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***Taenia solium* porcine cysticercosis in Madagascar: comparison of immuno-diagnostic techniques and estimation of the prevalence in pork carcasses traded in Antananarivo city**

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ABSTRACT

Taenia solium cysticercosis was reported in official veterinary and medical statistics to be highly prevalent in pigs and humans in Madagascar, but few estimates are available for pigs. This study aimed to estimate the seroprevalence of porcine cysticercosis among pigs slaughtered in Antananarivo abattoirs. Firstly, the diagnostic performance of two antigen-ELISA techniques (B158B60 Ag-ELISA and HP10 Ag-ELISA) and an immunoblotting method were compared with meat inspection procedures on a sample of pigs suspected to be infected with (group 1; n=250) or free of (group 2; n=250) *T. solium* based on direct veterinary inspection in Madagascar. Sensitivity and specificity of the different tests were then estimated using a Bayesian approach for detection of porcine cysticercosis in the absence of a gold standard. Then, a third set of pig sera (group 3, n=250) was randomly

collected in Antananarivo slaughterhouses and tested to estimate the overall prevalence of *T. solium* contamination in pork meat traded in Antananarivo.

The antigen ELISAs showed a high sensitivity (>84%), but the B158B60 Ag-ELISA appeared to be more specific than the HP10 Ag-ELISA (model 1: 95% vs 74%; model 2: 87% vs 71%). The overall prevalence of porcine cysticercosis in Antananarivo slaughterhouses was estimated at 2.3% (95% credibility interval [95%CrI]: 0.09–9.1%) to 2.6% (95%CrI: 0.1–10.3%) depending on the model and priors used. Since the sample used in this study is not representative of the national pig population, village-based surveys and longitudinal monitoring at slaughter are needed to better estimate the overall prevalence, geographical patterns and main risk factors for *T. solium* contamination, in order to improve control policies.

Keywords: *Taenia solium; cysticercosis; immunodiagnostic; Enzyme-linked immunoelectrotransfer blot; ELISA; pigs; Madagascar*

1. Introduction

Taenia solium cysticercosis is a neglected parasitic disease involving humans and pigs and is endemic in developing countries where pigs roam freely and scavenge human feces around villages (Torgerson, 2013). *T. solium* cysticercosis was reported to be highly prevalent in humans and pigs in Madagascar, with seroprevalences of cysticercosis in humans ranging from 7% to 21% in the 1990s and 7% to 48% in pigs (Andriantsimahavandy et al., 1997; Andriantsimahavandy et al., 2003; Michelet et al., 2010; Rasamoelina-Andriamanivo et al., 2013; Ribot and Coulanges, 1988). Cysticercosis has been described in other islands in the Indian Ocean, in particular in La Réunion during the 1990s (Michault et al., 1990; Michault et al., 1989).

Treatment of cysticercosis in humans is problematic, as the subsequent inflammatory response can be harmful for the patient. To reduce the need for treatment, prophylaxis should be improved through mass screening, treatment of adult-worm carriers and control of cysticercosis in pigs (Boussard et al., 2012). For this reason, continuous efforts are being made to develop rapid and efficient diagnostic tests, and evaluations of the performance of laboratory techniques for the detection of *T. solium* in humans are regularly reported (Carod et al., 2012; Deckers and Dorny, 2010; Hernandez et al., 2000; Hubert et al., 1999; Prasad et al., 2008; Simac et al., 1995; Villota et al., 2003). Several methods have been previously described to detect antibodies to *T. solium* infections in humans and in pigs, such as radioimmunoassay, hemagglutination, the complement fixation test, dipstick assay, latex agglutination, enzyme-linked immunosorbent assay (ELISA) and immunoblot techniques (Deckers and Dorny, 2010). These assays measure exposure to the parasite. In contrast, the ELISAs which have been developed to detect parasite antigens (Ag) circulating in the host demonstrate the presence of the living parasite. Such Ag-ELISAs have also been trialed in

both humans and pigs (Deckers and Dorny, 2010; Rodriguez et al., 2012; Sciutto et al., 1998).

In developing countries, the routine diagnosis of porcine cysticercosis in pigs is based (i) for live animals on lingual palpation that is efficient only when moderate to heavy infection occurs in individual animals (da Silva et al., 2012; Phiri et al., 2006), and (ii) for carcasses on visual postmortem and incisional examination during veterinary inspection at abattoirs.

Although several of the laboratory diagnostic techniques described above have been used to estimate the prevalence of the zoonotic *T. solium* cysts in pigs, the interpretation of test results can be difficult, especially in detecting cysticercosis in pigs with low levels of cysts (Dorny et al., 2004, Krecek et al., 2008, Krecek et al., 2012, Ramahefarisoa et al., 2010, and Sciutto et al., 1998b).

In the present study, we aimed to determine the diagnostic performance of different tests for detection of porcine cysticercosis in the absence of a gold standard and to estimate the prevalence of cysticercosis in pigs slaughtered in Antananarivo, Madagascar.

1. Materials and methods

1.1. Serum sample collection

From April to December 2010, blood was collected from pigs in the four main slaughterhouses in Antananarivo city, the capital of Madagascar, namely Ampasika, Ankadindratombo, Anosipatrana, and Anosizato. Information regarding sampling date, slaughterhouse, region of origin, breed, sex and age was recorded for each animal. Blood was sampled from the jugular vein directly into plain BD Vacutainer® tubes and allowed to clot at 4°C. Serum was obtained by centrifugation, dispensed into 2 ml aliquots, stored in labeled vials and kept at -80°C until shipped on dry ice for testing.

A total of 750 blood samples were collected from pigs raised in 11 different regions (out of a

total 22) in Madagascar. Samples were split into three groups: group 1 samples (n=250) came from animals considered to be infected based on visual inspection, group 2 (n=250) consisted of samples from animals considered free from infection based on absence of lesions on visual inspection, and group 3 consisted of blood samples (n=250) randomly collected from slaughtered pigs in November and December 2010.

1.2. Examination of pigs

The *T. solium* cysticercosis status of carcasses was determined by an extensive visual postmortem and incisional examination according to the local meat inspection regulations (Phiri et al., 2006; Phiri et al., 2002). Heart, masseters, diaphragm, and tongue were visually examined. Long and parallel incisions were made in external and internal masseter muscles. The tongue was palpated and a longitudinal incision was made at the base of the tongue to check for cysts. The heart was cut open to detect cysts in the septum (Boa et al., 2002). No information was recorded about the number of larvae in muscles making the investigation of the infection intensity impossible. The cysticerci stages, i.e. viable or degenerated, were not registered. Only the location of cysticerci lesions for animals considered in group 1 were registered. In group 1, cysticerci lesions were observed in limbs (100%), pork shoulder (49.6%), masseter (12.4%) , tongue (39.6%), heart and pericardium (5.2 %), as predilection sites. Cysticerci were reported in only one location (limbs) in 30.4% (n=76) of pigs in group 1.

1.3. Serological tests

Sera in groups 1 and 2 were analysed using three serological tests. Enzyme-linked immunoelectrotransfer blot (EITB) analysis was carried out using the Cysticercosis Western Blot Kit (LDBio Diagnostics, Lyon, France) according to the manufacturer's instructions. This test was considered positive if the pig serum detected at least two specific bands. Two different Ag-ELISAs were also used. ELISAs were performed in a single test and positive

samples were confirmed by duplicate test. The first was the Cysticercosis Ag-ELISA (ApDia Ltd., Turnhout, Belgium), which makes use of the B158C11A10 and B60H8A4 monoclonal antibodies to detect circulating antigens released by viable cysticerci (Brandt et al., 1992; Draelants et al., 1995). The assay was carried out according to the manufacturer's instructions, the optical density (OD) was read at 450 nm and the Ag index was calculated as described. The cut-offs recommended by the manufacturer were used, where an Ag index less than 0.8 was considered a negative result, an Ag index greater than 1.3 was classified as a positive result and values in between were considered "doubtful". The manufacturer reports that upon testing 99 animals infected with viable cysticerci of *Taenia* species using the B158B60 Ag-ELISA, all gave positive results. Based on repeated testing of 300 negative porcine samples, the manufacturer claims a specificity of 99.6% in diagnosis of porcine cysticercosis (Dorny et al., 2004; Nguekam et al., 2003). The second Ag-ELISA detects a metacestode antigen using the HP10 monoclonal antibody (Harrison et al., 1989), and was carried out according to the method described by Sciutto et al. (Sciutto et al., 1998). In this case an OD greater than 0.177 was considered a positive result, an OD less than 0.129 was classified as a negative result and ODs in between were considered "doubtful results". This latter Ag-ELISA was also used to screen the group 3 sera. All ELISAs were performed once and all positive samples were retested to confirm results.

1.4. *Statistical methods*

As a first step the diagnostic performance of the three immunodiagnostic tests was determined using carcass visual and incisional examination as the "gold standard". In addition, receiver operator characteristic (ROC) curve analysis was performed. "Doubtful results" were removed from the dataset (8 for HP10 Ag-ELISA and 1 for B158B60 Ag-ELISA). The statistical analysis was carried out in R v3.0.3 (R development core team, 2008)

using the caret and pROC packages.

However, carcass inspection is not a true gold standard for validation of diagnostic tests for porcine cysticercosis unless complete carcass dissection and enumeration of cysts is carried out, which is rarely logistically and economically feasible. Thus, a Bayesian approach (Markov chain Monte Carlo [MCMC] simulation with Gibbs sampling) was adopted to estimate test sensitivity and specificity in the absence of a gold standard (Berkvens et al., 2006; Branscum et al., 2005). To maximise the number of samples with complete test results, EITB results were excluded from the analysis. Data from groups 1 and 2 on carcass inspection and the two Ag-ELISAs were included in the analysis (n=117). As both ELISAs detect circulating parasite antigens, an assumption of conditional dependence between these two tests was made and two co-variance parameters were included in the model (Branscum, 2005). In contrast, carcass inspection was assumed to be conditionally independent of both ELISAs due to a biologically different outcome being measured (i.e. visible pathology rather than antigen). An initial model was constructed which included prior information about sensitivities and specificities of the three tests. The BetaBuster software (<http://www.epi.ucdavis.edu/diagnostictests/betabuster.html>) was employed to calculate beta (α , β) distributions based on published estimates (see Table 2). Two models (Models 1 and 2) were run using two sets of priors for the sensitivity and specificity of B158B60 Ag-ELISA, based on two different studies conducted in Africa (Dorny et al., 2004; Krecek et al., 2011). The priors for the other diagnostic tests were the same in the two models.

The Bayesian models were run using the WinBUGS software (v14) (Lunn et al., 2000). An initial burn-in of 5,000 iterations was discarded, and followed by 50,000 further iterations. The median and 95% credibility intervals of the posterior distributions of the parameters of interest were obtained using MCMC with Gibbs sampling. Model convergence was assessed

by running five chains simultaneously and visually inspecting time-series plots for each parameter. Models were validated by comparing the number of parameters estimated by the model (pD) and the Deviance Information Criterion (DIC) values calculated in the posterior mean of the multinomial probabilities and in the posterior mean of the parameters of the model (Berkvens et al., 2006). After running the initial models, a sensitivity analysis was carried out by replacing the informative priors with non-informative priors or partially informative priors. In the latter case, prior beta distributions were substituted with uniform (a, b) distributions. The two parameters, a and b , which are the minimum and maximum values of the random variable, were defined according tests and models: $a=0.5, b=1$ for HP10 Ag-ELISA sensitivity, B158B60 Ag-ELISA sensitivity (Model 1) and specificity and carcass inspection specificity; $a=0.4, b=0.9$ for HP10 Ag-ELISA specificity and B158B60 Ag-ELISA sensitivity (Model 2); $a=0, b=0.5$ for sensitivity of carcass inspection.

The true prevalence of porcine cysticercosis in pork carcasses slaughtered and retailed in Antananarivo was then estimated using a Bayesian approach based on the apparent prevalence determined through testing of sera from the group 3 pigs with the HP10 Ag-ELISA (<http://www.epi.ucdavis.edu/diagnostictests/aptoprev.html>). The sensitivity and specificity estimates for the HP10 Ag-ELISA from Models 1 and 2 were used to generate informative Beta priors. Models were run in WinBUGS as described above and median values and 95% credibility intervals for the true prevalence were estimated.

3. Results

Diagnostic test results for pigs which were deemed positive and negative for cysticercosis based on carcass visual and incisional examination are summarized in Table 2. In group 1, carcasses, cysticerci lesions were observed in limbs (100%), pork shoulder (49.6%), masseter (12.4%), tongue (39.6%), heart and pericardium (5.2%), as predilection sites. Cysticerci were

reported in only one location (limbs) in 30.4% (n = 76) of pigs in group 1.

EITB results were obtained for 108 pigs (64 in group 1 and 44 in group 2), B158B60 Ag-ELISA results for 145 pigs (128 in group 1 and 17 in group 2) and HP10 Ag-ELISA results for 288 pigs (159 in group 1, and 129 in group 2). HP10 Ag-ELISA results were also obtained for 175 pigs in group 3 (Table 2).

When diagnostic performance was assessed using visual and incisional examination of carcass as the “gold standard”, all three immunodiagnostic tests showed a high sensitivity (>90%). However, the HP10 Ag-ELISA was less specific than EITB and B158B60 Ag-ELISA (see Table 3). When ROC curve analysis was carried out, the area under the curve (AUC) was 0.916 for the EITB, 0.971 for the B158B60 Ag-ELISA, but 0.802 for the HP10. Of the 117 pigs for which complete results were available for three diagnostic tests (carcass visual and incisional examination, B158B60Ag-ELISA and HP10 Ag-ELISA), there was full agreement between all three tests in 111 cases (94.8%). The cross-classified results of the three tests are presented in Table 4. This dataset was used to estimate the sensitivity and specificity of the three diagnostic tests using Bayesian analysis. The results are summarized in Table 5. Similar estimates of diagnostic test performance were generated by the two models based on different prior distributions for the sensitivity and specificity of B158B60 Ag-ELISA. The median estimates of sensitivity and specificity for the B158B60 Ag-ELISA were somewhat higher for model 1 than for model 2. The B158B60 Ag-ELISA was the most sensitive test overall and also showed a high specificity. Visual inspection was very highly specific but showed a lower sensitivity, whereas the HP10 Ag-ELISA was the least specific of the three tests.

When sensitivity analysis was carried out using non-informative or partially informative priors, all median estimates of test sensitivity and specificity fell within 8% of the original values, except for the specificity of the HP10 Ag-ELISA in Model 1, which increased by

12% with a non-informative prior, and the sensitivity of the B158B60 Ag-ELISA in Model 2, which increased by 12% with a non-informative prior. The sensitivity of the visual and incisional inspection of carcasses increased by 46% with a non-informative prior and decreased by 22–26% with a partially informative prior.

Of the 175 pigs in group 3 tested with the HP10 Ag-ELISA, 19 were positive. Based on this result and the estimates of the diagnostic performance of this ELISA reported above, the prevalence of porcine cysticercosis was estimated as 2.3% (95% credibility interval [CrI]: 0.09–9.1%) if results from Model 1 were used to generate priors, and 2.6% (CrI: 0.1–10.3%) if results from Model 2 were used to generate priors.

4. Discussion

The aim of this study was to evaluate the performance of different diagnostic tests for porcine cysticercosis in Madagascar using samples collected from pigs upon slaughter and to estimate the prevalence of porcine cysticercosis among pigs slaughtered in Antananarivo, Madagascar. Since there can be variation in performance of diagnostic tests in different locations and populations, it is important to validate tests in the area in which they will be used (Deckers and Dorny, 2010).

The diagnosis of porcine cysticercosis remains challenging. The gold standard of detailed carcass dissection and cyst enumeration is time-consuming, expensive and requires skilled personnel, and so was not logistically feasible for this study. A Bayesian approach was thus adopted to estimate the sensitivity and specificity of diagnostic tests in the absence of a gold standard, as has been carried out for porcine cysticercosis in Zambia and South Africa (Dorny et al., 2004; Krecek et al., 2008).

Both Ag-ELISAs compared in this study were highly sensitive in diagnosis of porcine cysticercosis but the B158B60 Ag-ELISA was more specific than the HP10 Ag-ELISA,

probably due to the fact that the tests use different monoclonal antibodies, which likely target different circulating antigens or epitopes.

Serological test results should be interpreted carefully considering possible cross-reactions with other parasites. Recently concerns have been raised about the specificity of Ag-ELISA and EITB for diagnosis of porcine cysticercosis. Gavidia et al. (2013) and Jayashi et al. (2014) found that pigs from endemic areas that were EITB positive had no cysts upon necropsy. Similar results were reported by Devleeschauwer et al. (2013) using the B158B60 Ag-ELISA: in sentinel pigs that tested Ag-ELISA positive, no *T. solium* cysts could be found in the carcass. It is well-documented that infection with *T. hydatigena* causes false positives in B158B60 and HP10 Ag-ELISAs (Rodriguez et al., 2012), however other potential sources of false positive reactions in Ag-ELISAs in pigs have not been investigated (for example exposure to *T. saginata* or to the eggs of other taeniid cestodes). There is very little information on how much *T. hydatigena* exists in Madagascar. As some areas of the country have a serious problem with both household and feral dogs (Ratsitorahina et al., 2009), there is a possibility that *T. hydatigena* or *Echinococcus spp.* are circulating between dogs and pigs. However, the meat inspection noted the presence of no other parasites apart from *T. solium* cysts. In addition, when carcass inspection was used as a reference standard, the EITB assay and the B158B60 Ag-ELISA were found to highly specific for detection of porcine cysticercosis (specificities of 90.9% and 94.1%, respectively), suggesting that for these assays cross-reactivity with other parasites is not a major concern in this setting.

In the current Bayesian analysis, carcass inspection was found to be highly specific in diagnosis of porcine cysticercosis, consistent with previous reports (Dorny et al., 2004; Phiri et al., 2006). However, the sensitivity of this method was also surprisingly high in comparison with earlier estimates (Boa et al., 2002; Dorny et al., 2004). This suggests that

either the inspection was carried out more thoroughly in this study than in previous surveys, thus increasing the likelihood of detecting cysts. However, no information was recorded on cyst numbers or whether the pigs were considered heavily, medium or light infections, which is known to be related to the sensitivity of meat inspection and serological tests (Sciutto et al., 1998b).

There are limitations to the estimates of diagnostic performance obtained during this study. Firstly, since our 3 tests required significant amounts of sera, our samples were not systematically analyzed with the three laboratory-based diagnostic tests; this technical difficulty may have introduced bias if certain types of samples were more likely to have failed testing than others. Secondly, the sample size for the Bayesian analysis was small (n=117) and only 17 “negative” (by carcass inspection) samples were included, providing a potential further source of bias. The results of the sensitivity analysis fell within 8% of the original model results when partially informative priors were used and within 12% of the model results when non-informative priors were used. This suggests that the prior distributions employed were appropriate for the analysis. The one exception to this was the sensitivity of carcass inspection which showed a dramatic change when partially informative or non-informative priors were employed, indicating that the prior distribution very strongly influenced the posterior estimate of this parameter. The models each contained seven degrees of freedom (seven independent data cells) and were used to estimate nine parameters (sensitivity and specificity for each test, “prevalence” and two co-variance parameters). Thus, they were not “identifiable”, meaning that there were insufficient data to estimate the parameters of interest, unless prior information was included (Branscum et al., 2005). A possible explanation for the results of the sensitivity analysis is that the prior estimate of carcass inspection sensitivity was too low, perhaps because the inspection was conducted more carefully in this study than in previous surveys or due to a difference in infection

intensity (as discussed previously). Thus the actual estimate of the sensitivity of carcass inspection in this setting may actually be higher than reported here.

The prevalence of porcine cysticercosis in Madagascar estimated here is slightly higher than the official prevalence of 0.5-1% reported for the 2008-2012 period, which was based on visual inspection of carcasses in urban abattoirs (Direction des Services Veterinaires de Madagascar, 2012). Our estimate is surprisingly low given that Madagascar is considered to be a hotspot for human taeniasis and pig cysticercosis is often reported in local abattoirs and markets (Rasamoelina-Andriamanivo et al., 2013). Since the majority of pigs in Madagascar are not slaughtered at abattoirs, but rather in villages or at home (Rasamoelina-Andriamanivo et al., 2013), we could not estimate the overall prevalence of *T.solium* in pig population at country level; indeed, the samples in this study were not representative of the national pig population in Madagascar, but representative of the commercial pigs slaughtered during a short period of time in Antananarivo city for urban consumers only. Moreover, traders use lingual palpation to detect heavily infected animals at rural live pig markets. Although the efficacy of such control, and, in consequence, the prevalence of infected pigs at urban market, may be influenced by fluctuations in demand for pork through the year, it is likely that the most “healthy” pigs are sent for slaughter at abattoirs (Praet et al., 2010). Thus, abattoir-based surveys may underestimate the prevalence of porcine cysticercosis.

Recent village-based surveys in other African countries revealed porcine cysticercosis prevalence as high as 41% (Assana et al., 2010; Eshitera et al., 2012; Ganaba et al., 2011; Komba et al., 2013; Ngowi et al., 2010; Pondja et al., 2010; Praet et al., 2010). Thus, village-based studies may be necessary to gain a better understanding of the overall burden of porcine cysticercosis in Madagascar.

Results from the assessment of diagnostic tests performance reported here suggest that the B158B60 Ag-ELISA would be the most appropriate laboratory-based diagnostic for such

surveys. However, Ag-ELISA tests techniques remain challenging for farm-based testing as they require laboratories and trained staff. Detection of cysts through lingual palpation, the method which is used most widely in developing countries due to its simplicity and low cost, is notoriously insensitive for detecting low-intensity infections in individual animals (Phiri et al., 2006). Thus there is an urgent need for the development of simple, sensitive and inexpensive point-of-care tests which do not require additional equipment and can be deployed on-farm by farmers and animal health workers to inform treatment and control decisions on the ground.

In conclusion, our results provide a first laboratory-based description of the burden of cysticercosis in pigs slaughtered in Antananarivo city in Madagascar; they noted an apparent low percentage of pork carcasses contaminated with *T. solium* cysts at urban market level. To better define appropriate surveillance and control measures for cysticercosis in Madagascar several questions need to be investigated in further studies, including: (i) what is the prevalence of porcine cysticercosis in different Malagasy regions? (ii) What are the main risk factors for infection in farms? (iii) What is the seasonal variation in disease burden? (iv) How much understanding of the disease exists in rural communities? (v) How acceptable would potential new control measures be in rural communities?

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Conflict of interest

Authors declare that they have no conflicts of interest relating to this paper.

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References

Andriantsimahavandy, A., Lesbordes, J.L., Rasoaharimalala, B., Peghini, M., Rabarijaona,

L., Roux, J., Boisier, P., 1997. Neurocysticercosis: a major aetiological factor of late-onset epilepsy in Madagascar. *Trop Med Int Health* 2, 741-746.

Andriantsimahavandy, A., Ravaoalimalala, V.E., Rajaonarison, P., Ravoniarimbinina, P.,

Rakotondrazaka, M., Raharilaza, N., Rakotoarivelo, D., Ratsitorahina, M.,

Rabarijaona, L.P., Ramarokoto, C.E., Leutscher, P., Migliani, R., 2003. The current epidemiological situation of cysticercosis in Madagascar. *Arch Inst Pasteur Madagascar* 69, 46-51.

Assana, E., Amadou, F., Thys, E., Lightowers, M.W., Zoli, A.P., Dorny, P., Geerts, S., 2010.

Pig-farming systems and porcine cysticercosis in the north of Cameroon. *J Helminthol* 84, 441-446.

Berkvens, D., Speybroeck, N., Praet, N., Adel, A., Lesaffre, E., 2006. Estimating disease

prevalence in a Bayesian framework using probabilistic constraints. *Epidemiology* 17,

145-153.

Boa, M.E., Kassuku, A.A., Willingham, A.L., 3rd, Keyyu, J.D., Phiri, I.K., Nansen, P., 2002.

Distribution and density of cysticerci of *Taenia solium* by muscle groups and organs in naturally infected local finished pigs in Tanzania. *Vet Parasitol* 106, 155-164.

Boussard, M., Millon, L., Grenouillet, F., Jambou, R., 2012. Prevention and treatment of cysticercosis. *J Anti-Infect* 14, 143-150.

Brandt, J.R.A., Geerts, S., Dedeken, R., Kumar, V., Ceulemans, F., Brijs, L., Falla, N., 1992.

A monoclonal antibody-based ELISA for the detection of circulating excretory secretory antigens in *Taenia saginata* cysticercosis. *Int J Parasitol* 22, 471-477.

Branscum, A.J., Gardner, I.A., Johnson, W.O., 2005. Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. *Prev Vet Med* 68, 145-163.

Carod, J.F., Randrianarison, M., Razafimahefa, J., Ramahefarisoa, R.M., Rakotondrazaka,

M., Debruyne, M., Dautigny, M., Cazal, P., Andriantseheno, M.L., Charles, E.R.,

2012. Evaluation of the performance of 5 commercialized enzyme immunoassays for the detection of *Taenia solium* antibodies and for the diagnosis of neurocysticercosis.

Diagn Microbiol Infect Dis 72, 85-89.

da Silva, M.R.M., Uyehara, C.N.S., Silva, F.H., Espindola, N.M., Poleti, M.D., Vaz, A.J.,

Meirelles, F.V., Maia, A.A.M., 2012. Cysticercosis in experimentally and naturally infected pigs: Parasitological and immunological diagnosis. *Pesq Vet Bras* 32, 297-302.

Deckers, N., Dorny, P., 2010. Immunodiagnosis of *Taenia solium* taeniosis/cysticercosis.

Trends Parasitol 26, 137-144.

Direction des Services Veterinaires de Madagascar 2012. Situations zoosanitaires de

Madagascar de 2001 à 2011 (Ministere de l'Elevage).

- Dorny, P., Phiri, I.K., Vercruysse, J., Gabriel, S., Willingham, A.L., 3rd, Brandt, J., Victor, B., Speybroeck, N., Berkvens, D., 2004. A Bayesian approach for estimating values for prevalence and diagnostic test characteristics of porcine cysticercosis. *Int J Parasitol* 34, 569-576.
- Draelants, E., Brandt, J.R.A., Kumar, V., Geerts, S., 1995. Characterization of epitopes on excretory-secretory antigens of *Taenia saginata* metacestodes recognized by monoclonal-antibodies with immunodiagnostic potential. *Parasite Immunol* 17, 119-126.
- Eshitera, E.E., Githigia, S.M., Kitala, P., Thomas, L.F., Fevre, E.M., Harrison, L.J., Mwihi, E.W., Otieno, R.O., Ojiambo, F., Maingi, N., 2012. Prevalence of porcine cysticercosis and associated risk factors in Homa Bay District, Kenya. *BMC Vet Res* 8, 234.
- Ganaba, R., Praet, N., Carabin, H., Millogo, A., Tarnagda, Z., Dorny, P., Hounton, S., Sow, A., Nitiema, P., Cowan, L.D., 2011. Factors associated with the prevalence of circulating antigens to porcine cysticercosis in three villages of Burkina Faso. *PLoS Negl Trop Dis* 5, e927.
- Harrison, L.J.S., Joshua, G.W.P., Wright, S.H., Parkhouse, R.M.E., 1989. Specific detection of circulating surface secreted glycoproteins of viable cysticerci in *Taenia saginata* cysticercosis. *Parasite Immunol* 11, 351-370.
- Hernandez, M., Beltran, C., Garcia, E., Fragoso, G., Gevorkian, G., Fleury, A., Parkhouse, M., Harrison, L., Sotelo, J., Sciutto, E., 2000. Cysticercosis: towards the design of a diagnostic kit based on synthetic peptides. *Immunol Lett* 71, 13-17.
- Hubert, K., Andriantsimahavandy, A., Michault, A., Frosch, M., Muhlschlegel, F.A., 1999. Serological diagnosis of human cysticercosis by use of recombinant antigens from

Taenia solium cysticerci. Clin Diagn Lab Immunol 6, 479-482.

- Komba, E.V., Kimbi, E.C., Ngowi, H.A., Kimera, S.I., Mlangwa, J.E., Lekule, F.P., Sikasunge, C.S., Willingham, A.L., 3rd, Johansen, M.V., Thamsborg, S.M., 2013. Prevalence of porcine cysticercosis and associated risk factors in smallholder pig production systems in Mbeya region, southern highlands of Tanzania. Vet Parasitol 198, 284-291.
- Krecek, R.C., Michael, L.M., Schantz, P.M., Ntanjana, L., Smith, M.F., Dorny, P., Harrison, L.J.S., Grimm, F., Praet, N., Willingham, A.L., 2008. Prevalence of *Taenia solium* cysticercosis in swine from a community-based study in 21 villages of the Eastern Cape Province, South Africa. Vet Parasitol 154, 38-47.
- Krecek, R.C., Michael, L.M., Schantz, P.M., Ntanjana, L., Smith, M.F., Dorny, P., Harrison, L.J.S., Grimm, F., Praet, N., Willingham, A.L., 2011. Corrigendum to "Prevalence of *Taenia solium* cysticercosis in swine from a community-based study in 21 villages of the Eastern Cape Province, South Africa". Vet Parasitol 183, 198-200.
- Lunn, D.J., Thomas, A., Best, N., Spiegelhalter, D., 2000. WinBUGS - A Bayesian modelling framework: Concepts, structure, and extensibility. Stat Comput 10, 325-337.
- Michault, A., Duval, G., Bertil, G., Folio, G., 1990. [Sero-epidemiological study of cysticercosis in Reunion Island]. Bull Soc Pathol Exot 83, 82-92.
- Michault, A., Leroy, D., Coubes, P., Laporte, J.P., Bertil, G., Mignard, C., 1989. Immunological diagnosis in the cerebrospinal fluid and serum in patients with evolutive cerebral cysticercosis by ELISA. Trans Zool Soc London 37, 249-253.
- Michelet, L., Carod, J.F., Rakontondrazaka, M., Ma, L., Gay, F., Dauga, C., 2010. The pig tapeworm *Taenia solium*, the cause of cysticercosis: Biogeographic (temporal and spacial) origins in Madagascar. Mol Phylogenet Evol 55, 744-750.

- Ngowi, H.A., Kassuku, A.A., Carabin, H., Mlangwa, J.E., Mlozi, M.R., Mbilinyi, B.P., Willingham, A.L., 3rd, 2010. Spatial clustering of porcine cysticercosis in Mbulu district, northern Tanzania. *PLoS Negl Trop Dis* 4, e652.
- Nguekam, A., Zoli, A.P., Vondou, L., Pouedet, S.M., Assana, E., Dorny, P., Brandt, J., Losson, B., Geerts, S., 2003. Kinetics of circulating antigens in pigs experimentally infected with *Taenia solium* eggs. *Vet Parasitol* 111, 323-332.
- Phiri, I.K., Dorny, P., Gabriel, S., Willingham, A.L., 3rd, Sikasunge, C., Siziya, S., Vercruyse, J., 2006. Assessment of routine inspection methods for porcine cysticercosis in Zambian village pigs. *J Helminthol* 80, 69-72.
- Phiri, I.K., Dorny, P., Gabriel, S., Willingham, A.L., Speybroeck, N., Vercruyse, J., 2002. The prevalence of porcine cysticercosis in Eastern and Southern provinces of Zambia. *Vet Parasitol* 108, 31-39.
- Pondja, A., Neves, L., Mlangwa, J., Afonso, S., Fafetine, J., Willingham, A.L., 3rd, Thamsborg, S.M., Johansen, M.V., 2010. Prevalence and risk factors of porcine cysticercosis in Angonia District, Mozambique. *PLoS Negl Trop Dis* 4, e594.
- Praet, N., Kanobana, K., Kabwe, C., Maketa, V., Lukanu, P., Lutumba, P., Polman, K., Matondo, P., Speybroeck, N., Dorny, P., Sumbu, J., 2010. *Taenia solium* cysticercosis in the Democratic Republic of Congo: how does pork trade affect the transmission of the parasite? *PLoS Negl Trop Dis* 4.
- Prasad, A., Gupta, R.K., Pradhan, S., Tripathi, M., Pandey, C.M., Prasad, K.N., 2008. What triggers seizures in neurocysticercosis? A MRI-based study in pig farming community from a district of North India. *Parasitol Int* 57, 166-171.
- R development core team, 2008. R: A language and environment for statistical computing, In: <http://www.r-project.org>. Vienna.

- Rasamoelina-Andriamanivo, H., Porphyre, V., Jambou, R., 2013. Control of cysticercosis in Madagascar: beware of the pitfalls. *Trends Parasitol* 29, 538-547.
- Ribot, J.J., Coulanges, P., 1988. Malagasy zoonoses. *Rev Elev Med Vet Pays Trop* 41, 9-22.
- Rodriguez, S., Wilkins, P., Dorny, P., 2012. Immunological and molecular diagnosis of cysticercosis. *Pathog Glob Health* 106, 286-298.
- Sciutto, E., Martinez, J.J., Villalobos, N.M., Hernandez, M., Jose, M.V., Beltran, C., Rodarte, F., Flores, I., Bobadilla, J.R., Frago, G., Parkhouse, M.E., Harrison, L.J., de Aluja, A.S., 1998. Limitations of current diagnostic procedures for the diagnosis of *Taenia solium* cysticercosis in rural pigs. *Vet Parasitol* 79, 299-313.
- Simac, C., Michel, P., Andriantsimahavandy, A., Esterre, P., Michault, A., 1995. Use of enzyme-linked-immunosorbent-assay and enzyme-linked immunoelectrotransfer blot for the diagnosis and monitoring of neurocysticercosis. *Parasitol Res* 81, 132-136.
- Torgerson, P.R., 2013. One world health: Socioeconomic burden and parasitic disease control priorities. *Vet Parasitol* 195, 223-232.
- Villota, G.E., Gomez, D.I., Volcy, M., Franco, A.F., Cardona, E.A., Isaza, R., Sanzon, F., Teale, J.M., Restrepo, B.I., 2003. Similar diagnostic performance for neurocysticercosis of three glycoprotein preparations from *Taenia solium* metacestodes. *Am J Trop Med Hyg* 68, 276-280.

Figure Legends

Table 1. Values of priors and corresponding beta distributions used to estimate the performance of three diagnostic tests for cysticercosis in pigs.

Diagnostic test	Parameter	Mode ^a	2.5th– 7.5thpercentile range	Beta (α , β) prior distribution	Source of prior probabilities
Carcass inspection	Se	0.221	0.137, 0.337	15.24, 51.18	Dorny et al. (2004)
	Sp	1	0.895, 0.999	33.28, 1.00	Dorny et al. (2004)
HP10Ag-ELISA	Se	0.704	0.494, 0.851	16.73, 7.61	Krecek et al. (2011)
	Sp	0.661	0.408, 9.845	10.88, 6.07	Krecek et al. (2011)
B158B60Ag- ELISA(Model 1)	Se	0.867	0.575, 0.964	10.95, 2.53	Dorny et al. (2004)
	Sp	0.947	0.890, 0.975	111.96, 7.21	Dorny et al. (2004)
B158B60Ag- ELISA(Model 2)	Se	0.633	0.438, 0.792	17.00, 10.28	Krecek et al. (2011)
	Sp	0.87	0.765, 0.932	55.56, 9.15	Krecek et al. (2011)

a. Most likely value for parameter.

Table 2. Summary of samples tested and diagnostic assay results.

	Group 1 (33) ^a		Group 2 (21)		Group 3 (21)	
	N	(%) (95% CI) ^b	N	(%) (95% CI)	N	(%) (95% CI)
Carcass inspection	250	100	250	0	0	–
EITB	64	92.1 (82.7–97.4)	44	9.1 (2.5– 21.7)	0	–
HP10Ag-ELISA	158	98.1 (94.5–99.6)	122	37.7 (29.1– 46.9)	0	–
B158B60Ag- ELISA	127	100 (97.1–100)	17	5.9 (0.1– 28.7)	175	10.9 (6.7–16.4)

a. Number of different production areas from which sampled pigs originated.

b. 95% confidence interval.

Table 3. Summary of performance of three diagnostic tests for cysticercosis in pigs using visual and incisional examination of carcasses as the “gold standard”.

	Sensitivity(%) (95% CI) ^a	Specificity(%) (95% CI)	PPV ^b (%) (95% CI)	NPV ^c (%) (95% CI)	AUC ^d (95% CI)
EITB	92.2	90.9	93.7	88.9	0.916
(n = 108)	(82.7–97.4)	(78.3–97.5)	(84.5–98.2)	(75.9–96.3)	(0.861–0.970)
HP10 Ag-ELISA	98.1	62.3	77.1	96.2	0.802
(n = 180)	(94.6–99.6)	(53.1–70.9)	(70.7–82.7)	(89.3–99.2)	(0.758–0.847)
B158B60 Ag-ELISA	100	94.1	99.2	100	0.971
(n = 144)	(97.1–100)	(71.3–99.9)	(95.7–100.0)	(79.4–100)	(0.912–1)

a. 95% Confidence interval.

b. Positive predictive value.

c. Negative predictive value.

d. Area under the curve.

Figure 1. ROC plot for EITB (green line), HP10 Ag-ELISA (red line) and B158B60 Ag-ELISA (blue line) for detection of porcine cysticercosis using carcass inspection as the reference standard.

