Veterinary Parasitology 219 (2016) 77–83

Contents lists available at ScienceDirect

Veterinary Parasitology

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

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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ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 19 August 2015
Accepted 22 August 2015

Keywords:
Taenia solium
Cysticercosis
Immunodiagnostic
Enzyme-linked immunoelectrotransfer blot
ELISA
pigs
Madagascar

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 antigen ELISAs 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.

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, 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, 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

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http://dx.doi.org/10.1016/j.vetpar.2015.08.027
0304-4017/

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; Hernández 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., 1998a).

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, 2012; Ramahefarisoa et al., 2010; 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.

2. Materials and methods

2.1. 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 samples were taken at the bleeding post by official veterinary officers. 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 (1000 g for 10 min), 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.

2.2. 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, 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 and the cysticerci stages, i.e. viable or degenerated, were not registered. The location of cysticerci lesions were recorded for animals considered to be in group 1.

2.3. 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. 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 a sensitivity of 100% and a specificity of 99.6% in diagnosis of porcine cysticercosis, but no references are provided to support this claim. The second Ag-ELISA detects an antigen from the metacestode stage of *T. solium* using the HP10 monoclonal antibody (Harrison et al., 1989), and was carried out according to the method described by Sciutto et al. (1998a).

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". HP10 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.

2.4. 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. The statistical analysis was carried out in R v3.0.3 (R development core team, 2011) 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 ($n = 117$). Doubtful results were excluded from the dataset. 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 et al., 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 (Johnson and Chun-Lung, 2010) was employed to calculate beta (α,