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Porcine Cysticercosis Sero-prevalence and Factors Associated with its Occurrence in Southern Highlands, Tanzania

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ABSTRACT

Porcine cysticercosis is endemic in Tanzania, especially in the Southern Highlands, Central areas and Northern Highlands regions of the country. It is a condition that reduces pork quality and suitability for human consumption and hence affecting public health and the pig industry. The prevalence and possible associated factors for porcine cysticercosis occurrence were investigated to assist in the planning for sustainable control measures. This study was conducted in three wards of Iringa region, southern Tanzania. A blood sample was collected from 346 pigs in 88 households and analysed using an enzyme-linked immunosorbent assay (ELISA), to detect *Taenia solium* antigens (Ag). A questionnaire administered to farmers and an observational checklist tool assisted the researcher in collecting household information on pig management, sanitation and hygiene practices. On average, 22.3±3.44 per cent of pigs was seropositive for porcine cysticercosis in the three wards, and 53.4 per cent of the surveyed households had at least one seropositive pig. Statistically, porcine cysticercosis was associated with the scavenging pig rearing method (OR=2.426; $p \leq 0.05$) and poor quality pig housing (OR=1.75; $p \leq 0.05$). This means that scavenging pigs and those living in poor-quality pens had two times higher chances of being infected. Having more than half studied pig units with positive case(s) indicates limited inputs in pig feeding and sheltering thus exposing them to infection vulnerability. The poor-quality pens allowed pigs to escape and/or scavenge on *T. solium* eggs contaminated environment. Training and sensitising on improved pig management using locally available resources is

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essential. Also emphasising regular latrine use, hygiene and sanitation practices can support control.

Introduction

Pigs, whose population is estimated to be around 2 million, are kept in most parts of Tanzania, mostly under small-scale production or as a household farmyard activity [24]. It has been estimated that 22.4 per cent of livestock-keeping households in both rural and urban areas keep pigs [16]. Pig farming contributes significantly to rural livelihood and is an important source of protein, consumed in the form of pork [1]. Globally, pig production has shown an increasing trend in low-income and middle-income countries and was estimated to reach 60 per cent of the world's pig production by 2020 [42]. However, the pig business encounters various constraints including diseases that limit the expected outputs and expectations of farmers [23]. Porcine cysticercosis hinders pig production by reducing meat quality and creating fear of pork consumption [25]. The cysts in pork destroy pork's wholesomeness and good appearance and put consumers at risk of tapeworm infestation upon ingesting viable cysts in raw or undercooked meat. The viable cysts develop into worms in the human small intestine. Therefore, the infested humans and people living in the same household become vulnerable to cysticercosis, since human tapeworm carriers will shed eggs that may contaminate the environment including food and water. Accidentally, the ingestion of eggs in contaminated food and/or water exposes eggs to penetrating human intestinal mucosa to develop cysts in body tissues.

The causative agent of porcine cysticercosis is *Taenia solium,* a zoonotic parasite which also causes taeniosis and cysticercosis in humans [39]. Humans are definitive hosts, harbouring the adult forms of a parasite in their intestines, whereas pigs are intermediate hosts, harbouring the larval forms (cysts) in muscles and other body tissues [34]. The infection of humans by the larval form is possible through ingestion of *T. solium* eggs from the contaminated environment. The eggs are deposited in the environment by human tapeworm carrier(s), who previously consumed pork with viable cyst(s). Once larvae lodge in the central nervous system, they cause neurocysticercosis [39], which is the cause of up to 30 per cent of epilepsy cases in humans in endemic areas [38]. Porcine cysticercosis is among the poverty-related diseases; it affects farmers who allocate limited resource for pigs, while aiming at earning for household subsistence hence pigs are promoted to scavenge for food where they become exposed to infection. Pigs are infected when feeding on *T. solium* eggs contaminated environment. The disease is common in low-income and middle-income countries with sub-standard pig rearing systems, which promote fewer inputs and allow pigs to roam for their needs, hence they become vulnerable to infestation [2].

In Tanzania, porcine cysticercosis seroprevalence ranges from 1.5 to 33.3 per cent, varying by regional and rearing systems and depending with pig keeping traditional settings [30]. The most famous pig-keeping system is the free-roaming which exposes pigs to feeding on the human faecal contaminated environment. The environment is contaminated with pork tapeworm eggs by humans carrying the adult form of the parasite through indiscriminate defecation [20]. This is among the practices that make allow the high persistent prevalence of porcine cysticercosis in the Southern Highlands zone of Tanzania [30]. The Southern Highlands zone that covers Njombe, Iringa, Mbeya, Songwe and Ruvuma regions, together contributes about 54 per cent of pig production in the country [16]. In general, porcine cysticercosis can be reduced through multiple interventions: Regular animal and meat inspection, deworming of the infected cases, sanitation and good pig husbandry [12]. However, many pig-keeping communities inadequately abide by the given recommendations due to different reasons including lack of knowledge and ignorance. Several studies in Tanzania associate porcine cysticercosis with husbandry practices involving: Free-roaming pig rearing, drinking water from rivers and ponds and feeding pigs with contaminated food residues, among other factors [30]. Different programs have been continuously implemented in different parts of the country to reduce and eventually eradicate the disease.

Among the leading efforts in Tanzania for managing *T. solium* is education, which is focused on enabling farmers to use improved pig housing made from locally available resources, improve pig feeding by preparing the feeds and feeding animals from locally known and well-treated available resources, and also environmental hygiene and sanitation [5,18,30]. Strategically, education is more focused on the endemic communities which are practising free-roaming pig management, human open defecation and poor sanitation and hygiene [29,33]. In embracing the growing information technology, digitally-based education has increasingly been adopted in addition to conventional paper-based and face-to-face methods. Digital health education has been found to hasten knowledge transfer and effective disease control in public health education [31]. The Vicious Worm Educational Tool (VWET) is an example of the digital health education material, which was found effective in training the health and agricultural professionals in the control of *T. solium* in Mbeya region, Tanzania [18].

Monitoring the effectiveness of different methods employed in the efforts of reducing the occurrence of porcine cysticercosis, is an important evaluation step in guiding for improvement and further development. For instance, the effectiveness of digital health education on *T. solium* control in imparting knowledge and stimulating positive change in the community was

investigated in a pilot study in Iringa District [9]. The program reported improving community knowledge of good practices in pig management, sanitation and hygiene towards the control of the *T. solium* parasite [10]. The current study establishes the baseline regarding porcine cysticercosis prevalence and farmers' practices towards control of porcine cysticercosis in the context of testing a digital health education tool on *T. solium* cysticercosis/taeniois.

Materials and methods

Study area

The study was carried out in Iringa District (7^0 46′ 23.1384" S and 35^0 41′ 56.8320" E) in the Southern Highlands of Tanzania. The district occupies 20,576 km^2 at 1600-2700 m above sea level with average temperatures falling below 15°C in cold seasons and with rainfall of up to 704 mm per year. The wet season is from November to May and peaks in January [40]. The estimated population by 2012 was 254,032 [28]. Pigs are predominantly kept under small-scale farming, which contributes to about 14.5 per cent of the pig population in Tanzania [28]. The porcine cysticercosis prevalence estimated by lingual palpation in the area was 7.5 per cent by 2013 [41]. This study was conducted as part of the research activities in an innovation project called Non-Discriminating access for Digital Inclusion (the DigI-project), and carried out in nine villages in three wards (Izazi, Migoli and Mlowa). A digital health education intervention, developed by a team of Tanzanian and international experts, with a multi- and interdisciplinary approach (Holst *et al*., 2021a) was proposed and piloted to help control the disease in the community. The community was provided with free internet access to a digital health education platform that offered educational content for porcine cysticercosis control [9]. Izazi and Migoli wards were selected as interventions, while Mlowa Ward served as a control.

Sampling design

In a cross-sectional study design, samples were gathered between March and May of 2019. The number of pigs to be examined was given as "n," and the formula used to estimate the sample size was, therefore, $n = Z^2PQ/L^2$ [22]. "P" was a known or estimated prevalence of the factor in the study area, "Q" was 1-P, a proportion free of the factor and "L" was an acceptable estimation error [22]. The value of "Z" in this study was 1.96, calculated at a 95 per cent confidence level. The value of "P" was the estimated cysticercosis-infected pigs in a population (33% or 0.33) and "Q" was the proportion of pigs not infected with cysticercosis (1-0.33). The value of "L" was 5 per cent or 0.05. According to the computation, 339 pigs in total were needed, even though the study was able to collect a sample of 346 suitable pigs from the 88 households that were the subject of the study. On average, the studied pigs in Izazi and Migoli were five pigs per household and three pigs per household in Mlowa ward.

With information from ward officers that one household keeps approximately four pigs on average, a random sample of 100 pig-keeping households was selected. The selection was randomized from a list of 423 known pig-keeping households obtained from the ward office. Upon being informed, 88 out of the 100 selected households agreed to participate in the study and 12 did not participate due to lack of time (n = 7) or unavailability when visited or traced by phone call (n = 5).

Data collection

A structured questionnaire that was produced in English, translated to Kiswahili, and then presented as a face-to-face interview guide to respondents in the study households, was pretested by ten experts from Sokoine University of Agriculture. Then from the pretest amendments, households were visited once for the interview that followed with pig blood sampling. In each household, the interviewee was the family head or an adult representative. The basic collected information included: duration and experience in pig rearing, reasons for adopting the pig rearing system and the purpose of pig keeping. Upon permission from the household head or representative, the status of the latrine and pig pen was assessed for the basics as described on quality parameters [6]. The latrine and pig pen quality was scored according to a scaled evaluation chart.

Pig blood sampling

Before sampling, a brief animal history, including animal age and the likelihood of being in gestation for females was gathered from the respondents by using a checklist tool. Then blood samples were taken from all adult pigs, not in gestation and piglets above 2 months of age (weaned). Piglets less than 2 months of age pose difficulty in blood collection due to small blood vessels, but also they have a low chance of having a detectable cyst infestation, as cysts develop in 10 to 12 weeks after infection [32]. Gestating sows were avoided to reduce the chance of stress, which may have adverse results on the pregnancy. A pig was restrained using a pig snare and about 5 ml of blood was collected through the external jugular vein into a plain vacutainer tube as recommended by Swindle, [35]. The collected blood sample was immediately stored in a cool box in an upright position. Samples were then transported to a mini-laboratory at Migoli Ward Dispensary for centrifugation and serum harvest. The harvested serum samples were kept in a 2ml cryogenic tube per pig and temporarily stored at -20°C in Migoli, before they were transported to Sokoine University of Agriculture for further storage and analysis.

Ethical consideration and consent to participate

This research was approved by Sokoine University of Agriculture (Ref. No. SUA/ADM/R.1/8/316) and the Tanzania National Institute for Medical Research (Ref. No. NIMR/HQ/R.8a/Vol. IX/2947). Participants verbally consented to participate after being informed of the purpose and scope of the study. For confidentiality and data protection, an identification number was assigned to each household instead of names. Permission to carry out the study was also provided by the district, ward and village government authorities of Iringa District Council (Ref. No. FA.255/265/01/PART 'C;/72).

Data analysis

Each completed questionnaire transcript was assigned an identification code and its data, together with the related pig samples data were entered into a Microsoft Excel spreadsheet.

To get pig sample data, the frozen serum samples were thawed at room temperature for 30 min. and analyzed for *T. solium* antigens using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Dorny *et al.,* 2004). The pre-treated control and serum samples were added to the B158C11A10 monoclonal antibody-coated plate wells, which were then incubated. The positive samples were detected by absorbance read at the presented intense blue colour that turned yellow with 0.5M H_2SO_4. The circulating cysticerci antigen (excretory-secretory products glycoprotein) in the serum were bound to B158C11A10 monoclonal antibody (IgG isotype)-coated in plate wells. The bound-in wells of the kit form an antigen-antibody complex. The complexes become detected with specific peroxidase-conjugated B60H8A4 monoclonal antibodies that are added with a coloured chromogen solution (containing tetramethylbenzidine and hydrogen peroxidase) to bind the complex. Antigen index was calculated using optic density read at an absorbance of 450nm. Then antigen index values $\leq$0.8 and values $\geq$ 1.3 were deemed positive and negative, respectively, while values in between the two were considered doubtful and re-analyzed.

The frequency and distribution of cysticercosis positive samples were generated in the Microsoft Excel spreadsheet program, which was then incorporated with the questionnaire collected data. The association between disease prevalence and household factors was analysed using regression analysis in the Statistical Package for Social Sciences (SPSS) version 20 (Armonk, New York).

Results

Studied households and pig populations

According to Table 1, the distribution of respondents in the studied households among the three wards was 17, 38 and 45 per cent for Izazi, Migoli and Mlowa, respectively. The proportion of males and females among the respondents was 68.5 and 31.8 per cent respectively. Out of the surveyed households, 75 (85.2%) of them had pig pens while 13 (14.8%) households were practising free-roaming pig keeping.

The total number of pigs sampled from the three wards was 346, distributed as 70 (20%), 164 (47%), and 112 (33%) from Izazi, Migoli and Mlowa Wards, respectively. Taking care of the inclusion criteria that pigs for sampling were required to be at the age of above two months and non-gravid for females, 65 per cent of the sampled pigs were males and that13.9 per cent were pigs left to scavenge in the daytime and the rest were kept in pig pens.

Pig houses and human sanitation facilities

In all the households, there was at least a single pig pen used to confine the pigs either in the daytime or both day and night. Good as well as poor quality pig houses were observed in the study area. Poor quality pens were the ones characterized by weak walls, rough and wet muddy floors, but also lacking good drinkers, shade and enough space to accommodate the pigs. Overall, 168 (48.6%) pigs were kept in pig pens that qualified to be of good quality. Comparatively, Migoli Ward had a higher proportion (56.7%) of pigs kept in good-quality pig pens when compared to the other two wards, whereby 35.7 and 44.6 per cent of pigs for Izazi and Mlowa, respectively were kept in good-quality pig pens ($p \leq 0.05$). Of the pig pens, 84 per cent were old, meaning they were built five or more years ago, the rest were new pig pens. None of the pigs in Izazi was kept in new pig pens; the proportion was significantly lower ($p = 0.0233$) compared to the other wards, which were

Table 1
Studied households' respondents and pig populations

Respondent particulars	Gender		Ward of residence			Has the household had a pig pen?	
Number (n=88)	Male	Female	Izazi	Migoli	Mlowa	Yes	No
Percentage (%)	68.5	31.8	17.0	37.5	45.5	85.2	14.8
Animals particulars	**Animal sex**		**Animals from**			**Animal housed (day time)**	
Number (n=346)	Males	Females	Izazi	Migoli	Mlowa	Indoor	Scavenging
Percentage (%)	65	35	20.2	47.4	32.4	86.1	13.9

Table 2
Surveyed households and porcine cysticercosis status among three wards

Particular		Ward			X^2 analysis *(p-value)*
		Izazi	Migoli	Mlowa	
Number of households surveyed		15	33	40	-
Number of pigs sampled		70	164	112	-
The average number of pigs per household		5	5	3	-
Number of pigs in daytime					
	Indoor	49	142	107	< 0.0001*
	Scavenging (outdoor)	21	22	5	
Number of pigs kept in					
	Good quality pen	25	93	50	0.0079*
	Poor quality pen	45	71	62	
Number of pigs kept in					
	New pig pens	0	17	15	0.0233*
	Old pig pens	70	147	97	
Seropositive pig n (%)		18 (26)	35 (21)	24 (21)	0.7380
Households with at least one seropositive pig n (%)		11 (73.3)	16 (48.5)	20 (50.0)	0.2344

Table 3
Pig situations associated with porcine cysticercosis seropositivity

Pigs situation	Percentage seropositive (n = 77)	Odds ratio (Range)	p-value
Housing system			
Confined (n = 311)	20.6	2.43 (1.20 - 5.30)	0.026*
Scavenging (n = 35)	37.1		
Pigpen Quality kept in			
Good (n = 168)	18.5	1.75 (1.02 - 2.99)	0.042*
Poor (n = 178)	25.8		
Kept with farming experience			
>6 months (n = 324)	22.2	0.87 (0.47 - 3.91)	0.956
<6 months (n = 22)	22.7		

* indicates significant difference between situations

10.4 and 13.4 per cent in Migoli and Mlowa Wards respectively. Free-roaming pigs were observed around some households, including the one that reported raising their pigs in confinement during the daytime. It was also observed that latrines without closable doors entertained roaming pigs to scavenge. In most families, hand washing facilities made of locally available water containers were installed at latrine entrances. However, a notable four household latrines and almost all, 98 per cent of hand washing facilities indicated not being used regularly.

Sero-prevalence of Porcine Cysticercosis

The results in Table 2 indicate that 13.9 per cent of the pigs were kept under the scavenging system in the daytime and 86.1 per cent were full-time confined. In Izazi, 30 per cent of the sampled pigs lived by scavenging in the daytime, which is a significantly higher proportion ($p < 0.0001$) than the ones for pigs kept that way in Migoli (13.4%) and Mlowa (4.5%). About 77 (22.3%) out of the 346 tested pigs were seropositive for cysticercosis infection and 53.4 per cent of the household had at least one pig that tested positive. In both the seroprevalence status and the presence of at least one positive pig in a household, there were no significant differences between the three wards at a 95 per cent confidence level.

Prevalence of Porcine Cysticercosis in relation to pig raising practices

Among the 77 seropositive, 18 were scavenging in the daytime and inclosed in pens at night (Table 3) and 59 were full-time confined. The proportion of seropositivity among scavenging pigs was 37.5 per cent as compared to 19.7 per cent among the confined pigs. The comparative risk of testing positive was therefore 2.43 times higher in scavenging pigs than in confined pigs. The difference in the risk of infection between the scavenging and the confining pigs, was significant ($p = 0.026$) when the Odds Ratio was calculated. Similarly, pigs kept in low-quality pens had a higher risk of infection compared to pigs raised in good-quality pens (p=0.042). The relative experience of the farmers in keeping pigs did not pose a significant risk of pigs testing positive for cysticercosis.

Discussion

The results of this study showed that porcine cysticercosis sero-prevalence was within the previously reported range in the endemic regions of the southern highlands of Tanzania [30]. In 2013, the disease was reported to have a 7.5 per cent prevalence by lingual palpation method (Yoana *et al*., 2013), which is a less sensitive examination method compared to ELISA used in the current study. The reported 22.3 per cent of seroprevalence gives a clue that the disease is still endemic in the studied areas, comparable to the previously reported status in Mbeya and Ruvuma regions [3,33]. The endemic state indicates access of pigs to *T. solium* eggs from human faeces which is common in communities where improper use or lack of toilets is prevalent and pigs are allowed to scavenge or are raised in poor-quality pens [4]. Few farmers were reported to raise their pigs in a free-range system. However, pig houses in many households are poorly made, indicating that pigs kept indoors might still get a chance of escaping and roaming and hence increasing their vulnerability to infection. The household infection rate is greater than the overall prevalence, with more than half (53.4%) of the households with at least one pig infected. This is probably because pigs do not contract the infection from another infected pig directly.

Generally, sociocultural practices and lifestyles have been identified as key factors for *T. solium* epidemiology [13]. The reported and observed free-roaming pigs in the study area represents the community practice that increases the chance of pig infection [17,33]. The high infection rate among pigs is also suggestive of a high human infection rate [34,39]. The environment lacking sanitation and regular use of latrine exposes free-roaming pigs to *T. solium* eggs from infected human faeces [8].

Raising pigs in the scavenging system is among the characteristics of small-scale pig farming in some parts of Tanzania and is mostly perpetrated by low budget allocation due to low investment capital [14]. In addition, small-scale pig farming is mostly practised by low-income and illiterate families who may also fail to accommodate good quality latrine costs and often practice open defecation in the bushes [15,36]. It has been reported by the UN Social and Economic Council that only 15 per cent of the people in rural areas of Tanzania use improved sanitation facilities and up to 8 per cent practice open defecation [37]. The situation is propagated by illiteracy, age factors, rurality and poverty [21] and remains among the important factors in addressing the persistent and spread of porcine cysticercosis and human taeniosis.

Controlling porcine cysticercosis and human taeniosis can be achieved if the community is educated and enabled to practice good pig husbandry and human sanitation practices [26,27,29]. This study reports a moderate prevalence of porcine cysticercosis. However, when weighed against previous and recent studies [19,33], the reported prevalence and infection rate suggest the disease is persistent. The notable prevalence and infection rate and the suggestive persistent trend of porcine cysticercosis in the area are probably dependent on the observed pig rearing and human sanitation practices. Being one of the countries with the lowest levels of access to sanitation, Tanzania, through the government and non-government organs has engaged in extensive national sanitation and hygiene programs that have shown promising results in the targeted regions [7]. Advocating for the installation of improved sanitation facilities should go hand in hand with campaigning on the importance of their proper use. The presence of non-used sanitation facilities in this study was evidenced by latrines with no faecal matter in the pits, while some had dry floors with widespread cobwebs in containment and no clear footpath.

The findings of this study suggest that strong educational efforts should be directed toward pig farmers. Strategic health education on pig management, safety pork consumption, sanitation and hygiene is necessary for the control of *T. solium* and other hygiene and food-borne diseases. Pieces of training and sensitization on proper latrine use can promote latrine ownership and regular use. Digital health education is suggested to be included in knowledge dissemination due to the demonstrated efficacyin knowledge uptake in recent trials [10,18].

Conclusion and recommendation

Over half of the studied pig keeping household units were prevalent for porcine cysticercosis. This indicates that they may practice poor pig management which exposes them to infection. The study also demonstrates pigs to be raised poorly in terms of feeding and good sheltering which exposes them to infection vulnerability. The studied poor-quality pens allowed pigs to escape from total confinement, and the free-roaming pigs were infected with *T. solium* eggs through feeding in a contaminated environment. The studied communities need training and sensitization on improved pig management using locally available resources. Regular latrine use, hygiene and sanitation practices are essential in emphasizing and supporting disease control. Further studies are recommended to establish ways of incorporating the proper use of improved sanitation facilities in the cultural practices and mindsets of rural communities. Digital health education tools may represent an innovative way to share appropriate and tailored knowledge with affected communities.

Study limitations

The Ag-ELISA test for porcine cysticercosis was reported to be only 82.7 per cent sensitive and 86.3 per cent specific [11]. In pigs, the assay test may cross-react with *Taenia hydatigena* and *Taenia asiatica* if the pig is infected with these *Taenia* species although the latter has not been reported in Tanzania. The exclusion of pigs in gestation and piglets may have slightly affected randomization in sampling.

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Authors' contributions

FK performed all research activities: conception and design of the study, manuscript development and response to reviewers' comments. BJM moderated the idea conceptualization, supervision and manuscript revision. CH moderated the structured questionnaire and observation checklist. BN and FS were the fieldwork technical facilitators and provide inputs on research material and revised the manuscript. JN and AW improved the research idea, provided research material and manuscript revision and HN performed all supervision works and manuscript revisions.

Declaration of Competing Interest

The authors declared no competing interests. All authors have been approved for the final article submission.

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