authors, and indeed is why we did not ask slaughterhouses about meat inspection in our study, as the responses will be of questionable reliability.

I miss in the results of the sero-survey how the 29 seropositive pigs clustered within the 97 sampled units. Was there little or high between unit variance?

Response: Thank you for this suggestion, we agree this is worthy of further attention in the results. We have replaced Figure 2 with a new Table 4, which shows not only the seroprevalence, but also the degree of clustering (intraclass correlation coefficient, ICC) and design effect within each category. We have described these results with the following additional text in the results section: *"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 estimated 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."*

Why was region not considered as an explanatory variable in the analysis?

Response: Province is now considered as an explanatory variable in Table 5. It was not significantly associated with seroprevalence.

Discussion

L 307 please drop ‘somewhat’

Response: agreed and amended.

L 313 to BE representative

Response: agreed and amended.

L 319 please add that the 7.8% refers to smallholder farms

Response: agreed and amended.

L 325 ‘small, medium’ should be written as ‘small/medium’ to avoid confusion with smallholders.

Response: agreed and amended.

L 379 I do not find it useful to highlight the overall seropositivity in the conclusions. The fact that seroprevalences highly differed between sampling categories seems of greater interest.

Response. Agreed and amended. The conclusion now focuses on how the results indicate that porcine cystsicosis is endemic in this region, with infection associated with smallholder production.

The authors elaborate on the fact that traders informally slaughter pigs. The fact that seropositivity was highest amongst traders (and lower among smallholders and much lower in slaughterhouses with meat inspections) implies that traders may specifically target (cheaper?) infected pigs – knowing that they will not be controlled. And farmers may sell ‘good pigs’ direct to slaughterhouses and those with signs of cysticercosis to traders? Do slaughterhouses purchase pigs directly from smallholders?

Response: Thank you for these thoughts. Typically in this region, pigs from smallholders are sold to slaughterhouses via traders/middlemen, as noted in the Discussion. It is very possible that traders/middlemen tend to slaughter pigs with any signs of infection, rather than sell these on to slaughterhouses. However, we also emphasise that relatively few pigs were sampled from traders/middlemen; as such, the confidence intervals for seroprevalence for this category are quite wide, and overlap with the CIs for the seroprevalence estimates for smallholders and slaughterhouses. We apologise that this wasn’t clear for in the original manuscript, as we have noticed there was an error in the reporting of 95% CIs for these categories, which has now been corrected (slaughterhouses: 2.0%-7.5%; traders/middlemen: 4.4%-37.8%). Thus, we cannot conclude with any certainty that seroprevalence is indeed higher in pigs of traders/milddlemen than in pigs at slaughterhouses, and so have decided not to speculate on this in the Discussion. However, this is a potential hypothesis of interest for future work.

Was the chosen design effect of 1.5 reasonable for this study? Could the authors calculate a rho/ICC? If yes, this would be of interest for future studies.

Response: This is a very good point. As noted above, we now present the ICCs and design effects in the results section (new table 4). The actual design effect was in fact 1.5 for smallholders, and even lower for other categories, suggesting this was a reasonable assumption. The Discussion now includes the following addition: *"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."*

Tables and Figures

Table 4 I would drop coefficient and standard error. Confinement is here 'main confinement' thus with one category per unit (unlike Table 2 that presented multiple options for one unit). How was this new variable categorised?

Response: Following the addition of a new Table 4, this comment now refers to Table 5 in the revised manuscript. We have kept the coefficient and standard error for completeness, and because we refer to the large standard errors in the Discussion. However, we would also be ok with removing these from the table depending on the preference of the editors. "Main confinement type" was determined based on the type of confinement in which the majority of pigs at the unit were kept – this is now explained in a table footnote.

Figure 1 in its present design will most likely need to be printed in colour for differentiation of the elements

Response: We would be happy with colour printing of the figure, and also believe this should be fine as we expect a large majority of readers will access the article online.

Figure 2 is not referenced in the text. Seroprevalence in place of Prevalence.

Response: Agreed and amended. Figure 2 has been replaced with a table (new Table 4), in order to also indicate clustering (ICC and design effect) by category, to address the reviewer's other comments. The table is now cross-referenced in the results pig survey section, and prevalence has been changed to seroprevalence.

Supplementary information

Four authors have seemingly 'only' contributed to 'study design and/or questionnaire', resulting in a long co-authors list that is rather exceptional for such a study.

Response: We appreciate that the author list is rather long. This is a result of the cross-sectoral nature of the study, involving investigators/experts in the human health and livestock sectors, and also the fact that the cysticercosis analysis "piggy–backed" (if you'll excuse the pun!) on a swine

influenza project, thus bringing in additional investigators. All co-authors made significant contributions to the study and we therefore hope that this author list can be agreed as satisfactory by the editors.

Title: 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 husbandary 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 multistage 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 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.

Keywords: porcine cysticercosis; *Taenia*; livestock production; zoonosis; pigs; Cambodia.

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. asciatica* and *T. hydatigena* causes cysticercosis in pigs [1]. Despite having been declared a potentially eradicable disease in 1992 [2], it remains highly prevalent and a major public health and economic concern in many developing countries in Asia, Africa, and South America [3]. 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 [4]. Humans can also develop cysticercosis via fecal-oral transmission of *T. solium* eggs or proglottids, for example by ingesting contaminated food or water [5], and the cysts can develop in the brain or spinal cord causing neurocysticercosis [6]. 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 [7]. *T. asiatica* causes taeniasis in humans (the definitive host), but is not thought to cause human cysticercosis [8]. Meanwhile *T. hydatigena*, for which dogs and cats are the definitive hosts, is not thought to infect humans [7].

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 [9], and causing an estimated 2.78 million DALYs (Disability Adjusted Life Years) worldwide [11]. Economic losses result not only from the costs of diagnosis, treatment, and loss of productivity related to human disease [12–15], 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 [13, 16]. 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 [17, 18].

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 [19]. A vaccine against porcine cysticercosis has demonstrated effectiveness in preventing the disease in pigs in field trials [20]. 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 [21]. However, a seroprevalence of 10% has previously been estimated among humans [22], while an abattoir survey found that 10.9% of pig carcasses showed signs of cysticercosis [23], 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 [24], where conditions can be conducive for transmission [25–27]. 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.

METHODS

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 (Figure 1).

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 [24]. 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), whilst 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.

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.

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 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 [2], and allowing for a moderate design effect of 1.5 due to clustering at herd (household/farm/slaughterhouse)-level, we would be able to estimate seroprevalence within each category with precision of between +/-6.1% and +/-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).

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 hours of collection, and the plasma extracted by syringe and transferred to a new 1.5 ml tube and stored at -20°C until they could be processed.

A commercially available cysticercosis antigen enzyme linked immunosorbent assay (Ag-ELISA) was used for serological anlaysis (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 [28]. 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 minutes after stopping the reaction with 0.5M H_2SO_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 (ODsample) by the cut-

196 off value. An Ag Index ≥1.3 was considered a positive reaction, while an Ag Index ≤0.8 was considered
197 negative. Samples with an Ag Index between 0.8 and 1.3 were considered indeterminate and were
198 retested.

199

200 **Data analysis**

201 The data were entered and managed in a FileMaker Pro v14.0 relational database, and exported into
202 R version 3.2.1 [29] for analyses. Descriptive analysis of variables relating to pig husbandry practices
203 and cysticercosis knowledge and awareness of participants were conducted, and Fisher's exact test
204 was used to test for associations with category of pig sector unit. Pigs with a positive Ag-ELISA test
205 result were deemed as positive for porcine cysticercosis. Individual units (i.e., backyard farms,
206 small/medium commercial farms, large commercial farms, tradesmen/middlemen, slaughterhouses),
207 were deemed as positive if at least one of the pigs sampled was seropositive.

208 The pig survey data represent data from a complex survey design, with clustering of pigs by
209 herd, where a herd is here defined as all pigs held by the same producer, slaughterhouse, or
210 trader/middlemen at the time of sampling. To avoid underestimation of standard errors, analysis of
211 the seroprevalance data accounted for this clustering wherever possible. For seroprevalence in
212 categories where at least one pig was positive, 95% confidence intervals were calculated using Wald
213 type-intervals adjusting for survey design, using the R package "survey" version 3.30-3 [30, 31]. Since
214 this cannot be done for groups with an observed seroprevalence of zero, upper 95% confidence limits
215 for zero numerators were calculated using Hanley's "rule of three" i.e., 3/*n* [32].

216 To test for associations between pig-level cysticercosis infection and herd- and pig-level
217 covariates, generalised linear mixed-effect models (GLMMs) were used, adjusting for random effects
218 at herd-level. However, the number of porcine cysticercosis infections in the dataset was quite low
219 (see Results), which presents two challenges. First, maximum likelihood methods for parameter
220 estimation are known to be biased for rare events in small datasets [33]. 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 [34, 35]. To overcome these challenges in our clustered data, we applied a two-stage Bayesian approach for the GLMMs proposed by Abrahantes & Aerts [35]. In the first stage, Firth's penalized logistic regression was applied using the R package "logistf" [36], 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" [37], 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 penalised 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.

RESULTS

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).

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.

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 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.

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 carcases. 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,

291 and eighteen (69.2%) reported to have treated or received treatment for pigs that showed signs of
292 cysticercosis .

293

294 **Pig Survey**

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

299 The mean age of pigs sampled was 4.1 months (range: 2 to 17 months), with almost equal
300 numbers of male (308; 49.7%) and female (312; 50.3%) pigs. Most pigs (485; 78.2%) were crossbreed,
301 91 (14.7%) were exotic breeds, while 44 (7.1%) were local breeds. The majority (542; 87.4%) were
302 finishers or kept for fattening, 61 (9.8%) were weaners, twelve (1.9%) were sows kept for breeding,
303 five (0.8%) were piglets.

304 Out of 620 pigs, 29 (4.7%) tested positive for antigens to *Taenia spp*. (*T. solium*, *T. asiatica* or
305 *T. hydatigena*). The highest seroprevalence was observed in pigs sampled from traders/middlemen
306 (16.7%; 95% CI [adjusting for survey design]: 4.4%-37.8%), followed by those from smallholders (7.6%;
307 95% CI: 3.4%-14.1%) and slaughterhouses (4.1%; 95% CI: 2.0%-7.4%) (Table 4). None of the pigs
308 sampled from small/medium or large commercial farms tested positive for cysticercosis. Seropositive
309 pigs were found in 9 (15.2%), 5 (71.4%) and 3 (42.9%) of the sampled smallholders, slaughterhouses,
310 and traders/middlemen, respectively. Within these seropositive units, a median of 1 (range 1-3)
311 seropositive pigs were detected. The intraclass correlation coefficients (ICC) within each category
312 suggested a low but significant degree of clustering of seropositive pigs within smallholder units (ICC:
313 0.20; 95%CI: 0.03-0.34). ICC estimates for slaughterhouses and traders/middlemen were low with 95%
314 CIs not significantly above 0 (Table 4). The design effect of clustering of seropositive pigs within units

315 was calculated as 1.5, 0.5 and 1.2 for smallholders, slaughterhouses, and traders/middlemen,
316 respectively.

317 In bivariate analysis, using a 2-step Bayesian GLMM approach allowing for herd-level random
318 effects (see Methods), category of pig sector unit was a significant predictor of infection (*P*=0.008).
319 Specifically, odds ratios (OR) estimated from the fixed-effect coefficients from this model showed that
320 pigs from small/medium farms (OR=0.0; 95%CI=0.0-0.3) and large-commercial farms (OR=0.1;
321 95%CI=0.0-0.9) were significantly less likely to be seropositive than pigs from smallholders. None of
322 the other variables measured at pig-level or herd-level were significantly associated with infection in
323 pigs (Table 5), thus multivariable analysis was not carried out.

324

325 **DISCUSSION**

326 There is paucity of information on cysticercosis in Southeast Asia, particularly regarding how infection
327 among pigs and awareness of the disease among pig workers varies across the diverse pig production
328 systems in this region. In this study, 4.7% (95% CI: 2.9%-7.4%) of pigs tested positive for cysticercus
329 infection in south-central Cambodia. This is lower than seroprevalence estimates from nearby
330 countries of Laos (68.5%) [7], and Myanmar (15.9%) [38], as well as estimates from countries in sub-
331 Saharan Africa [39–41] and Latin America [42]. This might be because, in our study area, the vast
332 majority of pigs are fully confined in pens, even among smallholders, compared to more extensive
333 (i.e., free roaming and scavenging) pig husbandry practices which may predominate in the other study
334 settings. Indeed, it is important to emphasise that our results are unlikely to be representative of
335 Cambodia as a whole; one would expect porcine cysticercosis seroprevalence to be considerably
336 higher in the more remote northeastern provinces, for example, where pigs are often kept tethered
337 or free-roaming in scavenging-based systems [24]. Another explanation for the lower seroprevalence
338 observed in our study compared to some other settings may be due to differences in diagnostic

methods, since some of the aforementioned studies [38, 42] 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 [24], 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 [24].

363 The seroprevalence we observed in slaughterhouses (4.1%; 95% CI; 2.0-7.5%) is lower than
364 that estimated by Sovyra [23] in a previous survey in Cambodia, in which 10.9% (95% CI; 8.1-17.2%) of
365 pig carcasses showed signs of cysticercosis by meat inspection. Since the latter method has lower
366 sensitivity (estimated at ~40%) [21] than the serological assay we used, the study by Sovyra [23] is, if
367 anything, likely to have underestimated the true seroprevalence at that time. Thus, the differences
368 between our estimates may reflect a real decrease in seroprevalence of porcine cysticercosis in
369 Cambodia over the past decade. However, the variation in study areas, as well as diagnostic
370 techniques, between the two studies mean that our results are not entirely comparable. Interestingly,
371 one of the larger abattoirs sampled in our study, where seropositive pigs were also identified, is known
372 to mostly slaughter pigs that have been raised and imported from large scale producers in
373 neighbouring Thailand and Vietnam, suggesting that imported pigs could also present a risk for *Taenia*
374 spp. transmission in Cambodia.

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

382 Across our study smallholders and farms, several herd-level factors were prevalent which
383 could facilitate human-to-pig transmission of cysticercosis, such as use of household waste as pig feed,
384 lack of latrines, and use of surface water bodies as a water source. However, we did not find any
385 significant associations between specific pig husbandry practices and porcine cysticercosis infection
386 across the farms. This in contrast to studies in other countries where, for instance, lack of latrine
387 facilities have been identified a significant risk factor [43–46]. This might again relate to the

388 confinement of pigs in our study area compared to free-range system in some other settings. High
389 mobility of pigs due to frequent trading between production units in south-central Cambodia also
390 mean that the observed porcine infections could have been acquired at another site, and thus would
391 not be associated with the conditions or practices at the site where they were sampled. It is also
392 important to acknowledge that, due to limited numbers of pigs that could be sampled in some
393 categories, our study may simply have lacked sufficient power to detect such associations, as evident
394 from the large standard errors for several of our fixed-effect coefficients in our GLMM analysis.

395 Indeed, the complex and clustered design of animal sampling is not always accounted for in
396 other porcine cysticercosis surveys, which can result in underestimation of standard errors and bias in
397 tests for associations, for example due to random effects at herd level or other sampling levels. Here,
398 we used multi-level models to adjust for herd-level random effects in our analysis. However, as noted
399 in the methods, a challenge we encountered in doing this was quasi-complete separation of the binary
400 outcome for many covariates, due to relatively low seroprevalence of infection. To overcome this, we
401 applied a two-stage Bayesian GLMM approach proposed by Abrahantes & Aerts [35], although further
402 methodological research is needed to determine the best approaches for analysing clustered, rare
403 event data to reduce bias and deal with data separation issues.

404 Another important limitation of our study is that the Ag-ELISA test cannot differentiate
405 between infection by *T. solium*, *T. asiatica*, and *T. hydatigena*, all of which are found in pigs in South-
406 East Asia [21]; thus the species that infect the positive pigs in this study could not be determined. This
407 limits the interpretation of the results in terms of zoonotic risk, given that *T. hydatigena* is not known
408 to infect humans. It also highlights the need for validated diagnostic tests which can differentiate
409 between *Taenia* spp. infection in live pigs. Also, the assay may not have detected circulating antigen
410 in pigs with low viable cysticerci and/or pigs who had been previously treated before blood was
411 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

DECLARATIONS

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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584 **Tables**

585 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		
		No. (%) of units interviewed	No. (%) of units where pigs were sampled	Median (range) of no. of pigs sampled per unit	Total no. of pigs sampled in each category
Smallholders	117	115 (98.3)	59 (50.4)	2 (1-6)	171
Small/medium commercial farm	26	23 (88.5)	20 (76.9)	7 (1-14)	132
Large commercial farm	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

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587

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

	No. (%) of farms				
	All farms	Smallholders	Small/medium farms	Large commercial farms	P- value (Fisher's exact test)
N	143	115	23	5	
Types of pig confinement on farm:					
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
Types of pig feed:					
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
Water sources:					
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
Latrines:					
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

589

590

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

592 slaughterhouses

	Number (%) of participants						
	Total	Small-holders	Small/medium commercial farms	Large farms	Traders/ middlemen	Slaughter-houses	P-value (Fisher's Exact test)
N	163	115	23	5	14	6	
Disease awareness:							
Can name the disease	30 (18.4)	14 (12.2)	4 (17.4)	1 (20.0)	8 (57.1)	3 (50.0)	**<0.001**
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
Previously observed symptoms in pigs:							
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

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594

595 Table 4. Seroprevalence and clustering of porcine cysticercosis among pigs across different

596 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)[1]	ICC[2] (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

597 [1]For non-zero numerators, 95% CIs for seroprevalence are adjusted for clustered survey design. For zero
598 numerators (i.e. small/medium farms and large farms), upper 95%CIs are estimated using Hanley's rule of
599 three;

600 [2]ICC, intraclass correlation coefficient.

601 Table 5. Bivariate analysis of pig-level and herd-level factors with cysticercosis seropositivity in pigs.

602 Results were generated from 2-stageBayesian generalised linear mixed models adjusting for random

603 effects at herd-level

			Std.	Odds		
		β coefficient	**Error**	**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				**0.008**
	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[1,2]	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[1]	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[1]	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[1]	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	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
Province	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)	

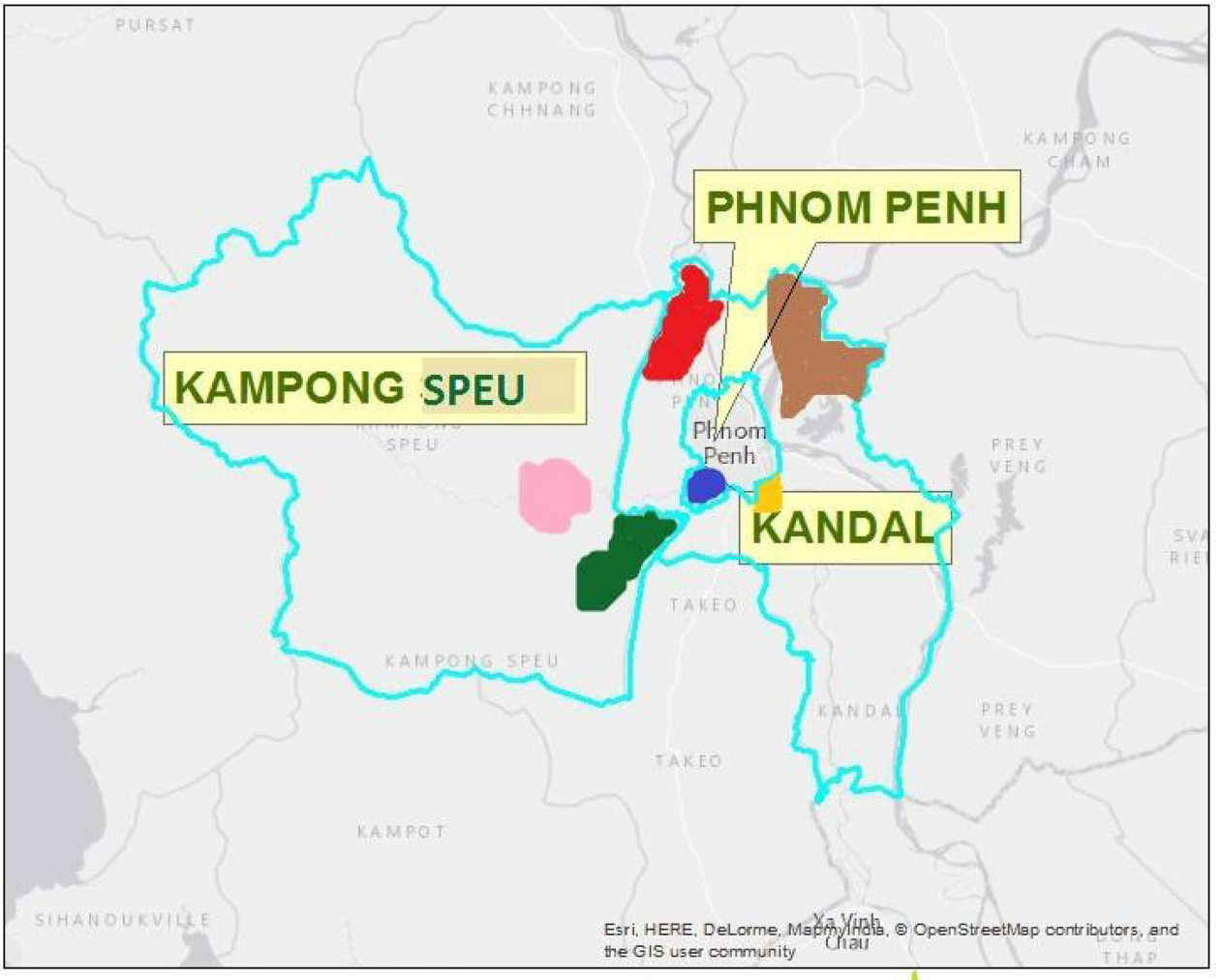
604 [1]Data from producers only (i.e. smallholders, small/medium and larger commercial farms)

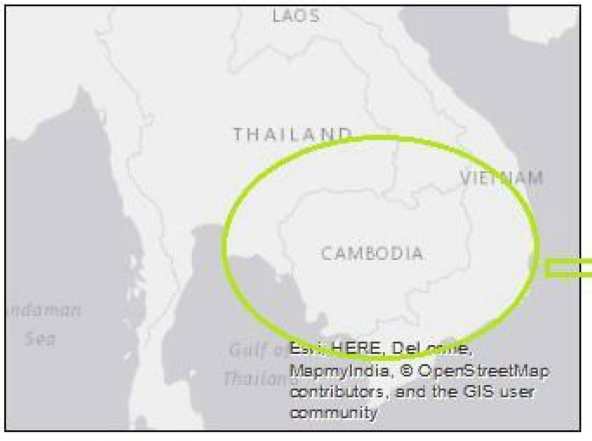
[2]Main confinement type was determined based on the type of confinement in which the majority of pigs in the unit were kept.

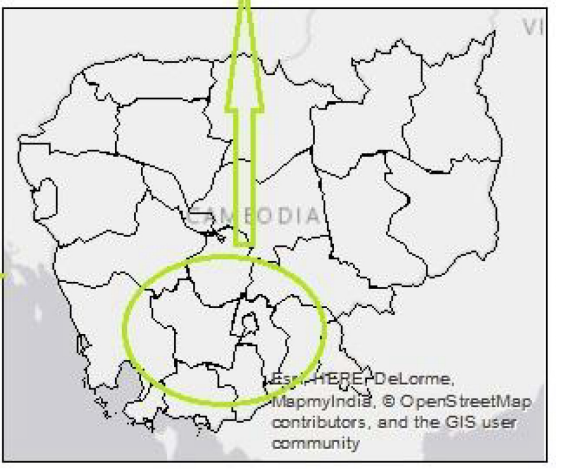
608 **Figure Legends**

609 Figure 1. Map of study area within Cambodia

610







KEY (DISTRICTS)

- DangKao
- Ponhea Leu
- Ta Khmao
- Khsach Kandal
- Samraong Tong
- Kong Pisei

0 5 10 20 30 40
Kilometers

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W E
S