Acta Tropica xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Sero-prevalence of *Taenia Solium* cysticercosis in rural and urban smallholder pig production settings in Uganda

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ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 12 January 2016
Accepted 15 January 2016
Available online xxx

Keywords:
Pig
Sero-prevalence
Taenia solium
Cysticercosis
Rural and urban production settings
Uganda

ABSTRACT

The pork tapeworm, *Taenia solium*, is prevalent in Uganda although the prevalence has not been determined in all areas of the country. A cross-sectional study, to determine the sero-prevalence of the parasite in pigs kept under rural and urban production settings, was carried out in three Ugandan districts, Masaka, Mukono and Kamuli. Serum samples from 1185 pigs were tested for the presence of *T. solium* cysticercosis antigen using the HP10 antigen-ELISA (Ag-ELISA) and the ApDia Ag-ELISA assays. Using parallel interpretation of the two tests showed lower levels of observed prevalence of *T. solium* in rural production settings (10.8%) compared to urban (17.1%). Additionally, Maximum Likelihood Estimation for evaluating assays in the absence of a gold standard, using TAGS on the R platform, estimated the true sero-prevalence to be lower in rural production setting, 0.0% [0.0–3.2%; 95% confidence interval (CI)] than in urban production setting, 12.3% (4.2–77.5% CI). When the sensitivity/specificity (Se/Sp) of the assays were estimated, assuming conditional independence of the tests, HP10 Ag-ELISA was more sensitive and specific [(Se = 53.9%; 10.1–100% CI), (Sp = 97.0%; 95.9-100% CI)] than the ApDia assay [(Se = 20.2%; 1.5–47.7% CI), (Sp = 92.2%; 90.5–93.9% CI)]. Subject to parasitological verification, these results indicate there may be a need to implement appropriate control measures for *T. solium* in the study areas.

1. Introduction

Taenia solium neurocysticercosis is considered a serious neglected, public health concern particularly in areas with poor standards of sanitation, public health and inappropriate animal husbandry practices (Secka et al., 2010; WHO, 2014). The pig is the primary intermediate host (porcine cysticercosis) and humans are the definitive hosts (taeniasis) (Soulsby, 1982). Dogs can also act as intermediate hosts (Ito et al., 2002), as can humans leading to human cysticercosis/neurocysticercosis; the latter being the leading cause of late onset epilepsy in pig-keeping communities in the developing countries (WHO, 2013).

In recent years the pig population in Uganda has grown (>15%), with an estimated total population of over 3.2 million pigs in 2008 (MAAIF, 2011). Factors including; their high fecundity and conversion rate, their early maturity, short generation interval and minimal space requirements, have made pigs an important source of livelihood for over 1.1 million resource-poor farmers in the rural and peri-urban communities as well as some urban centers in Uganda (Ouma et al., 2014a,b). This growth has resulted from increased demand for pork and pork products by consumers, with the consumption *per capita* of pork in Uganda being estimated at 3.4 kg/person/year (FAOSTAT, 2014). Various studies have associated growth in pig production and pork consumption in developing countries with increasing prevalence of *T. solium* cysticercosis, especially in pigs under poor management (Assana et al., 2010; García et al., 2003; Mwape et al., 2012; Praet et al., 2010). The parasite is known to be endemic in areas of Uganda (Nsadha et al., 2014; Waiswa et al., 2009).

In urban production settings in Uganda, pigs are commonly kept in corrals whereas pigs in rural areas are kept under extensive

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http://dx.doi.org/10.1016/j.actatropica.2016.01.016
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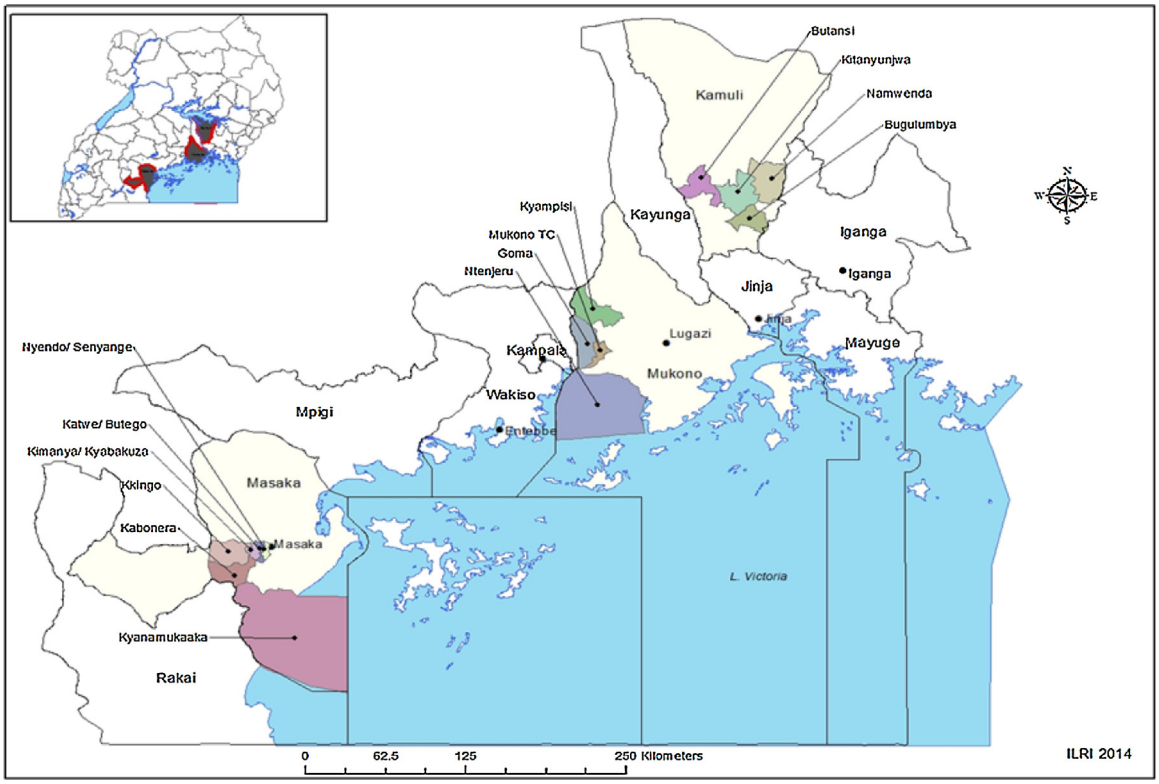

Fig. 1. A map of Uganda showing Masaka, Mukono and Kamuli districts and study areas within these.

management systems (Dione et al., 2014). The later system may promote parasite transmission (Eshitera et al., 2012; Pondja et al., 2010).

The main objective of this study was to determine and compare the sero-prevalence of *T. solium* cysticercosis in the rural and urban smallholder pig production settings of three districts in Uganda, where there was little previous knowledge regarding prevalence of the parasite.

The study has also demonstrated the use of the Maximum Livelihood Estimation (MLE) method to estimate prevalence, sensitivity and specificity in the absence a gold standard diagnostic test (Dohoo et al., 2009).

2. Materials and methods

2.1. Site selection

A cross-sectional survey was conducted from April to August 2013 in Masaka, Mukono and Kamuli districts of Uganda by the Smallholder Pig Value Chain Development (SPVCD) project and its partners (Ouma et al., 2014a,b). The selection of participating sites utilised a geographical targeting protocol to prioritise location based on their pig population density, human poverty levels and proximity to urban centers. Details of the site selection process was described elsewhere (Dione et al., 2015).

In order that we might compare rural and urban production settings, value chain domains that were identified by Ouma et al. (2014a,b) as rural production for rural consumption; and rural production for urban consumption were classified as "rural" and urban/peri-urban production for urban consumption was classified as "urban" production. Note that Kamuli district was not considered to include any form of urban production setting. Fig. 1 shows the districts and sub-counties where pigs were sampled.

2.2. Sample size determination

The original sample size was calculated to estimate district-level prevalence of undefined diseases, i.e., not specifically for this study, and assuming an infinite population (no recent census data) using the formula adopted from Thrusfield (2007) as follows: $n = [Z^2P(1\text{-}P)]/d^2$ Where: n is the required sample size; Z is the multiplier from a standard normal distribution (1.96) at a probability level of 0.05; *P* is the estimated prevalence which is most conservatively estimated to be 50% considering that there is no reliable prevalence data for *T. solium* cysticercosis in pigs in two of the districts under study (Mukono and Masaka) and d is the desired precision for the estimate (+/− 5%). A sample size of 384 pigs was required for the study in each district. A sample size of 384 pigs was required for the study in each district; in the field, a total of 375, 408, and 402 pigs were sampled in Masaka, Kamuli and Mukono, respectively. The actual number of pigs sampled in Masaka was lower than the required sample size because of the harsh working conditions experienced due to heavy rain. Post-hoc power calculations for this study, using Stata 10.0 (Stata Corp, LP USA) indicated a power of 75% to show significantly different prevalence levels between rural and urban production systems at the 5% level of significance. The study observed prevalence of 10.8% (100/927) in rural and 17.1% (44/258) in urban settings, implying that the selected sample size was reasonable. However, this power has not been adjusted for potential intra-cluster (village) correlation.

2.3. Household and pig selection

A list of all pig keeping households was generated by local partners in each village. The study households were then randomly selected using computer-generated random numbers. Apparently healthy pigs over three months of age were selected. Pregnant sows or sows with litters under two months old were excluded. One

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Table 1
Overall pig population and sample sizes in the surveyed villages.

Value-chain domain	District	Village	Number of households	Number of pigs (*N*)	Number of pigs sampled (*n*)
U-U	Masaka	Kyabakuza-B	74	357	36
		Kijjabwemi	90	265	45
		Senyange A	69	219	51
R-U		Kisoso	88	351	47
		Butego	63	217	30
		Kanoni-Bukunda	131	385	49
		Kyamuyimbwa-Kikalala	102	312	39
R-R		Ssenya	76	240	37
		Lukindu	54	226	41
Sub-total			971	3258	375
R-R	Kamuli	Butabala	84	191	40
		Ntansi	136	314	111
		Kantu	120	320	113
		Bukyonza	60	86	29
		Baluboinewa	62	113	46
		Isingo B	100	213	69
Sub-total			636	1336	408
U-U	Mukono	Jogo	103	684	68
		Kitete	63	379	58
R-U		Kyoga	80	153	56
		Dundu	69	224	61
R-R		Kazo/Kalagala	85	272	59
		Bugoye/Kabira	91	261	51
		Nsanja/Gonve	89	266	49
Sub-total			893	4232	402
Total			2503	8826	1185

R-R: Rural production for rural consumption; R-U: Rural production for urban consumption; U-U: Urban production for urban consumption.

pig fulfilling the inclusion criteria was randomly selected for blood collection.

2.4. Collection of blood samples

Pigs were restrained using a catcher and bled from the anterior vena cava using BDVaccutainer® needles (gauge 19) and BDVacutainer® plain tubes (10 ml). The blood samples were kept standing in an ice box at +4 °C to ensure no hemolysis occurred while in the field. At the laboratory, blood was centrifuged to separate serum from blood clot. Serum was harvested into barcoded 2 ml vials that were stored at −20 °C until processing.

2.5. Serological analysis

Serological analysis for *T. solium* cysticercosis antigen was carried out at the International Livestock Research Institute (ILRI) laboratories in Nairobi, Kenya employing the HP10Ag-ELISA (Harrison et al., 1989) with some modifications to that described by Krecek et al., 2008, 2011 and, the commercially available B158C11A10/B60H8A4 Ag-ELISA (apDIA Cysticercosis) following the manufacturer's instructions (ApDia n.v, 2004).

These tests detect the secretory and excretory products of viable cysticerci (Alcobedes et al., 2010). Although some authors reported having detected antigens in a pig with only one viable cyst, assay efficiency is positively relayed to cyst burden (Rodriguez-Hidalgo et al., 2006).

The cut-off values for the two assays differed, with that for the Ap-Dia assay (3.5 × mean ratio >1.3) being much more stringent than the HP10 Ag-ELISA [>(negative control mean + 3 standard deviation)]

The different cut-off calculations, potentially could have had an effect, on the overall results and therefore a comparison was made of the sero-prevalence values of HP10 and ApDia assays applying both cut-off determinations to both sets of assay results. This may have been the underlying cause of obtaining different results when the two ELISA tests are performed on the same samples (Krecek et al., 2008, 2011).

2.6. Statistical analyses

A McNemar chi-square test was used to evaluation the correlation between the proportion of positive results for HP10 and ApDia assays and a simple comparison of number of positive tests was used to estimate the level of independence of the tests. The two assays are not 'gold standard', as they do not have perfect specificity (i.e., Sp = 1) or sensitivity (i.e., Se = 1) (Enøe et al., 2000). In the absence of this gold standard test, Maximum Likelihood Estimation (MLE) (Dohoo et al., 2009), which can be used if at least two populations (rural and urban) have differing prevalence, was carried out using TAGS program on the R platform (Pouillot et al., 2002) to estimate and compare the sero-prevalence of *T. solium* cysticercosis in pigs in the rural and urban production settings as well as the sensitivity and specificity of the assays, with bootstrapped 95% confidence intervals (1000 samples), assuming conditional independence for the tests.

2.7. Ethical considerations

Ethical approval was granted by the Research and Ethics Committee of the College of Veterinary Medicine, Animal Resources and Biosciences of Makerere University (Reference No: VAB/REC/13/104) and by the Ugandan National Council for Science and Technology (UNCST) (Reference Number: HS1477). All farmers signed a consent form to participate in the study and to allow their pigs to be bled (Table 1).

3. Results

The number of positive and negative results of HP10 and ApDia Ag-ELISA assays and hence the observed (apparent) prevalence of *T. solium* cysticercosis for each assay in the two production settings, rural and urban, and overall is shown in Table 2.

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Table 2
The observed sero-prevalence of *Taenia solium* infection in pigs kept in rural and urban settings as determined by the HP10 and ApDia Ag-ELISAs.

Ag-ELISA result (+/−)		Production setting		
HP10	ApDia	Rural	Urban	Overall
−	−	827	214	1041
+	−	72	20	92
−	+	28	20	48
+	+	0	4	4
Total samples		927	258	1185
Observed prevalence (HP10)		72/927 = 7.8%	24/258 = 9.3%	96/1185 = 8.1%
Observed prevalence (B158/B60)		28/927 = 3.0%	24/258 = 9.3%	52/1185 = 4.4%

Table 3
Contingency Table indicting the level of agreement in the observed (apparent) sero-prevalences between the HP10 and ApDia Ag-ELISAs.

ApDia	HP10		
	Positive (+)	Negative (−)	Overall
Positive (+)	4	48	52
Negative (−)	92	1041	1133
Overall	96	1089	1185

Table 4
Apparent sero-prevalence of *Taenia solium* cysticercois in rural and urban settings for the districts of Kamuli, Masaka and Mukono when the results of the HP10 and ApDia Ag-ELISA's were interpreted in parallel.

District	Production setting (positive/total samples)		
	Rural	Urban	Overall
Kamuli	(55/408) 13.5%	–	(55/408) 13.5%
Masaka	(20/243) 8.2%	(24/132) 18.2%	(44/375) 11.7%
Mukono	(25/276) 9.1%	(20/126) 15.9%	(45/402) 11.2%
Overall	(100/927) 10.8%	(44/258) 17.1%	(144/1185) 12.2%

The observed (apparent) prevalence in the rural production setting is lower than in urban setting for both tests. However, the level of agreement in the tests appears to be low (Table 3) with 96/1185 (8.1%) positive by HP10 Ag-ELISA but only 52/1185 (4.4%) positive by ApDia Ag-ELISA and the significant McNemar chi-square test result confirms this ($\chi^2 = 13.83$, $p < 0.001$).

Additionally, the number of samples testing positive in both tests (=4) equals the expected number of positives if the tests are independent ($8.1\% \times 4.4\% \times 1185 = 4.2$) and while recognizing this evidence is not overwhelming, because of low numbers of positive observations, for the remainder of the analysis the two tests are considered to be conditionally independent. This conditional independence implied that we had to interpret the two tests in parallel, i.e., a sample was considered 'positive' if positive by either the ApDia or HP10 ELISA test.

Table 4 summarizes the apparent prevalence, interpreting the two tests in parallel (i.e., a sample was considered positive if positive in either or both of the two assays), of the infection in districts with rural and urban production settings. Similar to individual test interpretation (Table 2) the apparent prevalence in rural areas (10.8%) is lower than in urban areas (17.1%) although all estimates are higher overall because of the change in interpretation.

The breakdown by district is presented here to recognize that Kamuli district samples came only from the rural production setting and represent partial confounding of production setting with district. This may cause the comparison of rural with urban production to be overly influenced by the absence of the Kamuli urban production setting. Given the apparent prevalence in rural areas is highest in Kamuli (13.5%) it is most likely to be raising the overall rural estimate and hence representing prevalence of *T. solium* cysticercosis in more remote rural location because of the absence of the urban production setting as opposed to a rural setting closer to urban settings seen in Masaka and Mukono.

Table 6
Comparison of the observed (apparent) sero-prevalence using the HP10 and ApDia Ag-ELISA's and applying the HP10 and ApDia Ag-ELISA cut-off values.

Type of test	Cut-off applied for test	
	ApDia (3.5 x mean ratio >1.3)	HP10 (>negative control mean + 3 std. dev.)
ApDia	52/1185 (4.4%)	66/1185 (5.6%)
HP10	32/1185 (2.7%)	96/1185 (8.1%)

Maximum Likelihood Estimation (MLE) of both test results in both rural and urban production settings provide the estimates of true *T. solium* cysticercosis prevalence, sensitivity and specificity of the two tests (Table 5). Assuming conditional independence of the tests and applying the same parallel interpretation to the MLE results gives a combined sensitivity (Se) estimate of 63.2% (Se1 + Se2)–(Se1 × Se2) and specificity (Sp) of 89.4% (Sp1 × Sp2).

Given the concerns regarding Kamuli we attempted to run the MLE analysis without including this district, unfortunately the number of positive results was then too small to provide any sensible estimates of prevalence, sensitivity or specificity. However, applying the combined Se and Sp estimates to apparent prevalence (AP) of *T. solium* cysticercosis in rural production settings would provide a true prevalence (TP) of 5.5% in Kamuli (TP = AP + Sp-1/Se + Sp-1) and zero in Masaka and Mukono. Hence, even with the inclusion of Kamuli potentially raising the estimated true prevalence in rural areas, the confidence intervals for rural and urban production setting prevalence do not overlap.

Finally, although the findings of the two assays were different based on the specified protocols described here, this could be due to the different methods used to calculate cut-offs. Table 6 shows a comparison of the sero-prevalence values when the same cut-off calculation criteria were used for both tests. The cut-off calculation for ApDia (3.5 × mean ratio >1.3) was more stringent than the one for HP10. The application of the ApDia cut-off would result in a reduction of HP10 positives of 64 (96 down to 32), whereas the

Table 5
Estimation of true *Taenia solium* cysticercosis prevalence (%), sensitivity and specificity in rural and urban production settings by Maximum Likelihood Estimation (MLE).

	Prevalence in rural	Prevalence in urban	HP10 specificity	HP10 sensitivity	ApDia specificity	ApDia sensitivity
Estimate (%)	0.0	12.3	97.0	53.9	92.2	20.2
95% confidence interval	0.0–3.2	4.2–77.5	95.9–100	10.1–100	90.5–93.9	1.5–47.7

Note: test results were assumed to be independent conditional on infection or disease status and have constant sensitivity and specificity in all populations.

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HP10 cut-off would increase the ApDia positives by 14 (52 up to 66).

4. Discussion

This was the first cross-sectional survey of *T. solium* cysticercosis conducted on a large representative sample of pigs in Uganda, thus adding more information on previous studies (Waiswa et al., 2009; Nsadha et al., 2014). It is also the first study directly comparing *T. solium* infection in pigs reared in the rural versus urban settings.

The overall apparent sero-prevalence (12.2%) reported here was lower than previous reports (25.7%) by Nsadha et al. (2014) in Lake Kyoga basin. However, that study was based on a smaller sample size and the selected study sites were potentially high *T. solium* cysticercosis risk areas near the shore of Lake Kyoga and characterized by factors such as scavenging of pigs, open-air defecation.

Pigs kept under extensive management systems are prone to parasite infections/infestations (Eshitera et al., 2012; Pondja et al., 2010), as transmission risks are greater. Therefore, it might reasonably have been expected that the estimated sero-prevalence would have been higher in the rural setting where the pigs were kept either extensively, tethered or free range. In fact the reverse was observed in this study, both apparent and true prevalence estimates were lower in rural than urban setting where pigs were managed intensively. It is possible that this could be due to contaminated feeds or water given to pigs.

This study estimated the true sero-prevalence of *T. solium* cysticercosis, sensitivity and specificity of the HP10 and ApDia tests using MLE; including whole carcass cysticerci was not possible since dissection of pigs for whole carcass was not done due to financial constraints (Ngowi, et al., 2004). MLE was then selected as the method of analysis as opposed to the Bayesian statistical approach previously employed by Ngowi et al. (2004) and Krecek et al. (2008, 2011) to estimate true prevalence in absence of a gold standard, because of the low number of positives, lack of agreement between the two tests and absence of informative prior information.

The results indicated that the sensitivity for the HP10 Ag-ELISA was higher than that of the ApDia Ag-ELISA and that both tests do not detect the same antigen. It was suggested that this may be due to differences in the secretory and excretory products detected by the two assays, which are based on different monoclonal antibody reagents (Krecek et al., 2008, 2011). Variations in the findings of the two Ag-ELISA tests could also be due, to some extent, to differences in the cut-off calculation methods.

The study had some limitations as previously highlighted. One of these was the absence of an urban production setting in Kamuli which introduced partial confounding into the prevalence estimates.

The ELISA tests used here have been reported to cross-react with *T. hydatigena* which occurs in Uganda, but has only been reported in goats and sheep (Nyakarahuka, 2011; Venkata et al., 2012).

In conclusion, the serological evidence presented here indicates that *T. solium* cysticercosis may be present in the three districts examined in this study with the sero-prevalence being higher in urban rather than rural settings. Further parasitological confirmation, either through slaughterhouse studies or detailed post-mortem examination of pigs should be done in order to determine the true extent of the problem and verify that the parasite is present. The possible prevalence of this parasite in any population is a matter of concern, prompting further action with a view to instigating control measures. The HP10 Ag-ELISA was more sensitive and specific than the ApDia assay but both assays proved useful tools. In Uganda, uncontrolled slaughtering of pigs is still common practice. There is need to intensify awareness about the condition and deworming programs targeting both pigs and humans in the whole country regardless of the pig production setting. Strict inspection of pork and increased awareness on the importance of proper cooking of pork are needed to minimize transmission.

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgments

We thank the Smallholder Pig Value Chain Development project funded by IFAD-EU which is part of the CGIAR Consortium Research Program (CRP) on Livestock and Fish, as well as the CRP on Agriculture for Nutrition and Health; the German Academic Exchange Services (DAAD) and the Safe Food-Fair Food Project (GIZ funded) for their financial support. We are also indebted to Phil Toye, Eric Fevre, Lian Thomas, Alice Kiyonga, Velma Kivali, Evalyn Njiri and Alice Njeri for their technical support in the laboratory analysis of serum samples at ILRI. To all the ILRI-Uganda team, District veterinary staff and all participants that made field work possible. We thank Dr R.M.E. Parkhouse, Institute Gulbenkian de Ciencias, Oeiras, Portugal for supplying HP10 Ag-ELISA reagents. Thanks also to Tim Strugnell from the Veterinary Laboratories Agency (UK) for supplying the negative control sera for HP10-ELISA assay. Finally to Ian R. Dohoo, Professor Emeritus-Epidemiology, Department of Health Management, Atlantic Veterinary College University of P.E.I, Canada for technical advice on statistical analysis of the work.

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Please cite this article in press as: Kungu, J.M., et al., Sero-prevalence of *Taenia Solium* cysticercosis in rural and urban smallholder pig production settings in Uganda. Acta Trop. (2016), http://dx.doi.org/10.1016/j.actatropica.2016.01.016