1 Feedstuff and poor latrines may put pigs at risk of cysticercosis – a case-control study

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12
13 **Abstract**

14 Attempts to control *Taenia solium* in low-income countries have been unsuccessful or
15 unsustainable. This could indicate a ‘missing link’ in our understanding of the transmission
16 dynamics of the parasite and possibly the magnitude of environmental contamination. We aimed to
17 identify risk factors associated with porcine cysticercosis using a case-control study design, utilising
18 known information on persistent or multiple infections of porcine cysticercosis. The study, a
19 combination of questionnaire interviews and observational surveys, was conducted in July 2014 in
20 the two districts Mbeya and Mbozi, Tanzania. Study households were identified based on their
21 status regarding porcine cysticercosis prevalence and allocated into cases or controls based on
22 previous porcine cysticercosis presence. This resulted in 43 farmers in the case group and 50
23 farmers in the control group, from 20 villages. Potato peels were said to be given to pigs either raw

or boiled by 46% of the farmers. Based on logistic regression porcine cysticercosis could be associated with absence or a completely open latrine ($p=0.035$, OR 5.98, CI: 1.33-43.02) compared to an enclosed latrine. Feeding potato peels to pigs was also associated with increased risk of infection ($p=0.007$, OR 3.45, CI: 1.43-8.79). Logistic analysis including the pig management system indicated pigs kept in elevated pens ($p=0.049$, OR 5.33, CI: 1.08-32.27) and on a dirt floor ($p=0.041$, OR 9.87, CI: 1.29-114.55) were more likely to be infected compared to a cemented floor. Whether potato peels are contaminated with *Taenia* eggs before they reach the household or whether the contamination is from water or dirty hands during the process of peeling, remains to be confirmed. This study suggests that detailed assessment of a number of areas of pig management is essential for designing effective control programmes.

Keywords: *Taenia solium*; Porcine cysticercosis; Transmission; Risk factors; *Taenia* eggs; Environmental contamination

1. Introduction

The zoonotic tapeworm *Taenia solium* causing taeniosis/cysticercosis is a serious threat to both the public health and agricultural sector of countries where the parasite is endemic (Karesh et al., 2012). Attempts to control *T. solium* in developing countries have so far been unsuccessful or unsustainable, although a number of interventions including health education (Ngowi et al., 2008), treatment of humans (Sarti et al., 2000), treatment of pigs (Pondja et al., 2012), vaccination of pigs (Molinari et al., 1997), and treatment of pigs and humans combined (Garcia et al., 2006), have been trialled. Despite these attempts to break the life cycle and control the parasite, *T. solium* remains highly prevalent in many low and middle income countries. Failure to control *T. solium* could

indicate a ‘missing link’ in our understanding of the transmission dynamics and perhaps an underestimation of the effect of environmental contamination in the transmission of this parasite. Known risk factors for porcine cysticercosis have been identified to be insufficient number or usage of latrines (Ngowi et al., 2004), free-range production systems (Komba et al., 2013; Pondja et al., 2010), keeping pigs in elevated pens (Komba et al., 2013), increasing age (Komba et al., 2013; Pondja et al., 2010), and source of water (Komba et al., 2013). Increased risk with certain water sources indicates that some types of water sources could be contaminated with *Taenia* eggs, but studies confirming water contamination with *T. solium* eggs are still missing.

Free-range allowing pigs access to human faeces, appear to be the most prominent risk factor for porcine cysticercosis. However, recent data have shown that confinement of pigs can be insufficient in preventing transmission, indicating that pigs might become infected while being confined (Braae et al., 2014). We therefore hypothesised that pigs are infected with porcine cysticercosis through environmental contamination while being confined. To explore this, we used a case-control design, utilising known information on persistent or multiple infections of porcine cysticercosis in pigs, to identify risk factors responsible for transmission of *T. solium* from humans to pigs relating to human behaviour.

2. Methods

2.1 Study area

The study was conducted in July 2014 in the two districts Mbeya and Mbozi in Tanzania. Both districts are rural areas where pig production is almost exclusively on a smallholder level, with 31,190 pigs in Mbeya district and 117,483 pigs in Mbozi district according to the 2007/2008 census (URT, 2012), making it the most important pig producing region in Tanzania.

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71 **2.2 Study design and sample size**

72 Households were identified based on participation in a previous study investigating porcine
73 cysticercosis prevalence using Ag-ELISA (Brandt et al., 1992) at three time points over an
74 approximate period of 14 months among all pig keeping households from 22 villages (Braae et al.,
75 2014). Households were allocated into two different groups – case and control. Households
76 allocated to the control group had been visited at least twice during the 14-month period with no
77 cysticercosis positive pigs detected. Households allocated to the case group had one or more
78 cysticercosis positive pigs on at least two occasions during the 14-month period. Households that
79 had only been visited once (n=959) or had one or more positive pigs on only one occasion (n=82)
80 were excluded from the study. This resulted in 107 households from 20 villages, 10 villages from
81 each of the two districts, identified as suitable candidates for the study.

82

83 **2.3 Data collection**

84 Data collection comprised of a questionnaire survey and an observational survey. The questionnaire
85 was double blinded and interviews were performed by the same person and administered to a
86 member of each household (preferably the person responsible for the pigs) present at the time of
87 visit. The questionnaire was constructed to capture information about household characteristics,
88 drinking water, sanitation, pork consumption, pig production, and pig management. The
89 observational survey gathered information on latrine presence and usage, and presence and type of
90 pig pen. Latrines were categories based on whether pigs could gain access to the latrine; (1)
91 completely enclosed (latrine with a locked door and no entry points for pigs), (2) partially enclosed

(latrine with door, but not closed and pigs could possibly enter), and (3) latrine missing or open (door or latrine missing, easy access for pigs).

2.4 Ethical considerations

Permission to conduct the study was sought through Sokoine University of Agriculture in Morogoro, Tanzania, and regional, district, and local village authorities. Oral and written informed consent was also sought from all participating farmers after they were informed about the aim, risks, and benefits of the study. After the questionnaire had been administered the farmers were informed about ways to improve their pig management. The study was approved by the Imperial College Research Ethics Committee (ICREC), reference no. ICREC_11_3_6.

2.5 Statistical methods and analysis

Data were analysed in the statistical programme R (<http://www.r-project.org>). Univariate comparison of the groups was done using Student's *t*-test, Chi² test, or Fishers Exact test, depending on the variables. Logistic regression was performed with case-control as the dependent variable to control for association with the independent variables in a multivariate analysis. Odds ratios (OR) were calculated for all significant independent variables in the logistic regression models.

3. Results

From the 107 identified households, farmers participated from 93 households covering 20 villages in the survey area. Hence, fourteen farmers did not participate, 11 were unavailable at the time of visit, one declined to participate, one had moved to a neighbouring district, and one had passed

away. This resulted in 43 farmers in the case group and 50 farmers in the control group. There was no significant difference between the two groups in terms of household characteristics (Table 1).

Confinement of pigs was practised throughout the year by the majority of farmers according to their own statements (Table 2). However, more than half of the farmers said they left piglets to roam. Neither the production system ($p=0.81$) or farmers attitude towards letting their piglets roam ($p=0.51$) differed significantly between cases and control. Maize bran was the most common source of food within the area, but kitchen waste was used as pig feed by 91% of the farmers, and potato peels were said to be given to pigs by 46% of the farmers. Significant more farmers among the cases gave potato peels to their pigs compared with the controls (Chi^2 , $p=0.019$). Potato peels were given to the pigs either raw or boiled. There was no significant difference between the two groups in terms of giving boiled potato peels (Chi^2 , $p=0.91$), but more farmers from the cases group gave raw potato peels compared with farmers from the control group (Chi^2 , $p=0.067$). Potatoes were harvested from local fields and night soil was not commonly used in the region. However, the plough used for weeding and harvest is sometimes also used to dispose of children's faeces if defecation has taken place indiscriminately close to the household. Once harvested, potatoes, if moist, are laid out on the ground to dry within the household compound in close proximity to the latrine, before being stored in large bags. When peeled it is either done dry or wet, with the water leftover used for livestock.

All farmers, with the exception of one, had latrines near their houses. The types of latrine differed significantly between cases and controls (Fishers exact test, $p=0.043$). Most latrines had signs of recent usage, with no significant difference between the two groups (Chi^2 , $p=0.486$). Pigs were more likely to be kept in elevated pens if they belonged to the cases group (Chi^2 , $p=0.019$) and type

of flooring within the pens also differed significantly between cases and controls (Fishers exact test, $p=0.024$).

Multivariate logistic regression associated the absence/open type latrines ($p=0.035$, OR 5.98, CI: 1.33- 43.02) compared with completely enclosed latrines and the use of potato peels as feeds for pigs ($p=0.007$, OR 3.45, CI: 1.43-8.79) compared to none provided, as risk factors for porcine cysticercosis. The same model, but inclusive of boiled and raw potato peels, revealed that both raw ($p=0.011$, OR 7.76, CI: 1.80-46.05) and boiled ($p= 0.049$, OR 2.65 CI: 1.02-7.23) potato peels were risk factors for disease compared to no potato peels given. Pen variables were not included in the logistic regression above as it would eliminate a large proportion of the data due to missing information (households with no pen at the time of visit = 21). However, based on logistic regression including management parameters, pigs kept in elevated pens ($p=0.049$, OR 5.33, CI: 1.08-32.27) and on a dirt floor ($p=0.041$, OR 9.87, CI: 1.29-114.55) were more likely to be infected with porcine cysticercosis compared to a cemented floor when still controlling for the effect of the latrines and potato peel consumption. No interaction between feeds and latrines could be found using logistic regression, and no other independent variables were of significant importance.

4. Discussion

Confinement of pigs has shown not to decrease the risk of porcine cysticercosis within this research area (Braae et al., 2014). This could result from environmental contamination contributing to the transmission of *T. solium* to pigs. In this study, potato peels fed to pigs were found to be associated with porcine cysticercosis, both when given raw or boiled. This suggests contamination of the potato peels with *Taenia* eggs. Evidence point to *Taenia* eggs being inactivated at approximately 60°C, but gelatinization of starch from the potatoes might act as an insulator protecting the eggs

during cooking (Buttar et al., 2013). Therefore, potato peels containing eggs may yield some contribution to transmission even though they have been boiled. Whether potato peels actually contain *Taenia* eggs when harvested from the ground, contaminated when handled extensively by a tapeworm carrier such as when being peeled, or contaminated when put in water containing *Taenia* eggs, is speculative. Further investigation is needed to confirm the presence of eggs on potato peels within the *T. solium* endemic area, and if present, exactly where contamination takes place. In support of potatoes being contaminated on the fields before harvest, Yoder et al. (1994) found associations between cattle being fed potato by-products and infection with *T. saginata* in the US. Studies of *T. solium* epidemiology have included examination for *Taenia* eggs in soil samples collected near locations known to have *T. solium* tapeworm carriers to investigate environmental contamination and reports of finding *Taenia* eggs have occurred (Edia-Asuke et al., 2014; Gwebu et al., 2010). However, more studies based on detailed systematic collection strategies of soil samples and description of the quantities of soil examined, are needed. Contaminated feedstuff could explain why relatively many pigs within the research area are infected (Braae et al., 2014), although they are primarily kept confined.

Free-ranging of pigs is considered a major risk factor for porcine cysticercosis, but previous findings from the area suggested that production system might not be the only important factor for transmission under this particular setting (Braae et al., 2014). It was not possible to associate production system or whether or not farmers let piglets roam with infection in this study. However, presence of an open latrine was associated with infection regardless of production system used. Absence or very poor latrine facilities have been reported previously as a risk factor for porcine cysticercosis in Tanzania (Ngowi et al., 2004). Farmers' consumption habits of pork could not be associated with porcine cysticercosis infection in the pigs in this study. However cooking habits may be a more important risk indicator than consumption. *Taenia solium* is still widely prevalent

despite concerted attempts to break the life cycle of the parasite. The findings presented here should be taken into consideration when designing control programmes against *T. solium*. In order to block transmission to pigs, changes to both pig production and management systems will need to be implemented. Just assuring total confinement of pigs will be unlikely to be sufficient to stop transmission when it takes place within the pig pen. The association found here between porcine cysticercosis infection and earthed flooring and raised flooring also suggest that there is still something we are missing in terms of farmer management practises that relates to infection of porcine cysticercosis and therefore of importance in the control of porcine cysticercosis.

Unfortunately, information on the type of pen was unavailable from a large proportion of households, resulting in rather large confidence intervals. Caution should therefore be taken when interpreting the results relating to the type of pen used.

The study was also limited by the assumption that farmers had not changed husbandry practises between the initial blood sampling to the administration of the questionnaire and the observational survey. The results obtained in this study are strengthened by the case-control design, which is unique for porcine cysticercosis related surveys. In addition, there were no demographical difference or household characteristics that differed significantly between the case-control groups, minimising bias relating to these factors. Further studies are needed to determine whether certain feedstuff like potato peels and other tubers are contaminated on the fields or when being handled during peeling and washing.

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261 **7. Table legends**

262 **Table 1:** Comparison of household characteristics of cases and controls based on questionnaire
 263 data. P values are obtained from univariate analysis.

264
 265 **Table 2:** Household information on pig management based on questionnaire data. P values are
 266 obtained from univariate analysis.

Table 1: Comparison of household characteristics of cases and controls based on questionnaire data. P values are obtained from univariate analysis.

	Cases	Controls	p value
Participants (%)	43 (46)	50 (54)	
District (%)			0.3034 ^a
Mbeya	27 (52)	25 (48)	
Mbozi	16 (39)	25 (61)	
Sex (%)			0.2361 ^a
Male	26 (53)	23 (47)	
Female	17 (39)	27 (61)	
Mean age of the respondents (±SD)	40 (16)	42 (14)	0.5153 ^b
Is the respondent caretaker of the pigs			
Yes	39 (45)	48 (55)	0.4098 ^c
No	4 (67)	2 (33)	
Average number of adults within households (±SD)	3.1 (1.6)	3.3 (1.4)	0.4788 ^b
Average number of children within households (±SD)	2.6 (1.9)	2.4 (1.3)	0.5796 ^b
Education of the respondents (%)			0.9394 ^c
None	7 (50)	7 (50)	
Primary	32 (47)	36 (53)	
Secondary	4 (40)	6 (60)	
College	0 (0)	1 (100)	
Highest education within the households (%)			0.4246 ^c
None	2 (67)	1 (33)	
Primary	23 (53)	20 (47)	
Secondary	16 (42)	22 (58)	
High School	0 (0)	2 (100)	
College	2 (29)	5 (71)	
Type of latrine present at household (%)			0.0429 ^a
Completely enclosed	2 (18)	9 (82)	
Partially enclosed	3 (27)	8 (73)	
Open	38 (54)	33 (46)	

^a Chi² test

^b t-test

^c Fishers exact test

Table 2: Household information on pig management based on questionnaire data. P values are obtained from univariate analysis.

	Cases	Controls	p value
Pig production system used throughout the year (%) ^a			0.8071 ^b
Confinement	33 (45)	41 (55)	
Semi-confinement	5 (50)	5 (50)	
Free-range	5 (56)	4 (44)	
Leave piglets to roam (%) ^c			0.5133 ^d
Yes	21 (48)	23 (52)	
No	12 (38)	20 (63)	
Feed potato peels to the pigs (%)			0.0191 ^d
Yes	26 (60)	17 (40)	
No	17 (34)	33 (66)	
Feed boiled potato peels to the pigs (%)			0.3391 ^d
Yes	17 (55)	14 (45)	
No	26 (42)	36 (58)	
Feed raw potato peels to the pigs (%)			0.0671 ^d
Yes	9 (75)	3 (25)	
No	34 (42)	47 (58)	
Pen elevated if present (%)			0.0191 ^d
Yes	19 (61)	12 (39)	
No	13 (31)	29 (69)	
Type of flooring in pens (%)			0.0244 ^b
Cement	2 (14)	12 (86)	
Soil	6 (40)	9 (60)	
Wood	24 (56)	19 (44)	

^a The three production types practised during the different seasons have been ranked to one type as follows: Free-range, Semi-confinement, and Confinement

^b Fishers exact test

^c Of the farmers stated to keep piglets at any one time

^d Chi² test