Sveriges lantbruksuniversitet
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Faculty of Veterinary Medicine and Animal Science

Prevalence of the zoonotic diseases cysticercosis and trichinellosis among pigs in rural Cambodia

A cross-sectional study investigating prevalence and risk factors

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SUMMARY

Cysticercosis and trichinellosis are two parasitic zoonoses that are prevalent among pigs in Southeast Asia. The two diseases can cause serious illnesses in humans and human cysticercosis is a leading cause of death from food-borne diseases globally. In Cambodia, the majority of pigs are raised extensively in family backyards in households with between one and four pigs. Pork is the most importance source of meat in this region and is also an important source of income for the farmers. These factors could increase the risk for cysticercosis and trichinellosis in humans in Cambodia, but information regarding the prevalence in rural small-scale pig production is still very limited.

This study was conducted in four provinces in north-eastern Cambodia (Kampong Thom, Preah Vihear, Ratanakiri and Stung Treng). The objective was to determine the seroprevalence of porcine cysticercosis and trichinellosis in rural Cambodia, and to identify possible risk factors for both diseases. In total 139 households participated, and 242 blood samples were collected. Only households with less than 10 pigs above three months old were included in the study. For each pig, sex, age and breed were noted. For each household one person was interviewed about food and hygiene habits, management of the pigs, disease knowledge and practice of treatment with antiparasitic medicines. The serum samples were analysed with ELISA for presence of antigens for cysticercosis or antibodies towards trichinellosis. Univariable statistical analyses were used to identify associations between potential risk factors and positivity for cysticercosis and trichinellosis.

Positivity among the pigs was 11.2% (95% CI 7.5-15.8) for cysticercosis and 2.5% (95% CI 0.9-5.4) for trichinellosis. Cysticercosis was more common in the province Preah Vihear ($p<0.001$) than in the other provinces. Management systems for the pigs and access to human faeces were two risk factors significantly associated with porcine cysticercosis ($p<0.001$). Trichinellosis was more common in the province Ratanakiri ($p=0.001$). Feeding food waste to the pigs was identified as a risk factor for porcine trichinellosis ($p=0.048$). Treatment with antiparasitic medicine was identified as a protective factor for trichinellosis ($p=0.005$). Furthermore, the respondents that had heard of cysticercosis were more commonly men ($p=0.002$), and were also consuming undercooked pork meat to a greater extent ($p=0.004$). Province and gender were also significantly ($p<0.001$) associated with consumption of undercooked pork.

Although the present study is relatively small, several risk factors could be identified for porcine cysticercosis and trichinellosis. The results from this study can be used to guide future interventions and studies to improve both porcine but also human health in these provinces. It would however be interesting with further research on the situation in the other regions in Cambodia.

CONTENT

INTRODUCTION

Cambodia is a lower middle-income country in Southeast Asia with a population of approximately 16.9 million inhabitants (CIA, 2020). The economy has been growing rapidly in recent years and poverty in Cambodia has decreased from 47.8% in 2007 to 13.5% in 2014 (World Bank, 2019) and is believed to still be decreasing, although at a slower rate (Ly *et al.*, 2019). There is still a large proportion of the inhabitants, around 4.5 million, who remain near-poor and vulnerable to falling back into poverty if exposed to economic shock (World Bank, 2019). Approximately three quarters of the Cambodian population live in rural areas (CIA, 2020) and around 90% of the poor live in the countryside (World Bank, 2019).

Pork is the most important source of meat in this region (Huynh *et al.*, 2006) and in Cambodia 80% of approximately 1.7 million pigs (FAOSTAT, 2018) are raised extensively in family backyards (Sovann & San, 2002; Samkol *et al.*, 2006). The majority of these households keep between one and four pigs of mixed breeds and the pigs have an important role as a source of meat, income and to act as a family security asset (Sovann & San, 2002). The pigs are mainly fed with kitchen waste and rice bran (Samkol *et al.*, 2006).

Pig production in Cambodia suffer from high mortality losses caused by a number of diseases including various parasitic diseases (Sovann & San, 2002). This has many reasons including often inadequate feeding (Huynh *et al.*, 2006), insufficient veterinary and agricultural extension services (Sovann & San, 2002), poor knowledge about diseases and poor access to drugs and veterinary services (Samkol *et al.*, 2006). Two important parasitic diseases among pigs in Southeast Asia are cysticercosis and trichinellosis (Pozio, 2001; Dorny *et al.*, 2004) and these are also zoonoses, i.e. diseases that can also infect humans. Globally 2.6–8.3 million humans are estimated to suffer from neurocysticercosis and about 28,000 deaths were attributed to cysticercosis 2010, making it a leading cause of death from food-borne diseases (Havelaar *et al.*, 2015).

A major risk factor for porcine cysticercosis is free roaming pigs with access to human faeces (Pouedet *et al.*, 2002; Murrell *et al.*, 2005; Komba *et al.*, 2013), and a risk factor for porcine trichinellosis is feeding food waste containing meat to the pigs (Dopuoy-Camet *et al.*, 2007). These risk factors are both common in rural small-scale pig production in Cambodia and hence increase the risks for humans to acquire these zoonotic parasitic diseases (Samkol *et al.*, 2006; CIA, 2020).

The objectives of this study were to determine the prevalence of cysticercosis and trichinellosis among rural pigs in four provinces in north-eastern Cambodia and to identify possible associations between prevalence and different risk factors, such as food and hygiene habits, pig management and disease knowledge among the farmers.

LITERATURE REVIEW

Cysticercosis

The diseases cysticercosis and taeniasis are both caused by the tapeworm Taenia solium (CDC, 2019a). Cysticercosis is an infection caused by the larvae of Taenia solium and taeniasis is the name of infection with adult tapeworm when present in human intestine.

Taenia solium is a cyclophyllidean cestode belonging to the family Taeniidae (Taylor *et al.,* 2007). It is known as the human pork tapeworm since its final host is man and its intermediate host usually pig or wild boar. Humans and dogs can also act as intermediate hosts, although that is rarer. In the final host, the predilection site is the small intestine and for the intermediate host it is muscle tissue. The adult tapeworm can become three to five meters long and can survive in a human for many years. It consists of a scolex bearing the attachment organs, a short, unsegmented neck and a chain of segments that is known as a strobila (Murrell *et al.,* 2005; Taylor *et al.,* 2007). Each segment is known as a proglottid and these proglottids are continuously budding from the neck region. As they pass down the strobila they become sexually mature with one or two sets of reproductive organs, where both self-fertilisation and cross-fertilisations between proglottids can occur. The internal structure of the segments largely disappears as they mature and eventually the fully ripe or gravid proglottid only contains remnants of the branched uterus packed with eggs.

Life cycle

The life cycle of *Taenia solium* is host-dependent in two stages and free living in one; adult tapeworms in the final host (human), larvae (cysticercus) in the intermediate host, and eggs in the environment (Murrell *et al.,* 2005) (see Figure 1). Gravid segments, each containing about 40 000 eggs, are usually shed intact from the strobila and then passed out with the faeces of the human (Taylor *et al.,* 2007). As the gravid segments are non-motile, they tend to be concentrated over a small area (Taylor *et al.,* 2007). The eggs are then liberated outside the body of the human by disintegration of the segment or are shed through the genital pore of the segment. The eggs can resist destruction for a relatively long period of time (Taylor *et al.,* 2007; Geerts, 2015). After they are ingested by a susceptible pig, the oncospheres are activated by the gastric and intestinal secretions (Murrell *et al.,* 2005; Taylor *et al.,* 2007; Geerts, 2015). The oncosphere reaches the blood stream or lymphatics by using its hooks to tear through the mucosa of the intestinal wall and travels via the blood or lymphatics to its predilection site. There the oncosphere loses its hooks and develops into a cysticerci, which is a fluid-filled cyst containing the larvae (Taylor *et al.,* 2007). Striated muscle is the main location, but cysticerci can also develop in other organs such as the lungs, kidney, liver and brain. Humans may get infected by ingesting raw or inadequately cooked pork containing viable cysticerci (Murrell *et al.,* 2005; Taylor *et al.,* 2007; Geerts, 2015). The scolex of the cysticerci then attaches to the mucosa of the small intestine and a chain of proglottids begins to grow from the base of the scolex, completing the cycle for taeniasis (Taylor *et al.,* 2007). The prepatent period is two to three months and adult tapeworms can survive in humans for many years (Murrell *et al.,* 2005; Taylor *et al.,* 2007; Geerts, 2015).

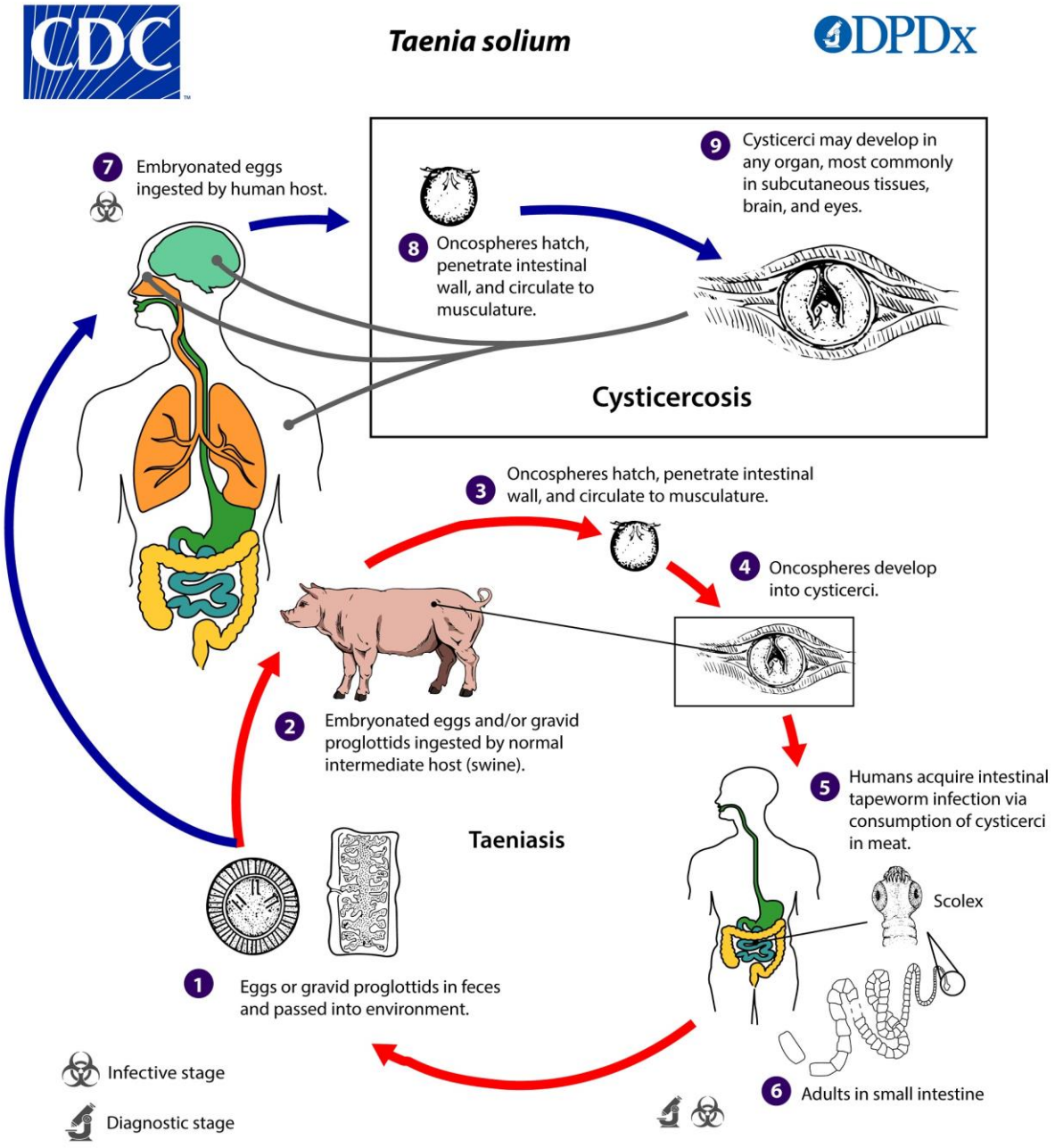

Figure 1. *Life cycle of Taenia solium* (CDC, 2019a).

https://www.cdc.gov/dpdx/cysticercosis/modules/Cysticercosis_LifeCycle_lg.jpg

Porcine cysticercosis

Pigs that are naturally infected with cysticerci are generally asymptomatic and clinical signs are usually inapparent for infected pigs (Taylor *et al.*, 2007; Geerts, 2015). Some clinical signs have only been described in massive infections (Geerts, 2015). Usually pigs are slaughtered at an age when all cysticerci still are viable (Taylor *et al.*, 2007). The cysticerci measure one to two centimetres and are easily visible between muscles fibres with their single large cyst.

Human cysticercosis

Humans acquire cysticercosis by ingestion of eggs through the faecal-oral route, not from ingesting cysticerci in undercooked pork (CDC, 2019a). The human then acts as an intermediate host and this is most likely to occur from the accidental ingestion of the eggs via contaminated food/water or unwashed hands, also called external autoinfection (Murrell *et al.*, 2005; Taylor *et al.*, 2007). Internal autoinfection by reverse peristalsis of the intestine to the stomach can also be a source of infection.

Although cysticerci can be found in every organ of the body in humans acting as the intermediate host, they are most commonly found in the subcutaneous tissue, eyes and brain of a human (Taylor *et al.*, 2007). For humans infected with cysticerci, various clinical signs may occur and the disease severity and clinical manifestations depends on the location, number and size of the cysts, and the intensity of the host's immune response (Taylor *et al.*, 2007; Garcia *et al.*, 2014). Larvae that reach the brain develop in the ventricles, and the most severe clinical signs are caused by cysticerci that develop in the central nervous system, producing mental disturbances or clinical signs of epilepsy or increased intracranial pressure which may be fatal. It is believed that the blood-brain barrier might protect the parasite from attack by the host's immune system, since the blood-brain barrier restricts access of the immune response to the brain (Garcia *et al.*, 2014). Consequent loss of vision may occur when cysticerci develop in the eye (Taylor *et al.*, 2007). A systematic review by Carabin *et al.*, (2011) showed that among patients with symptomatic neurocysticercosis (NCC) at neurological clinics, seizures or epilepsy was present in 78.8% of the patients and was the most common manifestation. The next common manifestation was headache, present in 37.9% of the symptomatic NCC patients, followed by focal deficits (16%) and signs of increased intracranial pressure (11.7%). Other symptoms occurred in less than 10% of patients with symptomatic NCC, including meningitis symptoms (7.9%) and visual changes (5.6%).

Adult tapeworms in humans with taeniasis may occasionally cause abdominal discomfort and diarrhoea, although infection generally is inapparent (Taylor *et al.*, 2007).

Epidemiology

Risk factors

There are several different risk factors related to cysticercosis and the spread of the infection (Taylor *et al.*, 2007). Free-ranging pigs with unrestricted access to human faeces due to lacking latrines and outdoor human defecation nearby the pig rearing areas, are major risk factors in the transmission of eggs from humans to pigs (Pouedet *et al.*, 2002; Murrell *et al.*, 2005; Taylor *et al.*, 2007; Komba *et al.*, 2013). In a study by Braae *et al.*, (2015), it was not possible to associate production system with infection, however they found an association between presence of an open latrine and infection, regardless of production system used, and suggested that it could be a result of environmental contamination contributing to the transmission of the parasite to pigs (Braae *et al.*, 2015). Other risk factors are allowing pigs to scavenge and eat human faeces as a way to improve the human sanitary conditions, or to intentionally use human faeces as pig feed (Murrell *et al.*, 2005; Willingham *et al.*, 2010). A human carrier involved in the management of the pigs is also a risk factor for porcine cysticercosis (Murrell *et al.*, 2005).

Inadequate meat-inspection at pig slaughter is an important contributor to the transmission of cysticerci from pigs to humans, as well as illicit marketing or trading of uninspected pork meat (Murrell *et al.*, 2005; Taylor *et al.*, 2007). In a study in Cameroon, the only risk factor identified for human cysticercosis was eating pork from home-slaughtered pigs (Nkouawa *et al.*, 2017). Another risk factor is eating raw or improperly cooked pork, e.g. as a cultural preference (Murrell *et al.*, 2005). A study in Vietnam found frequent consumption of undercooked pork as a risk factor for infection with *Taenia* spp. (Ng-Nguyen *et al.*, 2018).

The risk factors for human-to-human transmission differ slightly from the transmission of cysticerci to humans from pigs (Murrell *et al.*, 2005). Frequent pork consumption is one risk factor. Other risk factors include low economic status, low level of household sanitation, low personal hygiene standards, and use of night soil ("human dung") in production of vegetables. In a Vietnamese study, location of outdoor defaecation, consumption of raw vegetables, and source of drinking water were significantly associated with an increase in *Taenia solium* cysticerci exposure, where outdoor defaecation results in the contamination of the environment, including water and vegetables (Ng-Nguyen *et al.*, 2018).

Prevalence of cysticercosis in Southeast Asia

Cambodia

The epidemiological data in the scientific literature on *Taenia solium* taeniasis/cysticercosis in Cambodia, Lao PDR and Vietnam is rather limited compared with other regions where the diseases are present (Dorny *et al.*, 2004). Adenuga *et al.* (2018) mention as well that there is paucity of information on cysticercosis in Southeast Asia, particularly regarding infection among pigs and awareness of the disease among pig workers, and how it varies across the diverse pig production systems in this region. In a cross-sectional study among different pig production systems in south-central Cambodia, 4.7% of the pigs in the study tested positive for cysticercus infection (Adenuga *et al.*, 2018). The highest prevalence was among pigs sampled from traders/middlemen (16.7%), followed by smallholders (7.6%) and slaughterhouses (4.1%), while none of the pigs tested positive from the small/medium or large commercial farms. Practices that might facilitate human-to-pig transmission were prevalent among the smallholders, but not found to be significantly associated with infection. In the studied area, the majority of the pigs were kept confined in pens rather than in free-roaming systems, and the authors concluded that porcine cysticercosis is endemic among pigs in this region, with infection associated to smallholder production.

Vietnam and Laos

In a systematic review by Ng-Nguyen *et al.* (2017), human taeniasis and human *Taenia solium* cysticercosis occurred in 60 of the 63 provinces of Vietnam. The data on the prevalence of porcine cysticercosis were lacking, fragmented and/or out of date (Ng-Nguyen *et al.*, 2017). In a cross-sectional study on humans conducted in Dak Lak province in the Central Highlands of Vietnam, antibodies against *Taenia solium* cysticerci were identified in 17 individuals of 339 serum samples, resulting in a study prevalence of 5.0% (Ng-Nguyen *et al.*, 2018).

Reports of human *Taenia* infections range from 0% to 17% in Lao PDR and data on the prevalence of human cysticercosis are limited (Okello *et al.,* 2015). Cases of porcine cysticercosis have been reported, but the prevalence data are rarely available. Holt *et al.* (2016) conducted a cross-sectional study in one upland province (Luang Prabang) and one lowland province (Savannakhet) of Lao PDR. Humans and pigs were tested for antibodies against different zoonotic pathogens, including cysticercosis and *Taenia* spp. In Luang Prabang province, 2.3% of the humans in the study tested seropositive for *Taenia* spp. and 6.1% for cysticercosis, while in Savannakhet the seroprevalence in humans was 2.9% for *Taenia* spp. and 1.5 % for cysticercosis. Pigs were not tested for cysticercosis in the study.

Trichinellosis

Trichinella spiralis, also known as "the muscle worm", is a nematode belonging to the superfamily Trichuroidea (Taylor *et al.,* 2007). It has a wide host range including pig, man, rat and most mammals, with the small intestine and muscle as its predilection site.

Life cycle

The sexes are separate, and the male worm is about 1.5 mm long and smaller than the 3.5-4.0 mm long female (Taylor *et al.,* 2007). A peculiarity of the life cycle of *Trichinella* is the development of two generations within a single host (Dopuoy-Camet *et al.,* 2007; Taylor *et al.,* 2007; Pozio, 2015). *Trichinella* does not have a free-living stage and the adult parasites and infective larvae (muscle trichinae) are unusual in being present in the same host. Between the villi of the small intestine lies the developing adults until fertilisation. After fertilisation the males die while the females burrow deeper into the intestinal mucosa to start to produce new-born larvae (NBL) about a week later (Taylor *et al.,* 2007). The NBL enter the lymphatic vessels and travel to skeletal muscles via the bloodstream (Dopuoy-Camet *et al.,* 2007; Taylor *et al.,* 2007; Pozio, 2015). There they penetrate striated muscle cells, still as NBL, and become encapsulated by the host. The diaphragmatic, masseter and intercostal muscles, and the tongue, are considered the main predilection sites of the striated muscles (Taylor *et al.,* 2007). The parasitized muscle cell transforms by micro-vascularisation into a "nurse cell", and here the larvae grow and assume a characteristic coiled position. In the muscle cell the NBL develops to the L1 infective stage (Dopuoy-Camet *et al.,* 2007; Pozio, 2015). The larvae may remain infective for many years, up to 40 years in humans. Development of the larvae will be resumed when muscles containing the encysted trichinae is ingested by another hosts (Dopuoy-Camet *et al.*, 2007; Taylor *et al.*, 2007; Pozio, 2015). In the stomach, the L1 is liberated from the muscle cells after exposure to gastric acid and pepsin, and then move to the intestine where the larvae penetrate the mucosa (Dopuoy-Camet *et al.*, 2007; Pozio, 2015; CDC, 2019b) and undergo four moults to become sexually mature within about a week (Dopuoy-Camet *et al.*, 2007; Taylor *et al.*, 2007; Pozio, 2015).

Porcine trichinellosis

Infection with *Trichinella spiralis* in young pigs can induce inappetence, diarrhoea and weakness, while older pigs generally are more tolerant of infection (Taylor *et al.,* 2007).

Human trichinellosis

Human trichinellosis is manifested as a syndrome with specific clinical signs and symptoms of variable intensity (Kocięcka, 2000; CDC, 2019c). The intensity of the symptoms depends upon the extent of invasion, as well as the immune response of the host (man) and can range from asymptomatic to severe. The acute stage of human trichinellosis begins in most persons with the sudden appearance of a sensation of general discomfort and headache, fever that increase, chills and excessive sweating (Kocięcka, 2000; Dopuoy-Camet *et al.,* 2007). This occurs when the adults and the migrating larvae provoke the symptoms and clinical signs (Dopuoy-Camet *et al.,* 2007). The typical symptoms in the acute stage consist of persistent pyrexia, myalgia, facial oedema and severe asthenia, which last for several weeks (Kocięcka, 2000; Dopuoy-Camet *et al.,* 2007; Taylor *et al.,* 2007; CDC, 2019c). Periorbital oedema is characteristic for trichinellosis. Diarrhoea and conjunctival and sub-ungual haemorrhages are also sometimes observed, but less frequently (Kocięcka, 2000; Dopuoy-Camet *et al.,* 2007). Also, less frequently can neurological disturbances such as transient dizziness, tinnitus and nausea occur. Cardiovascular disturbances may also appear, but are particularly evident in moderate or severe trichinellosis and usually they develop later in the infection (Kocięcka, 2000). Trichinellosis may be fatal as a consequence of paralysis of the respiratory muscles, unless treated with anthelmintic and anti-inflammatory drugs (Taylor *et al.,* 2007). In the fifth to seventh week after contracting the disease, the signs and symptoms of the disease begin to disappear and the laboratory parameters return to normal values (Kocięcka, 2000).

Risk factors

The risk factors for *Trichinella* infection in pigs are rather few, but well documented (Dopuoy-Camet *et al.,* 2007). To feed food waste containing raw or undercooked meat to food-producing animals is one of the most important risk factors. Other risk factors are exposure of pigs to infected rodents, exposure to infected wildlife and exposure to infected pig carcasses (Dopuoy-Camet *et al.,* 2007; Momoh *et al.,* 2013) since the encapsulated larvae are capable of surviving for several months in decomposing flesh (Taylor *et al.,* 2007; Gottstein *et al.,* 2009). Another mode of transmission is tail biting in pigs (Taylor *et al.,* 2007). Pigs raised in outdoor farming is a condition that expose them to wildlife and is a clear risk factor (Dopuoy-Camet *et al.,* 2007; Momoh *et al.,* 2013), but depends on the infection level in the local wildlife (Dopuoy-Camet *et al.,* 2007). Dopuoy-Camet *et al.* (2007) describe a scenario with conditions where the pigs are at greatest risk in areas where *Trichinella* infections are endemic in wildlife and mention that this scenario is rather typical for developing countries. 'Backyard pigs' (pigs raised on small holdings with minimal confinement) are often fed food scraps and other food waste containing meat and have ready access to rodents and other wildlife (Dopuoy-Camet *et al.,* 2007). These pigs are generally not subjected to reliable methods of veterinary inspection since pigs raised in this manner are often not sold through retail marketing channels, which compounds the problems.

Infection with *Trichinella spiralis* in man is acquired from the ingestion of raw or inadequately cooked infected pork or its by-products, such as sausages, salami and ham (Taylor *et al.,* 2007; Gottstein *et al.,* 2009; CDC, 2019d). Drying, smoking or curing pork do not necessarily kill larvae in the pork products (Taylor *et al.,* 2007). It is even a risk for infection when tasting very small amounts of undercooked pork meat during preparation or cooking (CDC, 2019d).

Prevalence of trichinellosis in South East Asia

Cambodia

The information on the prevalence of trichinellosis in Cambodia is limited. Anti-*Trichinella* antibodies have been detected in serum samples from asymptomatic persons in a rural village of Cambodia, but otherwise it is a lack of documented infections in humans and animals (Pozio, 2001; 2007). However, in September 2017 the Ministry of Health of the Kingdom of Cambodia informed and confirmed an outbreak of trichinellosis among humans in Kampong Thom province with 33 ill people and eight deaths (Khmer Times, 2017)

Vietnam and Laos

Since 1968, when the first human case of trichinellosis was identified in Vietnam, there have been five reported outbreaks of trichinellosis in four provinces of Vietnam (Ng-Nguyen *et al.*, 2017). Thi *et al.* (2013) conducted a study in two provinces of northwestern Vietnam (Dien Bien province and Son La province), investigating the seroprevalence of trichinellosis in domestic animals, such as pigs, dogs and cats. Among the 558 pigs included in the study, the prevalence of *Trichinella* spp. was 5.6%. This is an overall lower seroprevalence in pigs compared to a previous study conducted in Son La province where the seroprevalence of porcine trichinellosis was 19.9% (Vu Thi *et al.*, 2010).

In Lao PDR, trichinellosis is endemic (Okello *et al.,* 2015). However, in the review by Okello *et al.* (2015) the majority of the reported human outbreaks occurred in the northern and central regions. Only limited data exist for the prevalence of *Trichinella* infections in pigs *(Okello et al.,* 2015). The prevalence of porcine trichinellosis has been found to be 2.1% in northern Lao PDR (Conlan *et al.,* 2014) and in another survey in the country the seroprevalence was found to be 13.7% (Okello *et al.,* 2015). In a cross-sectional study conducted in the upland province Luang Prabang and the lowland province Savannakhet of Lao PDR, there was a high seroprevalence of *Trichinella spiralis* in humans; 59.0% in Luang Prabang and 40.5% in Savannakhet (Holt *et al.,* 2016). In Luang Prabang 14.4% of the pigs tested seropositive for *T. spiralis*, and in Savannakhet the seroprevalence was 9.3% among pigs.

MATERIAL AND METHODS

Study design

The study was conducted in four different high-risk provinces for cysticercosis and trichinellosis, identified by the National Animal Health and Production Research Institute (NAHPRI), in the northeastern Cambodia (Figure 2). The provinces were Kampong Thom, Preah Vihear, Ratanakiri and Stung Treng. Within each province three to four districts were selected by the provincial veterinarian. There were not any specific selection criteria, although districts with a lot of pigs, and especially free roaming, were purposefully targeted, and an even geographic distribution of the districts within the province was desired. The aim was to collect 252 blood samples; 63 samples in each province. In Kampong Thom blood samples from 63 pigs were collected, in Preah Vihear 51 samples, in Ratanakiri 63 samples and in Stung Treng 65 samples; in total 242 blood samples. The intention of the study was to target pig farms with less than 10 pigs above three months old, where the pigs were kept either free roaming or partly confined. However, because of the current situation of African swine fever in Cambodia at the time of the study (September to November 2019), tethered pigs and pigs confined in pens were also included in the study to increase the chance of getting the intended number of samples. The households within each village in the district were intended to be selected through the method of snowball sampling, meaning that after the first household had been identified, that household would provide information about other households of interest (Goodman, 1961). Those household would then do the same and this would continue until enough samples had been obtained. In practice, however, the head of the village did locate the farms with pigs within the village and the households were selected based on that. The farmers were informed about the project and were asked for their consent to voluntarily participate.

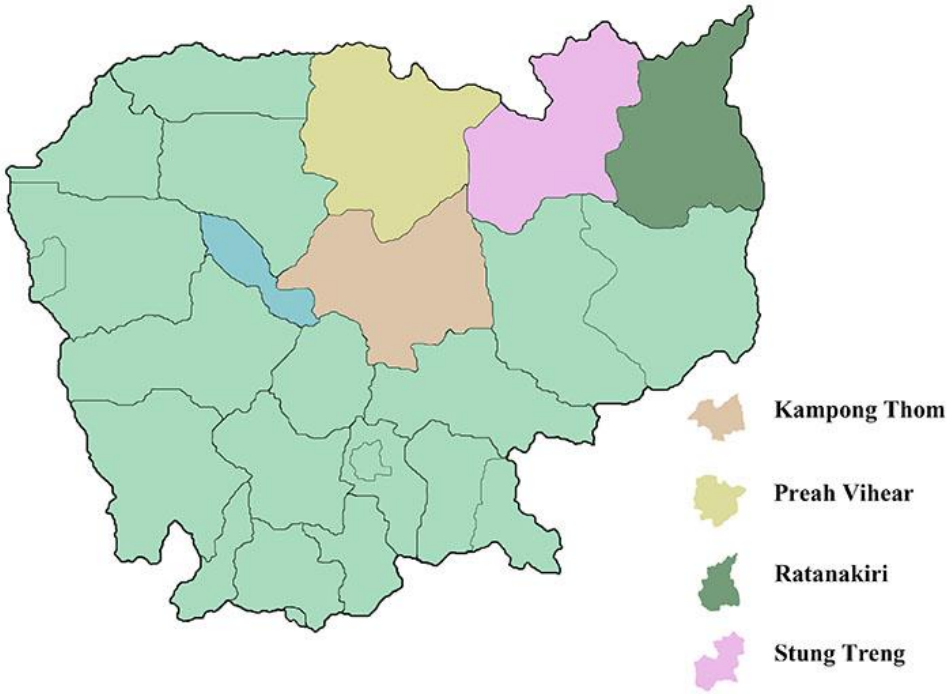

Figure 2. *The four provinces in Cambodia where the study was conducted.*

Collection of samples

The blood samples were collected during four weeks in October 2019; one week in each province. In each household one to three pigs were sampled, if possible from different age groups. Only pigs above three months of age were sample to minimize the risk of interference with maternal antibodies. Pigs with signs of sickness were not sampled and sampling of pregnant sows was avoided to decrease the stress and risk of spontaneous abortion. The blood was collected from the jugular vein or from the ear vein into a vacuum serum blood collection tube and was labelled with farm number and the serial number for the pig. An individual blood sample form was filled in for each sampled pig. The blood sample form contained sex, age and breed of the pig. After labelling, the samples were kept in a cooling box with ice during the day until they were centrifuged. Centrifugation was performed within 24 hours of sampling. The sera were transferred into cryotubes labelled with farm and pig number and stored in a cooling box until moved to a freezer (-18°C) in the province. When transported to Phnom Penh the serum tubes were kept in a cooling box until re-frozen at NAHPRI.

Questionnaire

In each household, one adult person (>15 years old) responsible for the pigs was interviewed regarding food and hygiene habits, management of the pigs and disease knowledge and practice of treatment with antiparasitic medicines. Prior to the study, the questionnaire (see Appendix 1) was translated by a Cambodian veterinary student (student 1) to Khmer (the official language of Cambodia). The same student and two other Cambodian veterinary students did the interpretation during the interviews with the farmers; in the first province by student 1 and student 2, in the second and third province by student 2, and in the fourth province by student 2 and student 3.

ELISA

All samples were analysed at NAHPRI the first week of November 2019 through enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions, with all samples run in duplicates. For cysticercosis the apDia Cysticercosis Antigen (Ag) ELISA kit (apDia bvba, Turnhout, Belgium) was used for determination of viable cysticerci of *Taenia* spp. According to the manufacturer, test performance had been evaluated in a study with 31 infected animals where all the samples gave positive results indicating a high sensitivity and the specificity have been demonstrated in another study where it was 99.6% (apDia, ND). For trichinella the kit PrioCHECK Trichinella Ab (Thermofisher Scientific) was used to detect presence antibodies against *Trichinella* spp. with a demonstrated sensitivity of 97.1-97.8% and a specificity of 99.5-99.8% (Frey *et al.*, 2009). None of the assays are species specific for infections in pigs, which has been in consideration since e.g. *Taenia asiatica* are common in pigs in Asia. Another limitation of the cysticercosis assay is that the assay does not detect degenerated or calcified cysticerci, only viable cysticerci (apDia, ND). One living cyst should be sufficient for detection with the cysticercosis assay. Circulating antigens has been first detected between two and six weeks in experimentally infected cysticercosis pigs and remained present generally throughout the observation period of six months.

Statistical analysis

Data was entered into Ms Excel and descriptive statistics was done using Excel. Possible risk factors for positivity among the pigs but also possible risk factors for humans, and factors affecting human knowledge and behaviours, were investigated with STATA 14.2 (StataCorp, College Station, Texas). Pearson chi2 was used for statistic between two categorical variables and Fischer's exact was used when Pearson chi2 was not applicable. T-test was used for analysing age as a continuous variable. Significance level was set to $p<0.05$. Question C4, C5, D5 and D11 were however excluded from the analyses due to misinterpretation by the interviewers.

RESULTS

Households and sample distribution

The total number of participated households was 139 and the total number of pigs that were sampled was 242. The distribution of the 139 households and the 242 samples among the provinces can be seen in Figure 3. Of the pigs sampled, 59.9% were females, 38.4% were males and for 1.7%, the sex of the pig was not specified. Of the pigs that were sampled, the youngest was three months old and the oldest was five years old. Most of the pigs were between three to five months old (Figure 4). Most pigs (73.1%) were of indigenous breeds, see Table 1.

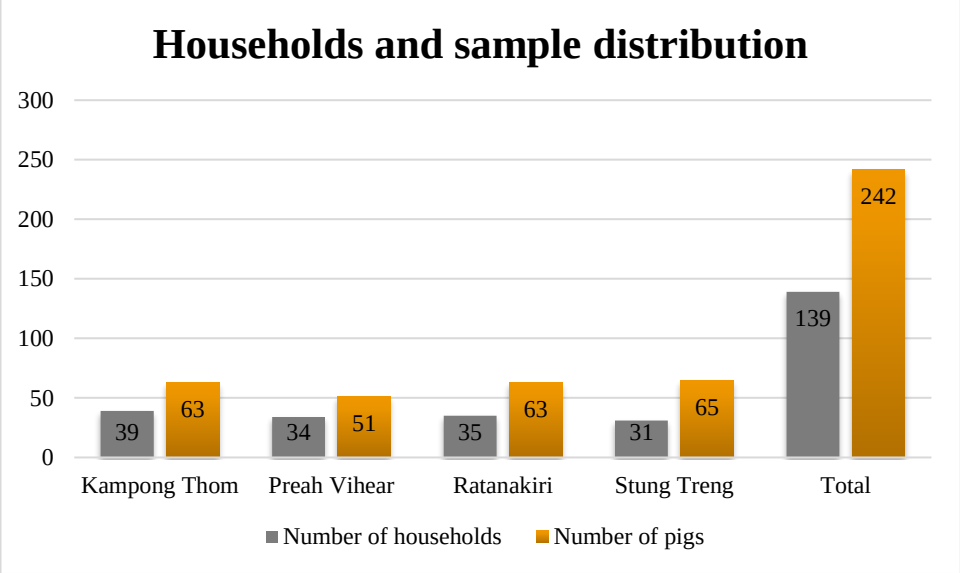

Figure 3. *Household and sample distribution in four provinces in Cambodia.*

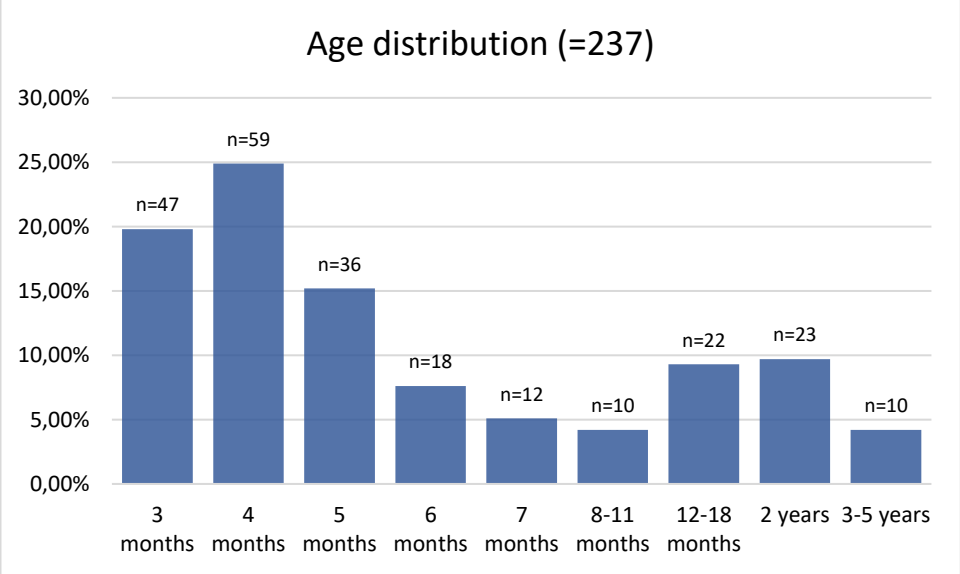

Figure 4. *Age distribution among the sampled pigs in four provinces in Cambodia.*

Table 1. *Pig breeds of the sampled pigs in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Pig breed										
Indigenous	37	58.7%	51	100.0%	29	46.0%	60	92.3%	177	73.1%
Commercial	24	38.1%	0	0.0%	34	54.0%	4	6.2%	62	25.6%
Mix	2	3.2%	0	0.0%	0	0.0%	1	1.5%	3	1.2%
Total	63	100.0%	51	100.0%	63	100.0%	65	100.0%	242	100.0%

Questionnaire

Out of the 139 households that were included in the study, all answered the questionnaire. Some questions were however not answered by all participants.

Household demographics

The results except the age of the respondent are described in Table 2. Most respondents (75.4%) were female and 24.6% were male. The mean age of the respondent was 41 years old (Table 3). For 42.6% of the respondents, primary school was the highest level of education and 34.6% did not have an education. Only one person (0.7%) had attended college or university.

Table 2. *Gender and level of education among the respondent in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Respondent	No.	%	No.	%	No.	%	No.	%	No.	%
Sex										
Female	35	89.7%	26	78.8%	17	54.8%	23	74.2%	101	75.4%
Male	4	10.3%	7	21.2%	14	45.2%	8	25.8%	33	24.6%
Total	39	100.0%	33	100.0%	31	100.0%	31	100.0%	134	100.0%
Highest education										
No education	19	48.7%	10	29.4%	14	43.8%	4	12.9%	47	34.6%
Primary school	13	33.3%	15	44.1%	13	40.6%	17	54.8%	58	42.6%
Lower Secondary	3	7.7%	6	17.6%	2	6.3%	4	12.9%	15	11.0%
Upper Secondary	4	10.3%	2	5.9%	3	9.4%	6	19.4%	15	11.0%
College /university	0	0.0%	1	2.9%	0	0.0%	0	0.0%	1	0.7%
Total	39	100.0%	34	100.0%	32	100.0%	31	100.0%	136	100.0%

Table 3. *Age of the respondent in four provinces in Cambodia*

	Kampong Thom	Preah Vihear	Ratanakiri	Stung Treng	Total
	(n=39)	(n=34)	(n=35)	(n=31)	(n=139)
Age y/o					
Mean	40 (19-65)	38 (18-57)	42 (25-74)	45 (21-64)	41 (18-74)

Food and hygiene habits

The majority of the households (98.6%) ate pork, and of these it was most common to eat pork two to five times per week (41.9%). The majority of the ones that ate pork (96.3%) cooked the pork meat until it was brown/grey throughout before they ate it, while 20.7% answered that they ate pork meat that either was red/pink in the middle or uncooked. Of the people that answered that they ate uncooked or red/pink pork meat, 96.2% only ate it sometimes and 3.8% ate it often (Table 4).

Table 4. *Pork consumption habits among respondent in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Pork consumption										
Every day	2	5.4%	10	29.4%	9	26.5%	3	9.7%	24	17.6%
2-5 times/week	8	21.6%	14	41.2%	15	44.1%	20	64.5%	57	41.9%
Once a week	4	10.8%	1	2.9%	3	8.8%	2	6.5%	10	7.4%
2-3 times/month	5	13.5%	7	20.6%	0	0.0%	5	16.1%	17	12.5%
Once a month	18	48.6%	2	5.9%	7	20.6%	1	3.2%	28	20.6%
Total	37	100.0%	34	100.0%	34	100.0%	31	100.0%	136	100.0%
Cooking level pork*										
Cooked (brown/grey)	37	100%	34	100%	31	93.9%	28	90.3%	130	96.3%
Cooked (red/pink)	0	0.0%	0	0.0%	25	75.8%	2	6.5%	27	20.0%
Uncooked (raw)	0	0.0%	0	0.0%	0	0.0%	1	3.2%	1	0.7%
Frequency of eating raw/red/pink										
Sometimes					24	96.0%	1	100.0%	25	96.2%
Often					1	4.0%	0	0.0%	1	3.8%
Total					25	100.0%	1	100.0%	26	100.0%

**not limited to one answer*

The majority (92%) of the participating families got the pork meat from the market or the mobile market (a motorbike driving around in the villages selling meat), see Table 5. One-third (33.8%) reported slaughtering pigs at home, of which 89.4% used the meat for own consumption and 25.5% sold the meat to others. When a picture of meat with cysts was shown, 28.9% answered "yes" on the question if they ever had seen that kind of cyst while preparing the pork meat. Of the people who answered that they had seen cysts when preparing the meat, 89.7% did not eat any of the meat, 7.7% did cut away the bad part and ate the rest, and 2.7% cooked the meat extra well before eating it.

Table 5. *Origin and quality of pork meat in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Origin of the meat										
Market	30	81.1%	31	91.2%	34	97.1%	30	96.8%	125	91.2%
Other	7	18.9%	3	8.8%	1	2.9%	1	3.2%	12	8.8%
Total	37	100.0%	34	100.0%	35	100.0%	31	100.0%	137	100.0%
Slaughter at home										
Yes	12	30.8%	22	64.7%	8	22.9%	5	16.1%	47	33.8%
No	27	69.2%	12	35.3%	27	77.1%	26	83.9%	92	66.2%
Total	39	100.0%	34	100.0%	35	100.0%	31	100.0%	139	100.0%
If slaughtering at home, what do with meat*										
Own consumption	7	58.3%	22	100.0%	8	100.0%	5	100.0%	42	89.4%
Sell to others	4	33.3%	1	4.5%	2	25.0%	5	100.0%	12	25.5%
Seen cysts										
Yes	3	8.1%	14	41.2%	5	15.2%	17	54.8%	39	28.9%
No	34	91.9%	20	58.8%	28	84.8%	14	45.2%	96	71.1%
Total	37	100.0%	34	100.0%	33	100.0%	31	100.0%	135	100.0%
If seen cysts, what did										
Didn't eat it	1	33.3%	12	85.7%	5	100.0%	17	100.0%	35	89.7%
Cut away bad part	2	66.7%	1	7.1%	0	0.0%	0	0.0%	3	7.7%
Cooked it extra well	0	0.0%	1	7.1%	0	0.0%	0	0.0%	1	2.6%
Total	3	100.0%	14	100.0%	5	100.0%	17	100.0%	39	100.0%

**not limited to one answer*

The majority (74.0%) of the households in the study had a pit latrine, while 19.7% used bush/field as a toilet (Table 6). At 31.9% of the farms, the pigs could at least at some point come in contact with the toilet or stool. Eighty-nine percent said they and their family always or at least most of the times, washed their hands before eating. Only 11% answered that they not so often or never, washed their hands in the family before eating.

Table 6. *Sanitary and hygiene habits in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
What kind of toilet										
Flushing toilet	1	2.6%	1	3.0%	0	0.0%	0	0.0%	2	1.6%
Pit latrine	32	82.1%	22	66.7%	17	58.6%	23	88.5%	94	74.0%
Bush/field	1	2.6%	10	30.3%	12	41.4%	2	7.7%	25	19.7%
Other	5	12.8%	0	0.0%	0	0.0%	1	3.8%	6	4.8%
Total	39	100.0%	33	100.0%	29	100.0%	26	100.0%	127	100.0%
Pigs contact with stool										
Yes	14	35.9%	21	63.6%	4	12.5%	4	12.9%	43	31.9%
No	25	64.1%	12	36.4%%	28	87.5%	27	87.1%	92	68.1%
Total	39	100.0%	33	100.0%	32	100.0%	31	100.0%	135	100.0%
Washed hands										
Yes, always	8	20.5%	20	58.8%	30	90.9%	25	80.6%	83	60.6%
Most of the times	19	48.7%	12	35.3%	3	9.1%	5	16.1%	39	28.5%
Not so often	12	30.8%	1	2.9%	0	0.0%	1	3.2%	14	10.2%
Never	0	0.0%	1	2.9%	0	0.0%	0	0.0%	1	0.7%
Total	39	100.0%	34	100.0%	33	100.0%	31	100.0%	137	100.0%

Farm details

The mean farm size was 4.2 pigs. The mean number of the different age categories of the pigs can be seen in Table 7. Piglets were defined as pigs under the age of one month, growers were defined as pigs between one to three months and fatteners were defined as pigs above three months of age. Confinement in pen was the most common housing system for pigs, with 66.2% reporting this for at least part of the pigs, 40.3% had pigs tethered, 25.9% had pigs partly confined, and 11.5% had pigs free roaming. Fifty percent kept their pigs free roaming during the dry season, and 12.5% during the rainy season, while 37.5% kept their pigs free roaming during both seasons (Table 8).

Table 7. *Number of pigs at the farms in four provinces in Cambodia*

	Kampong Thom	Preah Vihear	Ratanakiri	Stung Treng	Total
	(n=39)	(n=34)	(n=35)	(n=31)	(n=139)
Number of pigs	Mean	Mean	Mean	Mean	Mean
Piglets	1.8 (0-12)	1.2 (0-10)	0.3 (0-5)	0.7 (0-10)	1.0 (0-12)
Growers	1.0 (0-9)	1.4 (0-9)	1.1 (0-9)	2.4 (0-9)	1.4 (0-9)
Fatteners	1.3 (0-6)	0.6 (0-4)	1.8 (0-10)	0.1 (0-2)	1.0 (0-10)
Breeding sows	0.7 (0-2)	0.8 (0-3)	0.3 (0-2)	0.5 (0-3)	0.6 (0-3)
Breeding boars	0.0 (0-1)	0.1 (0-1)	0.1 (0-1)	0.2 (0-2)	0.1 (0-2)

Table 8. *Housing systems for the pigs in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Housing system pigs*										
Confined in pen	26	66.7%	13	38.2%	27	77.1%	26	83.9%	92	66.2%
Tethered	19	48.7%	14	41.2%	10	28.6%	13	41.9%	56	40.3%
Partly confined	2	5.1%	24	70.6%	8	22.9%	2	6.5%	36	25.9%
Free roaming	4	10.3%	8	23.5%	2	5.7%	2	6.5%	16	11.5%
Season free										
Dry	0	0.0%	19	79.2%	0	0.0%	1	33.3%	20	50.0%
Rainy	0	0.0%	0	0.0%	3	33.3%	2	66.7%	5	12.5%
Both	4	100.0%	5	20.8%	6	66.7%	0	0.0%	15	37.5%
Total	4	100.0%	24	100.0%	9	100.0%	3	100.0%	40	100.0%

**not limited to one answer*

Almost 80% of the households in the study reported that they fed their pigs kitchen waste or other food waste from markets/restaurants. Of these, 70.0% said that the food waste could contain meat, and 39.0% did not cook the food waste before feeding it to the pigs (Table 9).

Table 9. *Pig feeding habits in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Fed pigs waste										
Yes	24	61.5%	31	91.2%	27	77.1%	28	93.3%	110	79.7%
No	15	38.5%	3	8.8%	8	22.9%	2	6.7%	28	20.3%
Total	39	100.0%	34	100.0%	35	100.0%	30	100.0%	138	100.0%
Ever contained meat										
Yes	13	54.2%	18	58.1%	25	96.2%	21	72.4%	77	70.0%
Didn't know	2	8.3%	1	3.2%	1	3.8%	7	24.1%	11	10.0%
No	9	37.5%	12	38.7%	0	0.0%	1	3.4%	22	20.0%
Total	24	100.0%	31	100.0%	26	100.0%	29	100.0%	110	100.0%
Cooked before fed										
Yes	7	30.4%	15	55.6%	4	14.8%	8	28.6%	34	32.4%
Sometimes	6	26.1%	4	14.8%	14	51.9%	6	21.4%	30	28.6%
No	10	43.5%	8	29.6%	9	33.3%	14	50.0%	41	39.0%
Total	23	100.0%	27	100.0%	27	100.0%	28	100.0%	105	100.0%

Disease knowledge and practice of treatment with antiparasitic medicines

Of the participated households, 75.5% had heard of cysticercosis, of which 76.5% said they could explain what it was. Not many, 22.1%, knew humans can get infected but 98.1% knew pigs can get infected (Table 10). Of the people that knew humans can get infected, 91.3% said they knew how people get infected. Of the people that knew pigs can get infected, 22.4% said they knew how pigs get infected with the disease.

Table 10. *Knowledge of cysticercosis among the respondent in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Heard of cysticercosis										
Yes	31	79.5%	20	58.8%	32	91.4%	22	71.0%	105	75.5%
No	8	20.5%	14	41.2%	3	8.6%	9	29.0%	34	24.5%
Total	39	100.0%	34	100.0%	35	100.0%	31	100.0%	139	100.0%
Could explain										
Yes	29	93.5%	16	80.0%	27	84.4%	6	31.6%	78	76.5%
No	2	6.5%	4	20.0%	5	15.6%	13	68.4%	24	23.5%
Total	31	100.0%	20	100.0%	32	100.0%	19	100.0%	102	100.0%
Knew humans can get infected*	2	6.5%	15	75.0%	4	12.9%	2	9.1%	23	22.1%
Knew how humans get infected										
Yes	2	100.0%	14	93.3%	4	100.0%	1	50.0%	21	91.3%
No	0	0.0%	1	6.7%	0	0.0%	1	50.0%	2	8.7%
Total	2	100.0%	15	100.0%	4	100.0%	2	100.0%	23	100.0%
Knew pigs can get infected*	31	100.0%	19	95.0%	31	100.0%	21	95.5%	102	98.1%
Knew how pigs get infected										
Yes	3	9.7%	11	57.9%	5	17.2%	3	15.8%	22	22.4%
No	28	90.3%	8	42.1%	24	82.8%	16	84.2%	76	77.6%
Total	31	100.0%	19	100.0%	29	100.0%	19	100.0%	98	100.0%

**not limited to one answer*

Among the participating households, fewer people had heard of trichinellosis compared to cysticercosis (Table 11). Only 5.0% of the respondents reported to have heard of trichinellosis, and only half of them could also explain what it was. Of the people that had heard of the disease, half of the respondent knew humans can get infected and all of them knew pigs can get infected. Of the people that knew that humans can get infected with trichinellosis, all of them also said they knew how humans get infected. While of the people that had answered that they knew that pigs can get infected, only 20% said they knew how pigs get infected.

Table 11. *Knowledge of trichinellosis among the respondent in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Heard of trichinellosis										
Yes	0	0.0%	6	17.6%	1	2.9%	0	0.0%	7	5.0%
No	39	100.0%	28	82.4%	34	97.1%	31	100.0%	132	95.0%
Total	39	100.0%	34	100.0%	35	100.0%	31	100.0%	139	100.0%
Could explain										
Yes			2	40.0%	1	100.0%			3	50,0%
No			3	60.0%	0	0.0%			3	50,0%
Total			5	100.0%	1	100.0%			6	100,0%
Knew humans can get infected*			3	50.0%	0	0.0%			3	50.0%
Knew how humans get infected										
Yes			3	100.0%					3	100.0%
No			0	0.0%					0	0.0%
Total			3	100.0%					3	100.0%
Knew pigs can get infected*			5	83.3%	1	100.0%			6	100.0%
Knew how pigs get infected										
Yes			1	25.0%	0	0.0%			1	20.0%
No			3	75.0%	1	100.0%			4	80.0%
Total			4	100.0%	1	100.0%			5	100.0%

**not limited to one answer*

Approximately half of the participating households (54.7%) reported to treat their pigs with antiparasitic medicines and most of them did it either one time during a pig's life (48.0%) or every year (33.3%) (Table 12).

Table 12. *Practice of treatment with antiparasitic medicines in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
Household	No.	%	No.	%	No.	%	No.	%	No.	%
Treated pigs with antiparasitic										
Yes	30	76.9%	17	50.0%	8	23.5%	20	66.7%	75	54.7%
No	9	23.1%	17	50.0%	26	76.5%	10	33.3%	62	45.3%
Total	39	100.0%	34	100.0%	34	100.0%	30	100.0%	137	100.0%
How often										
Every year	14	46.7%	10	62.5%	0	0.0%	0	0.0%	24	33.3%
One time/life	8	26.7%	4	25.0%	5	71.4%	17	89.5%	34	47.2%
Other	8	26.7%	2	12.5%	2	28.6%	2	10.5%	14	19.4%
Total	30	100.0%	16	100.0%	7	100.0%	19	100.0%	72	100.0%

Prevalence and risk factors

Prevalence

Cysticercosis

The cysticercosis results from the ELISA tests are presented in Table 13. In total, 11.2% (95% confidence interval (CI) 7.5-15.8) of the 242 sampled pigs were positive for cysticercosis and 0.8% were suspected to be positive. Out of the 27 positive blood samples, 16 samples were from Preah Vihear province. There was a significant ($p<0.001$) difference of positive cysticercosis pigs between the provinces, where Preah Vihear had the highest result for a province with 31.4% (95% CI 19.1- 45.9) positive pigs of the sampled pigs in the province.

Table 13. *ELISA results for porcine cysticercosis in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Cysticercosis										
Positive	8	12.7%	16	31.4%	3	4.8%	0	0.0%	27	11.2%
Suspect	0	0.0%	1	2.0%	0	0.0%	1	1.5%	2	0.8%
Negative	55	87.3%	34	66.7%	60	95.2%	64	98.5%	213	88.0%
Total	63	100.0%	51	100.0%	63	100.0%	65	100.0%	242	100.0%

Trichinellosis

The trichinellosis results from the ELISA tests presented in Table 14. Four ELISA results are missing. In total, 2.5% (95% CI 0.9-5.4) of 238 blood samples were positive for trichinellosis. Three of the six seropositive pigs were sampled in Ratanakiri which had significantly (p=0.001) higher prevalence compared to the other provinces. No farm had more than one seropositive pig.

Table 14. *ELISA results for porcine trichinellosis in four provinces in Cambodia*

	Kampong Thom		Preah Vihear		Ratanakiri		Stung Treng		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Trichinellosis										
Positive	1	1.6%	1	2.0%	3	4.9%	1	1.5%	6	2.5%
Negative	60	98.4%	50	98.0%	58	95.1%	64	98.5%	232	97.5%
Total	61	100.0%	51	100.0%	61	100.0%	65	100.0%	238	100.0%

Risk factors

Cysticercosis

Positive pigs were significantly older (14.1 months compared to 8.2 months, p=0.002). Management systems showed a significant association with cysticercosis, see Figure 5. Free-roaming and partly confined pigs were both significantly (p<0.001) associated with cysticercosis, while there was a significant (p=0.004) decreased risk for infection with cysticercosis for the pigs confined in pens. If the pigs could come in contact with the toilet/stool the prevalence was significantly (p<0.001) higher, but no association between what kind of toilet the people in the households used and cysticercosis was shown. Neither was there any significant association between if the respondent ever had seen cysts in the pork meat when preparing it and porcine cysticercosis.

A significant (p=0.002) association between gender of the respondent and if the respondent had heard of cysticercosis was found, where males were more likely to have heard of the disease. No association could be found between knowledge of cysticercosis and if the pigs could come in contact with the stool, what kind of toilet the people in the households used, if the pigs were confined in pens or education level.

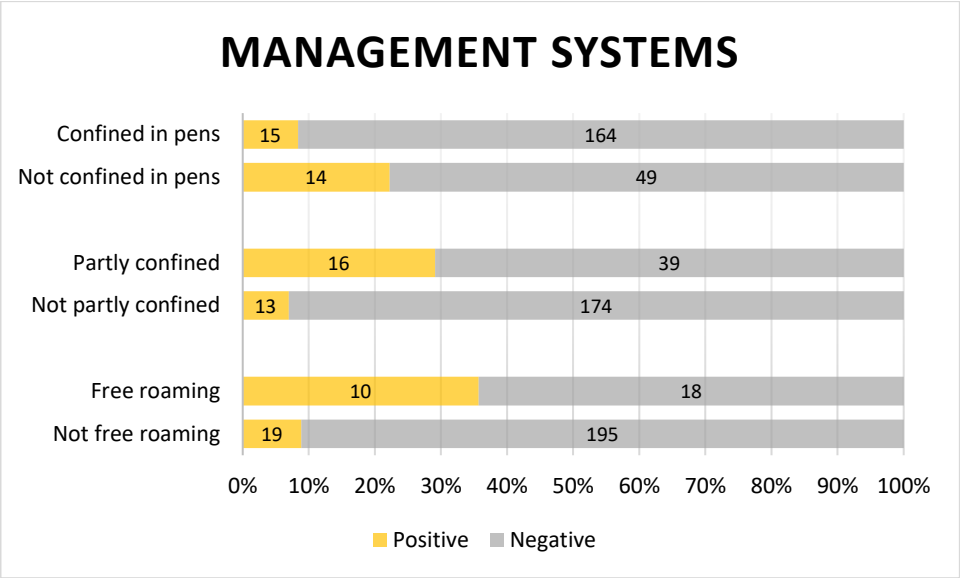

Figure 5. *Management systems and seroprevalence of cysticercosis.*

Trichinellosis

There was a significant (p=0.048) association between feeding food waste to the pigs and porcine trichinellosis. The lowest risk for trichinellosis were among those who did not feed food waste containing meat (Figure 6). The number seropositive pigs were low, but it was shown that the *Trichinella*-positive pigs had a tendency to be older (9.8 months compared to 8.8, p=0.8). There was no association between management systems and trichinellosis.

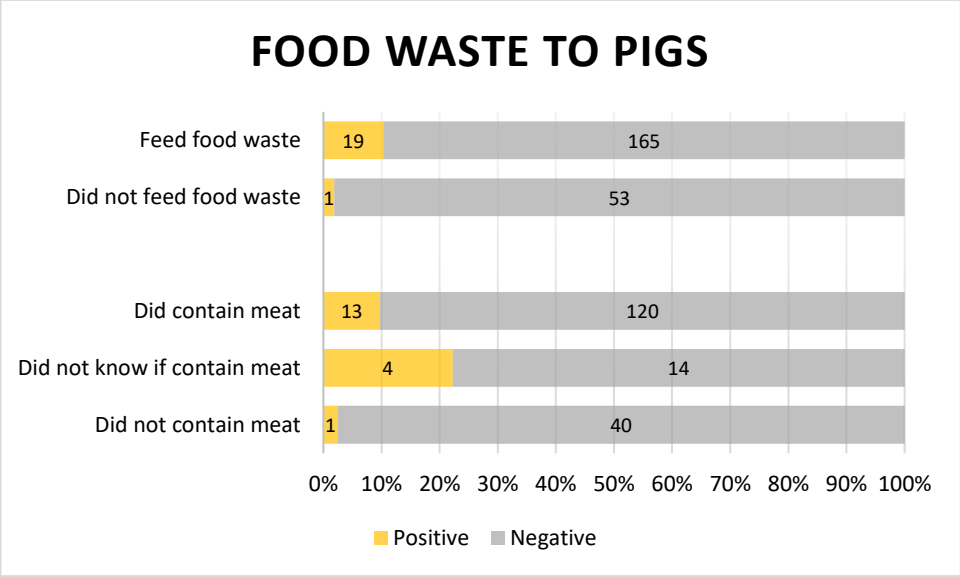

Figure 6. *Food waste to the pigs and seroprevalence of cysticercosis.*

Eating habits

Province and gender showed both a significant ($p<0.001$) association with eating pink/red/raw pork meat, where the province Ratanakiri and males showed a significantly increased tendency to eat raw or inadequately cooked pork meat. Also, the respondents that had heard of cysticercosis were significantly ($p=0.004$) associated with eating pink/red/raw pork meat.

Antiparasitic treatment

Treatment with antiparasitic medicines showed a significant ($p=0.005$) negative association with trichinellosis, with more pigs positive if they had not been treated with antiparasitic medicines. For cysticercosis no association could be found between treatment with antiparasitic medicines and infection.

DISCUSSION

Cysticercosis

In this study the seroprevalence of porcine cysticercosis was 11.2%. Preah Vihear had a significantly higher seroprevalence ($p<0.001$) of cysticercosis (31.4%) compared to other provinces. Preah Vihear was also the province with the highest percent within a province of pigs in contact with toilet/stool (63.6%), partly confined (70.6%) and free-roaming (23.5%) pigs, and respondents that had not heard of cysticercosis (41.2%) compared with the other provinces in the study. Management systems and pigs in contact with toilet/stool showed a significant ($p<0.001$) association with porcine cysticercosis, which confirm that these risk factors are of importance for the disease. Based on these results, Preah Vihear can be seen as a high-risk province for cysticercosis in this study compared to the other provinces. Interventions to improve the situation should therefore initially be conducted in Preah Vihear.

Preah Vihear was also the province where most of the households slaughtered pigs at home, primarily for own consumption. In this province, 41.2% had at some time seen cysts in the pork meat but only 58.8% of the respondents had heard of cysticercosis, which was the lowest knowledge level compared to the other provinces in the study. Absence of adequate meat-inspection performed by a veterinarian at the slaughterhouse, in combination with lack of knowledge of the disease in a province with presence of porcine cysticercosis is a problem that could be of importance for human health. Proper education for the veterinarians responsible for the meat inspection at the slaughterhouses is of importance, but the common occurrence of home slaughter as shown in this study points to the importance also to educate or at least inform the farmers about the disease. In each household in this study one person responsible for the pigs was interviewed, in total 75.4% of the respondents were women and 24.6% were men. Men were significantly more likely to have heard of cysticercosis, which could suggest that women should be initially targeted to inform about the disease to decrease the risk for cysticercosis.

The prevalence of cysticercosis was higher in this study compared to a previous study conducted in south-central Cambodia where the prevalence among pigs from smallholders was 7.6% (Adenuga *et al.*, 2018). One reason to the higher prevalence may be due to the management systems which purposively selected high-risk areas and farms. In the study by Adenuga *et al.* (2018) there was only one smallholder farm (0.9%, n=115) that had free-roaming pigs, compared to this study where 16 farms (11.5%, n=139) had free roaming pigs and 36 farms (25.9%, n=139) had partly free-roaming pigs. However, porcine cysticercosis was also prevalent among confined pigs that should not have access to human faeces. In this study 8.4% of the confined pigs were positive for cysticercosis, which was a lower prevalence compared to not confined pigs (22.2%). An explanation to this could be because of environmental contamination, which also Braae *et al.* (2015) discuss. Many of the farms that were visited had poultry roaming around free at the farm and it was not uncommon that the poultry entered the pig pens. This could contribute to transmission of the parasite in the stool to the pigs if the poultry e.g. have been walking in the stool and bring stool to the pigs via their feet. Also, piglets were often free roaming and sometimes the pigs at the farms escaped the pens, which can

signify that the pigs that are considered to be confined might have temporarily been free roaming at some time and thus had access to toilets/stool.

In a Tanzanian study, 93% of the pig keepers were aware of porcine cysticercosis but only 23% knew how pigs got infected (Komba *et al.*, 2013). Assana *et al.* (2013) also discuss the situation with cysticercosis in Africa and concludes that most farmers in endemic areas know about the cysts in infected pigs, but few are aware of how pigs get infected. Similar issues could be seen in this study. Most people (89.7%) did not eat meat if cysts could be seen in it, which may imply that they were aware of the risks with cysts in infected pigs. Of the people that had heard of cysticercosis, 99.0% knew pigs can get infected, but only 22.4% of these said they knew how the pigs get infected. It is not certain though that the respondents for sure could explain what the diseases were or how humans and/or pigs can get infected. Only a few of the respondents that did answer 'yes' on those questions did also actually explain with their own words what cysticercosis/trichinellosis were and how humans and/or pigs can get infected. Some respondents might believe they knew but might be mistaken. People that think they know will probably not be as inclined to seek information about the disease compared to the ones that have no knowledge at all. Reaching out with correct information about the disease cysticercosis to everyone could therefore be of importance, as an attempt to avoid misunderstandings and incorrect knowledge about the disease.

The respondents that had heard of cysticercosis showed a significantly (p=0.004) increased tendency to eat raw or undercooked pork meat, although that is a risk factor for infection with *Taenia solium* (Murrell *et al.*, 2005; Ng-Nguyen *et al.*, 2018). This might imply that "heard of" a disease not is a satisfying question to measure the knowledge level among the respondents. Again, it is of importance to reach out with correct information about the disease cysticercosis to the inhabitants to improve human health in Cambodia.

Trichinellosis

The seroprevalence of porcine trichinellosis was 2.5% in this study, but only results from 238 animals could be used. Since the information on the prevalence of trichinellosis in Cambodia is limited, it is not possible to compare these results with others. However, in the neighbouring countries Vietnam and Lao PDR the seroprevalence of porcine trichinellosis has been reported to be 5.6% in Vietnam (Thi *et al.*, 2013) and range between 2.1% to 14.4% in different provinces of Lao PDR (Conlan *et al.*, 2014; Holt *et al.*, 2016). Similar results were found in this study regarding the seroprevalence but also the significant difference between the provinces.

The highest seroprevalence of trichinellosis within a province was in Ratanakiri (4.9%), which was significant (p=0.001) higher compared to the other provinces in the study. Also, the respondent in Ratanakiri showed a significantly (p<0.001) higher tendency to eat pink/red/raw pork meat, which is how humans acquire trichinellosis (Taylor *et al.*, 2007; Gottstein *et al.*, 2009; CDC, 2019d). This is of interest, because even though this study only investigated the seroprevalence of porcine trichinellosis, it can still give us a better understanding of the risks for humans within these provinces. In this study Ratanakiri is a province that can be seen to be at greater risk for trichinellosis, not only among the pigs but also among the humans. Men

showed a significantly increased tendency to eat raw or undercooked pork meat compared to women. Generally, the majority of the respondents were women, but in Ratanakiri almost half of the respondents were men. Since only one of the respondents in Ratanakiri had heard of trichinellosis it could mean that more people in this province are inclined to eat raw or undercooked pork meat. This could be because of men's increased tendency to eat raw or inadequately cooked pork, in combination with low knowledge level among both genders. People with those eating habits are clearly at greater risk when living in a province as Ratanakiri with a higher seroprevalence of porcine trichinellosis compared to humans living in other provinces with a lower seroprevalence.

Only 5.0% of the respondent in the study had heard of the disease trichinellosis, compared to cysticercosis that 75.5% of the respondent had heard of. This might be since viable cysts can be seen in infected cysticercosis pork meat, while infected trichinellosis meat look indifferent for the human eye and can slip by undetected. It is of importance to educate and inform the farmers about trichinellosis and the risk factors for the disease to decrease the risk for porcine and human trichinellosis in the studied provinces.

Although this was a relatively small study, clear differences regarding the seroprevalence of trichinellosis were shown between the four provinces. Similar results were reported in a study in Lao PDR by Conlan *et al.* (2014), where the prevalence of porcine trichinellosis varied significantly by province. One of the provinces in the study was Luang Prabang, which had no positive samples at the time of the study (Conlan *et al.*, 2014). However, in another study by Holt *et al.*, (2016) in the same province (Luang Prabang), 14.4% of the pigs tested positive for trichinellosis. This might indicate that prevalence not only differ between provinces, but also within different regions in the provinces. Further studies in Cambodia are therefore needed to identify high risk areas in the country. By identifying them, interventions can be made to improve both porcine and human health.

One known risk factor for *Trichinella* infection in pigs is the practice of feeding food waste containing raw or inadequately cooked meat to the pigs (Dopuoy-Camet *et al.*, 2007). This practice was recognized as a risk factor for porcine trichinellosis in the present study as well. Ratanakiri was the province with the highest percentage within a province with households that fed the pigs food waste that contained meat, and it was also the province with the highest seroprevalence of trichinellosis. Even though it is not statistically confirmed, it is not un-reasonable to assume that it may have affected the seroprevalence results.

The majority of households in the study (66.2%) had pigs that were confined in pens and those pigs were expected to show a significantly decreased risk for porcine trichinellosis since they were assumed to not be as exposed to rodents and wildlife, which are two other risk factors for trichinellosis (Dopuoy-Camet *et al.*, 2007; Momoh *et al.*, 2013). However, no significant association could be found between management systems and trichinellosis. This could be explained by that even though the pigs were confined in pens, at many farms it did not exclude access for e.g. rodents inside the pens. Many of the pen had holes or gaps that rodents easily could get through (see Figure 7). In that way, pigs could still be exposed to them. Also, because of the situation with African swine fever at the time of the study, many farmers had confined their pigs that previously had been free roaming. It is therefore not certain that the confined

pigs in the study had been confined their whole lifetime and thus they could have been exposed to infected rodents, infected pig carcasses and infected wildlife. Confined pigs might have escaped the pens at some time as well.

Figure 7. *Pigs confined in a pen in Cambodia. Photo by author Rebecca Söderberg.*

Treatment with antiparasitic medicines were shown to be a protective factor for trichinellosis, with significantly (p=0.005) more pigs positive if they had not been treated with antiparasitic medicines. However, for cysticercosis infection no association could be found with antiparasitic treatment. This study did not investigate what kind of antiparasitic medicines that had been used, how the medicines were administrated or dosage, which are factors that probably affected the antiparasitic treatment results. It would be of interest to know what kind of antiparasitic medicines the farmers in this study had used and if treatment with antiparasitic medicines could be an option for porcine cysticercosis as well.

Questionnaire

The questionnaire was translated from English to the official language of Cambodia (Khmer) by a Cambodian veterinary student. Unfortunately, the questionnaire was not pre-tested prior to the study and during the field study it was discovered that a couple of the questions had been misinterpreted by the translator and therefore translated incorrectly. Question C4, C5, D5 and D11 in the questionnaire have therefore been excluded from the results.

Three veterinary students performed the interviews. It cannot be certain that the different interviewers had understood and asked the farmers the questions in the same way. The results could possible differ because of that. The answers to how often the farmers treat their pigs with

antiparasitic medicine (question D14) may have been interpreted differently among the interviewers. The two main answers were one time during a pig's life (48.0%) or every year (33.3%) but could potentially mean the same. This could however also differ depending on what kind of pig production the farmers had. The answers were not always filled in the questionnaire during the interview, some answers have consequently been forgotten and lost. When asking the disease knowledge questions about cysticercosis and trichinellosis and the farmer said they had not heard of the disease, one of the interviewers did explain a bit about the disease before asking the remaining follow-up questions, which could have affected some answers.

Some farmers had problems to remember e.g. how old the pigs were, which constitutes a possible recall bias. However, the majority of the questions were about current habits and situation, recall bias should therefore not be a major error in this study. It is also ineluctable that some farmers might not answered honestly on all questions. Some respondents may have answered based on their perceptions of what is "correct" or socially acceptable instead of actual practices, so-called social desirability bias (Fisher, 1993).

Sampling and lab work

Pigs with signs of sickness and pregnant sows were not sampled, not only to decrease the stress and risk of spontaneous abortion, but also to avoid potential selection bias since sick pigs would have been easier to catch. This might however be another possible selection bias as the pigs with signs of sickness may have had a different parasitic infection status. It is not unreasonable to assume that these pigs are generally more susceptible to infection and would have these parasitic infections to a higher extent compared to heathy pigs. Also, pregnant sows were often older than the other pig groups. Older pigs might have had an increased risk to come in contact with the parasites due to a longer life and this could be another selection bias as well. In this study it was shown that pigs positive to cysticercosis were significantly older. These exclusion criteria may have caused our results to show a lower prevalence than if sampling had been completely random.

Another possible selection bias is the selection of households. The intention was to select households through snowball sampling, but in practice the head of the village often had already located the farms. The contact with the head of the village was managed by the provincial veterinarian, thus it cannot be sure how much the head of the village was informed about the project. This could mean that, depending on how well the head of the village knew the households, the head of the village might have chosen the farms with the 'best' pig management practices, such as households with e.g. confined pigs with no access to stool, or the opposite, households with a pig management that did include several risk factors for the diseases. It would have been better if the snowball sampling had been practiced, but it could not be strictly adhered to in this study area. This might however be of less importance since the province all had less pigs because of the situation of African swine fever, and thus in practice all eligible farms were included.

When doing the ELISA tests for cysticercosis, the first four of the six plates had incorrect mixed positive and negative controls. When this was discovered, the samples with positive results

from these four plates were repeated with corrected positive and negative controls, to make sure that the results were correct.

CONCLUSIONS

In this study positivity for cysticercosis and trichinellosis among pigs in rural Cambodia were 11.2% and 2.5% respectively. The prevalence of the diseases varied significantly by province. Management systems for the pigs and pigs' access to toilet/stool were two risk factors significantly associated with positivity for porcine cysticercosis. Seropositivity for porcine trichinellosis were significantly associated with feeding food waste to the pigs. Also, treatment with antiparasitic medicines was identified as a protective factor for trichinellosis. Some previously demonstrated risk factors for human cysticercosis and trichinellosis were prevalent. Although the present study is relatively small, several different risk factors could be identified for porcine cysticercosis and trichinellosis. The results of this study can be used to give recommendations to improve both porcine but also human health in these provinces, especially in the provinces at higher risk. Further research in the rest of Cambodia would be of interest to get a better understanding of the situation and distribution of these two zoonotic diseases.

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POPULAR SCIENCE SUMMARY

Cysticercosis and trichinellosis are two parasitic diseases that can transmit between pig and human. The two diseases can cause serious illness in humans and human cysticercosis is a leading cause of death from food-borne diseases globally. Both cysticercosis and trichinellosis are widespread in south-eastern Asia, but information regarding the situation in Cambodia is still very limited. Pork is the most importance source of meat in this region and is also an important source of income. In Cambodia, the majority of pigs are raised in the countryside in family backyards with between one and four pigs. This kind of small-scale pig production is often at risk acquiring these two parasites.

Cysticercosis is an infection caused by the larvae of the tapeworm *Taenia solium.* Pigs and humans get infected by swallowing the eggs of the parasite. The eggs are produced by the tapeworm in the human intestine and are spread with the stool. Once ingested by human or pig the larvae spreads throughout the body and creates cysts that can create several different symptoms depending on location. Humans typically get symptoms from the central nervous system such as epilepsy, headache, convulsions and the disease can be fatal in many cases. To get the tapeworm the human must ingest the cysts, typically by eating undercooked pork, but this does not cause cysticercosis, but cause infection with the tapeworm in the human intestine. It is when a human ingests eggs from the tapeworm, they risk acquiring cysticercosis.

Naturally free-ranging pigs with access to human stool are at greater risk to get infected with cysticercosis than confined pigs with no access to human stool. Humans typically get cysticercosis by eating food contaminated with the eggs or when putting contaminated fingers in their mouth.

Trichinellosis is an infection caused by the parasite *Trichinella spiralis*, also known as “the muscle worm”. It can infect most mammals, including pigs and humans. Infection is acquired through eating infected meat. Pigs fed with food waste containing meat are at greater risk of getting the infection. Humans typically acquire the infection when eating raw or undercooked infected pork. Swelling of the eyes is a characteristically symptom for human trichinellosis, also fever, muscle pain and weakness, which last for several weeks.

The present study was carried out in four provinces of north-eastern Cambodia. The purposes of the study were to investigate the spread of cysticercosis and trichinella among pigs on the countryside in Cambodia and identify risk factors for the diseases. Blood samples were collected from in total 242 pigs in 139 households with less than 10 pigs above three months old. For each household one person were asked questions about food and hygiene habits, management of the pigs, disease knowledge and vaccination. The interview was done by a Cambodian veterinary student in Khmer (the official language of Cambodia).

The blood samples were analysed for cysticercosis-antigens (parts of the parasite) and for antibodies for trichinella. If antigen or antibodies are present the pig has been infected. This showed that 11.2% of the sampled pigs had cysticercosis and 2.5% had trichinellosis. Cysticercosis among the pigs were more common in one of the provinces (Preah Vihear), while trichinellosis were more common in another province (Ratanakiri). It was shown that pigs that were free roaming or partly free roaming were more likely to be infected with cysticercosis.

Pigs with access to human stool showed also an increased risk to be infected with cysticercosis. Trichinellosis was most common among the pigs that were fed with food waste. Pigs treated with medicines against parasites were less likely to be infected with trichinellosis. Further, the respondents that had heard of cysticercosis were more commonly men and were also eating undercooked pork meat to a greater degree. The province Ratanakiri and males showed an increased tendency to eat raw or inadequately cooked pork meat.

In summary, several different risk factors could be identified for cysticercosis and trichinellosis among pigs even though this was a relatively small study. The results of this study can be used give recommendations to improve both health for the pigs and the humans in the studied provinces. It would however be of interest with further research on the situation in the rest of Cambodia.

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APPENDICES

Appendix 1 – Questionnaire

Questionnaire/ តារាងបញ្ជីសំនួរ

◯ Used for questions with only one possible answer/ សំរាប់សំនួរជាមួយចម្លើយតែមួយ

☐ Used for questions with more than one possible answer/ សំរាប់សំនួរជាមួយម្លើយច្រើន

Farm no/ លេខកសិដ្ឋាន: …….

No	***A. Household demographics***/ទីតាំងភូមិសាស្ត្រលំនៅដ្ឋាន			
A1	Geographic location ទីតាំងភូមិសាស្ត្រ កន្លែង	Province/ខេត្ត:		
		District/ស្រុក:		
		Commune/ភូមិ:		
		Village/ឃុំ:		
A2	Sex of respondent ភេទអ្នកជួបសំភាស	Female /ស្រី	◯	
		Male/ ប្រុស	◯	
A3	Age of respondent/អាយុ	..... years/ឆ្នាំ		
A4	Highest level of education of respondent/កំរិតនៃការសិក្សាអ្នកជួបសម្ភាស	College/University /មហាវិទ្យាល័យ	◯	
		Upper secondary school/សាលាកំរិតខ្ពស់	◯	
		Lower secondary school/សាលាកំរិតទាប	◯	
		Primary school/បថមសិក្សា	◯	
		No education/អត់បានរៀន	◯	
A5	Number of people living in the household ចំនួនសមាជិកក្នុងគ្រួសារ	Adults/ជំទង់: (15-60 years): …….		
		Children/កូនក្មេង (< 15 years): …….		

		Elderly/មនុស្សចាស់ (> 60 years): …….		
No	***B. Food and hygiene habits***/ការអនាម័យអាហារនិងការរស់នៅ/កន្លែងស្នាក់នៅ			
B1	Do you eat pork meat in the family?តើសមាជិកគ្រួសារអ្នកបរិភោគសាច់ជ្រូកដែររឺទេ?	Yes បាទ/ចាស	◯	
		No/ ទេ	◯	**Go to B8**
B2	If yes, how often? បើបរិភោគ, បរិភោគញឹកញាប់ដែរឬទេ?	Every day /រាល់ថ្ងៃ	◯	
		2-5 times/week/ ២-៥ ដង/សប្តាហ៍	◯	
		Once a week/ មួយសប្តាហ៍ម្តង	◯	
		2-3 times/month/២-៣ ដង/មួយខែ	◯	
		Once a month/មួយខែម្តង	◯	
		Other: …….	◯	
B3	How well cooked is the pork meat that you eat? តើសាច់ជ្រូកដែលអ្នកបរិភោគត្រូវបានចំអិនត្រឹមត្រូវល្អដែររឺទេ?	Uncooked (raw) /ឆៅៗ	☐	
		Cooked (red/pink in the middle) ឆ្អិនខ្លះ អត់ខ្លះ	☐	**Go to B5**
		Cooked (brown/grey throughout) /ឆ្អិនល្អ	☐	
B4	If uncooked, how often do you eat uncooked meat?តើអ្នកបរិភោគសាច់ជ្រូកឆៅញឹកញាប់ដែរឬទេ?	Always /រៀងរាល់ពេលបរិភោគ	◯	
		Often/ញឹកញាប់ដែរ	◯	
		Sometimes/ពេលខ្លះ ម្តងម្កាល	◯	
B5	From where do you get the pork meat that your family consumes?	Own pigs/ សាច់ជ្រូកខ្លួនឯង	☐	
		Neighbors' pigs/ សាច់ជ្រូកអ្នកជិតខាង	☐	

	តើអ្នកទិញសាច់ជ្រូកពីណាមកបរិភោគ?	Market/ទិញនៅទីផ្សារ	☐	
		Other/ផ្សេងៗ: …….	☐	
B6	Have you ever seen cysts in the pork meat when preparing it? *(Picture)* តើអ្នកដែលធ្លាប់ឃើញពងព្រូន(ចុងអង្ករ)នៅក្នុងសាច់ជ្រូកដែលបរិភោគដែររឺទេ?	Yes /បាទ-ចាស	◯	
		No /ទេ	◯	**Go to B8**
B7	If yes, what do you do with the meat?ប្រសិនជាធ្លាប់ តើអ្នកធ្វើយ៉ាងម៉េចជាមួយសាច់ជ្រូក?	Eat it like normal meat/ញុំដូចធម្មតា	◯	
		Cook it extra well /ចំអិនអោយឆ្អិនល្អសិន	◯	
		Cut off the bad part and eat the rest កាត់ផ្នែកដែលមានចុងអង្ករចេញហើយបរិភោគផ្នែកដែលនៅសល់	◯	
		Don't eat any of it/អត់ញុំទាំងអស់	◯	
B8	What kind of toilet do you have? តើអ្នកប្រើប្រាស់បង្គន់ប្រភេទណា?	Flushing toilet connected to sewer បង្គន់អនាម័យទំនើប	◯	
		Pit latrine/បង្គន់ជីករណ្តៅ	◯	
		Bucket toilet/ កន្ទោ	◯	
		Bush/field toilet/ បន្ទបង់តាមវាល	◯	
		Don't have one/អត់មាន	◯	
		Other/ផ្សេងៗ:	◯	
B9	*Observational question:* Can the pigs come in contact with the toilet/stool? តើសត្វជ្រូកអាចមកប៉ះពាល់ផ្ទាល់ជាមួយបង្គន់ រឺលាមកដែររឺទេ?	Yes /បាទ/ចាស	◯	
		No/ ទេ	◯	

		Most of the times/ភាគច្រើន	◯	
		Not so often/មិនញឹកញាប់ទេ	◯	
		No, never/ មិនដែលឃើញសោះ	◯	
B10	Do you and your family wash your hands before eating? តើអ្នកលាងសំអាតដៃរបស់អ្នកមុនពេលបរិភោគដែររឺទេ?	Yes, always/រៀងរាល់ពេល	◯	
		Most of the times/ភាគច្រើន	◯	
		Not so often/មិនញឹកញាប់ដែរ	◯	
		No, never/ មិនដែល	◯	
No	***C. Farm details***/លំអិតពីកសិដ្ឋាន			
C1	Number of pigs (at time of visit) ចំនួនសត្វជ្រូក(កំលុងពេលចុះសិក្សា)	Piglets/កូនជ្រូក (< 1 month):		
		Growers/ជ្រូកជំទង់(1-3 months):		
		Fatteners/ជ្រូកសាច់ (> 3 months):		
		Breeding sows/ជ្រូកមេ:		
		Breeding boars/ជ្រូកបា:		
C2	Pig breed(s) on the farm ពូជជ្រូកនៅក្នុងកសិដ្ឋាន	Indigenous pigs/ពូជក្នុងស្រុក	☐	
		Commercial pigs/ពូជសំរាប់ពាណិជ្ជកម្ម	☐	
C3	Housing system for the pigs ប្រព័ន្ធនៃការចិញ្ចឹមសត្វជ្រូក	Tethered/ ចងក/ជើង	☐	
		Confined in pens/ដាក់ទ្រុង	☐	
		Partly confined/ ពាក់កណ្តាលប្រលេង	☐	

		Free roaming/ ប្រលែងដោយសេរី	☐	**Go to C6**
C4	Which group(s) of pigs are tethered/confined? តើជ្រូកនៅអាយុប៉ុន្មានដែលអ្នកចិញ្ចឹមចង/រឺដាក់ទ្រុង	Piglets/កូនជ្រូក (< 1 month)	☐	
		Growers/ជ្រូកជំទង់ (1-3 months)	☐	
		Fatteners/ជ្រូកសាច់ (> 3 months)	☐	
		Breeding sows/ជ្រូកមេ	☐	
		Breeding boars/ជ្រូកបា	☐	
C5	If partly confined, what time of the day are the pigs kept confined? ប្រសិនបើជាករណីជ្រូកចិញ្ចឹមពាក់កណ្តាលប្រលែង តើពួកគេប្រលែងនៅពេលណា?	Day time/ពេលថ្ងៃ	☐	
		Dusk/ ព្រលប់	☐	
		Night time/ពេលយប់	☐	
		Other/ផ្សេងៗ: …….	☐	
C6	If partly confined/free roaming, during which season do the pigs roam free? ប្រសិនបើជ្រូកចិញ្ចឹមពាក់កណ្តាលប្រលែង រឺដោយសេរី តើក្នុងរដូវមួយណា?	Dry season/រដូវក្តៅ	◯	
		Rainy season/រដូវភ្លៀង	◯	
		Both/រដូវទាំងពីរ	◯	
C7	Do you feed your pigs with kitchen waste or other food waste from markets/restaurants? តើអ្នកចិញ្ចឹមសត្វជ្រូកដោយផ្តល់សំណល់ផ្ទះបាយ រឺក៍សំណល់ផ្សេងៗពីទីផ្សារ/ ភោជនីដ្ឋាន?	Yes/បាទ/ចាស	◯	
		Sometimes /ពេលខ្លះ	◯	
		No/ទេ	◯	**Go to D1**

C8	If yes, does it ever contain meat?ប្រសិនបើមាន តើមានផ្គុកសាច់រឺទេ?	Don't know /អត់ដឹង	◯	
		Yes/បាទ/ចាស	◯	
		No/ទេ	◯	**Go to D1**
C9	Do you cook the food waste before feeding it to the pigs? តើអ្នកចំអិនសំណល់អាហារមុនពេលផ្តល់ទៅអោយសត្វជ្រូកដែរឬទេ?	Yes/បាទ/ចាស	◯	
		Sometimes /ពេលខ្លះ	◯	
		No/ទេ	◯	

No	***D. Disease knowledge and vaccination***/ចំណេះដឹងពីជម្ងឺនឹងការធ្វើវ៉ាក់សាំង			
D1	Have you heard about Trichinellosis? តើអ្នកដែលធ្លាប់ស្គាល់ជំងឺទ្រីស៊ីនែលឡាស៊ីសដែររឺទេ?	Yes /បាទ/ចាស	◯	
		No/ទេ	◯	**Go to D7**
D2	Can you explain what it is? អាចពន្យល់បន្តិចបានទេថាជាអ្វី?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	
D3	Who can get infected? *Don't read options out loud* អ្នកណាខ្លះដែលអាចកើតជំងឺនេះ?	Humans/ មនុស្ស	☐	
		Pigs/សត្វជ្រូក	☐	
		Other animals/សត្វផ្សេងៗ: …….	☐	
D4	*If answering "humans":* *ប្រសិនបើចំលើយថាមនុស្ស* Do you know how people get infected? តើអ្នកដឹងទេថាមនុស្សឆ្លងតាមរបៀបណា?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	

D5	Has anyone in your family or anyone else you know had Trichinellosis? តើមាននរណាម្នាក់ផ្សេងទៀតនៅក្នុងគ្រួសាររបស់អ្នកស្គាល់ពីជំងឺទ្រីស៊ីនែលូស៊ុសដែររឺទេ?	Yes, someone in the family បាទ មានអ្នកផ្សេងនៅក្នុងគ្រួសារ	☐	
		Yes, someone else បាទ មានអ្នកផ្សេងទៀត	☐	
		No/ទេ	◯	
D6	*If answering "pigs":* *ប្រសិនបើចំលើយថាសត្វជ្រូក* Do you know how pigs get infected? តើអ្នកដឹងទេថាសត្វជ្រូកអាចឆ្លងជំងឺតាមរបៀបណា?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	
D7	Have you heard about Cysticercosis? តើអ្នកដែលធ្លាប់ស្គាល់ជំងឺចុងអង្ករ (តេញ)ដែររឺទេ?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	**Go to D13**
D8	Can you explain what it is? តើអ្នកអាចពន្យល់ថាជាអ្វីបានដែររឺទេ	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	
D9	Who can get infected? *Don't read options out loud* អ្នកណាខ្លះដែលអាចកើតជំងឺនេះ? (ស្ងាត់!!!!)	Humans/មនុស្ស	☐	
		Pigs/ជ្រូក	☐	
		Other animals/សត្វផ្សេងៗ: …….	☐	
D10	*If answering "humans":* *ប្រសិនបើចំលើយថាមនុស្ស* Do you know how people get infected?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	

	តើអ្នកដឹងទេថាជំងឺនេះអាចឆ្លងមកមនុស្សតាមរបៀបណា?			
D11	Has anyone in your family or anyone else you know had Cysticercosis? តើមាននរណាម្នាក់ផ្សេងទៀតនៅក្នុងគ្រួសាររបស់អ្នកស្គាល់ពីជំងឺចុងអង្ករ(តេញ៉ា)ដែរឬទេ?	Yes, someone in the family បាទ មានអ្នកផ្សេងនៅក្នុងគ្រួសារ	☐	
		Yes, someone else បាទ មានអ្នកផ្សេងទៀត	☐	
		No/ទេ	◯	
D12	*If answering "pigs":* *ប្រសិនបើចំលើយថាសត្វជ្រូក* Do you know how pigs get infected? តើអ្នកដឹងទេថាសត្វជ្រូកអាចឆ្លងតាមរបៀបណា?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	
D13	Do you treat the pigs with antiparasitic medicines? តើសត្វជ្រូកត្រូវបានប្រើប្រាស់ថ្នាំប្រឆាំងនឹងជំងឺប៉ារ៉ាសិតដែរឬទេ?	Yes/បាទ/ចាស: …….	◯	
		No/ទេ	◯	**Go To D15**
		Don't know/មិនដឹង	◯	
D14	If yes, how often? បើប្រើ ប្រើញឹកពាប់ដែរឬទេ?	Every week/រៀងរាល់សប្តាហ៍	◯	
		Every month/រៀងរាល់ខែ	◯	
		Every year/រាល់ឆ្នាំ	◯	
		Other/ផ្សេងៗ: …….	◯	
No	***G. Animal movement***/ចលនាសត្វ			
G8	Do you slaughter pigs at home?	Yes/បាទ/ចាស: …….	◯	

	តើអ្នកអត្តឃាតសត្វនៅផ្ទះដែរឬទេ?	No/ទេ	◯	**Go to G10**
G9	If yes, what do you do with the meat? បើធ្លាប់ តើអ្នកធ្វើអ្វីជាមួយនឹងសាច់ជ្រូក?	Own consumption/ញុំខ្លួនឯង	☐	
		Sell to others/លក់អោយអ្នកដទៃ	☐	
		Other: …….	☐	