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Comparison of Serologic Diagnosis of Taeniosis and Cysticercosis in Field Samples from Eastern Zambia

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Abstract

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Background: Neurocysticercosis is a leading cause of epilepsy in *Taenia solium* endemic regions of the world accounting for about 30% of all epileptic cases. The main aim of this study was to do a Comparison of Serologic Diagnosis of Taeniosis and Cysticercosis in Field Samples from Eastern Zambia.
Methods: Retrospectively collected Samples through community-based cross sectional and longitudinal studies which looked at the prevalence and incidence of human *T. solium* infections, respectively, and described in earlier reports were selected for this laboratory based study. Samples, with coproantigen ELISA and serum antigen ELISA which the results were known were randomly selected for this study.
Results: A total of 886 serum samples were analyzed. The rT24 /rES33 EITB detected a taeniosis and cysticercosis prevalence of 5.9% and 9.5%, respectively. On performance the Kappa statistics revealed a fair agreement of rT4/rES33 EITB (Kappa value of 0.2781- 0.2117) compared to coproantigen ELISA and serum antigen ELISA. Although there is not a good agreement between the antibody and antigen test.
Conclusion: This study found that the performance of rT4/rES33 EITB compared to coproantigen ELISA and serum antigen ELISA were fair and because the agreement was not good between the antibody and antigen test the, selection of a test must be carefully made, with consideration of what is needed.

Keywords: *Taenia solium diagnosis; coproantigen ELISA; immunodiagnosis; Ag-ELISA; recombinant T24/ES33 EITB*

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Introduction

Human *Taenia solium* infections, namely taeniosis and cysticercosis, are parasitic diseases that in the past have not always been recognized for their importance [1]. However, it is becoming increasingly clear that greater priority should be given to these zoonoses because of their economic impact, particularly in resource poor countries, and their public health burden [2].

Adult intestinal tapeworm infection (taeniosis) in man is acquired by eating undercooked infected pork [3]. Pigs are the intermediate host and are infected by ingestion of infective eggs (or proglottids), which develop into cysticerci (porcine cysticercosis). Humans acquire cysticercosis when the metacestodes invade the brain tissues resulting in neurocysticercosis (NCC) that is the leading known cause of epilepsy in human populations of the developing countries [2].

An important risk factor for cysticercosis infection in both pigs and humans is an adult tapeworm carrier [4]. The control of this important parasite depends on the availability of cheap and reliable diagnostic tools which are currently lacking [5]. Identification and treatment of taeniosis infections can be difficult since infected individuals remain asymptomatic apart from the occasional gastro intestinal complaints, loss of appetite and weight loss [6]. The true impact of this disease has been obscured by the lack of sensitive and specific diagnostic tools, especially in low income countries, for the collection of epidemiological data [7]. Classically, taeniosis has been detected by direct parasitologic examination of stool samples. Detection methods based on microscopic observation of *Taenia* eggs in faecal samples and coproantigen detection assays have been developed. The diagnostic sensitivity of the microscopic techniques, however, is not optimal, with reports ranging from 38 to 69% [8]. Such low sensitivity is primarily due to the intermittent nature of egg release, which leads to an underestimation of the prevalence of taeniosis [9]. The coproscopic studies from patients with active tapeworm infection are commonly negative because, firstly, eggs may not appear in feces every day, and secondly, eggs are not uniformly distributed in feces [10]. The coproantigen assay is an antigen-capture ELISA [11, 12] that uses hyper immune rabbit sera produced against somatic extracts of tapeworms to capture and detect parasite antigens excreted in stool samples. This assay is reported to detect immature tapeworm stages hence able to detect carriers that are of epidemiological importance. have been developed based on the detection of parasite. Differentiation of human Taenia spp. by molecular assays is normally done on proglottids expelled from carriers after treatment [13, 14, 15]. In recent years, polymerase chain reaction (PCR) tests for species-specific confirmation of Taenia sppin the faeces [16]. Several methods and loci have been used for differentiating Taenia spp. [14] designated primers have been used these in multiplex PCR giving differential detection of T. saginata and T. solium However, current DNA extraction methods are too expensive to be used as a routine test and developing countries lack well equipped laboratories needed for molecular tests [17], which makes their use under field conditions questionable

The diagnosis of cysticercosis is based on serological detection of antibodies against cysticerci [18]. Enzyme-linked immunoassay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) are the most frequently used immunodiagnostic tests for human cysticercosis [19]. Further, the use of different immunodiagnostics tests (EITB, AbELISA and Ag-ELISA) on CSF has been shown to give indications of the location of the cysticerci and when combined with PCR can diagnose cases missed by the immunodiagnostic tests applied on CSF [20] Antigen detection has provided a suitable alternative for the drawbacks associated with antibody detection [21,22,23].Most importantly the circulating antigen detecting technique offers the advantage over antibody detecting tests of confirming the presence of live cysts and is reported to give a better correlation between the actual presence of viable infective cysticerci and antigen positive cases [24, 23,25]. It has been demonstrated that there is a positive correlation between the number of viable cysts and cysticercus antigen concentration [26]. Thus, there is a continuing need for alternative diagnostic methods with improved performances. This will also provide impetus toward research in making the test inexpensive and cost effective so as to make it available in resource poor communities. The EITB

recombinant which uses an upper (rES33; for taeniosis detection) and lower band (T24; for cysticercosis) was used in this study [27] and [19]. Our main objective was to do a comparison of Serologic Diagnosis of Taeniosis and Cysticercosis using EITB against coproAg for taeniosis and Ag-ELISA for cysticercosis in the concurrent diagnosis of human *T. solium* infections.

Materials and methods

An Enzyme-linked immunoelectrotransfer blot (EITB) with two recombinant antigens, rES33 and rT24, was used to detect serum antibodies for taeniosis and cysticercosis, respectively. Recombinant antigens were electrophoretically separated using SDS-PAGE and western blot; 2.5 mm strips in width were cut and then stored at -70oC until used. Detection of antibodies was performed using 5 uL of serum per strip at a 1:100 dilution, as described [28]. The visible observation of the rES33 or rT24 protein band was considered a positive result for taeniosis or cysticercosis, respectively. Although both antigens were on a single strip, analysis for each antigen (referred to as the rES33-EITB or rT24-EITB tests) was conducted separately.

Study design

A community-based cross sectional survey studies were conducted in the dry season between July and August 2009 and October 2010. The Kakwiya in Petauke and Vulamkoko, Katete District of the Eastern province of Zambia were selected because they are a pig keeping communities without any active ongoing sanitation programmes and cysticerci were observed in slaughtered pigs. The willingness of the community to participate in the study and the RHC to collaborate was also taken into account together with the availability of an adequate working space in the clinics and staff for the collection of samples. Only villages (n=20) within a radius of 7 km from Rural Health Centres were selected. The selected villages were visited and all persons invited to participate in the study. Each willing participant was, after written informed consent, given two plastic sample bottles and requested to submit a stool and a urine sample at the RHC.

The submitted samples were divided into two aliquots, one placed in 10% formalin (for coprology and copro-Ag ELISA) and the other in 70% ethanol (for copro-PCR), and were stored at 4 °C until use. The samples were examined for taeniosis and cysticercosis antibodies using the EITB Recombinant strips with an upper (rES33; for taeniosis detection) and lower band (rT24; for cysticercosis) was used.

Laboratory analysis of samples

The first tests were done in previous studies conducted in Katete district of the Eastern Province.

Copro-antigen-enzyme-linked-immunosorbent assay (Copro-Ag ELISA)

A copro-antigen detection ELISA (Copro-Ag ELISA) as described by [11], and modified by [29], was performed on the stool samples for the diagnosis of taeniosis. The plates were read using an automated spectrophotometer (Labsystem Multiskan RC) at 492 nm optical density. To determine the test results, the optical density of each stool sample was compared with the mean of a series of eight *Taenia* negative stool samples plus 3 standard deviations (cutoff).

Serum antigen enzyme-linked-immunosorbent assay (Sero-Ag ELISA)

The serum samples were examined for the presence of cysticerci antigens using a double monoclonal antigen-based B158/B60 Ag-ELISA as described by [30] with minor modification. All positive and serum samples were done in duplicate. The two wells containing the same sample were checked that they gave roughly the same optical density. The average optical density was calculated for every sample. The cut off was calculated based on the optical densities of the negative samples using a variation of the student T-test [31]. The cut off that was determined was used to calculate a ratio (Ratio = average optical densities/cut off). When the ratio was greater than one, the sample was considered positive with 99.9% certainty.

Enzyme-linked immunoelectrotransfer blot (EITB) for detection of taeniosis and Cysticercosis

An Enzyme-linked immune transfer blot (EITB) to detect for presence of *Taenia* antibodies in serum samples was carried out as described by [27]. However, this essay uses one strip. The recombinant EITB which has two bands on one strip was conducted on all the

serum samples to detect the presence of antibodies against taeniosis and cysticercosis. Fifty milliliters of Phosphate buffered saline (PBS)-0.3%Tw-5% nonfat milk was prepared (150ul of Tween20 was added into stirring PBS, for 10 to 15 minutes, then added 2.5gm of non-fat dry milk in PBS-Tw solution, stirred for 15 minutes).500 ul milk solution was added to each well and 5ul of sera was added into each well. They were rocked for few minutes back and forth to mix. Strips were added in. They were rocked and incubated for at least 2 hours or overnight.

Half a liter of PBS-Tween (Phosphate buffered saline-Tween) 0.3% was prepared by adding 1.5 mls of Tween20 into 500 mls of PBS and stirred. Forty eight milliliters of PBS-Tw was used to dilute the conjugate diluent and 6 ul (1:8000) of GAHG-POD-AC was added. The remainder of PBS-Tw was heated in microwave for 2 minutes – swirl to dissipate heat. The wells of the strips were washed 4 times and incubated for 5 minutes after each washing with the warmed PBS-Tw with all liquid aspirated after fourth wash. Five hundred microlitres of the diluted conjugate was added into each well and incubated for 1 hour. Then Diaminobenzidine dehydrochloride (DAB) substrate was prepared (or TMB peroxidase membrane substrate) - 25miligrams of DAB for 50mls of PBS only with 10ul of 30% hydrogen perioxide. Wells were washed with PBS-Tw 3x 5 minutes each. Wells were later washed with PBS only 2x 5 minutes each. 500 ul of DAB was added in each well and incubated for 10 minutes. They were washed with H2O 10x 1 minute each to stop the reaction. Strips were taken out for water to dry up. To determine the test results, bands for both taeniosis and cysticercosis were observed.

Statistical analysis

All data was entered and cleaned in the excel spread sheet and analysed using SPSS version 20. Multiple regressions were used to determine association between the disease status and factors. The Kappa statistic was used to determine the agreements between the rES33/rT24, EITB results and the other tests.

Results

A total number of 886 serum samples were examined. The results for Copro-Ag ELISA detected 69 positives representing (7.8%). Sixty-nine (7.8%) were detected on Serum Ag-ELISA for cysticercosis. The EITB strips detected 52 taeniosis positives and 84 cysticercosis positives representing (5.9%) and (9.5%) respectively.

Agreement of the serum Ag-ELISA and EITB in detecting cysticercosis

Table 1 shows the performance between the rT24 EITB and Ag-ELISA in detecting cysticercosis. Out of 886, the Ag-ELISA detected 69 positives in which 43 were negative on rT24, EITB. The rT24, EITB detected 84 positives out of which 58 were negative on Ag-ELISA respectively. The tests were in agreement on 26 samples giving a Kappa value of 0.2781. According to [32], such a Kappa value indicates a fair agreement between the two tests.

Table 1: Agreement between the rT24 EITB and the Ag - ELISA in detection of cysticercosis

Test		Ag-ELISA		
	Result	Positive	Negative	Total
rT24, EITB	Positive	26	58	84
	Negative	43	759	802
	Total	69	817	886
Kappa Value (p)		0.3 ($p < 0.001$)		

rT24, EITB-Enzyme-linked immunoelectrotransfer blot. Sero-Antigen-ELISA)-Enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium* in serum

Agreement of the copro Ag - ELISA and EITB in detecting taeniosis. The rES33, EITB detected 52 positives out of which 36 were negative on Copro Ag-ELISA respectively. Copro Ag-ELISA detected 69 positives out of which 53 were negative on rT24, EITB. The tests were in agreement on 16 samples giving a Kappa value of 0.2117. According to [32], such a Kappa value indicates a fair agreement between the two tests.

Table 2: rES33, **EITB** versus the Copro Ag-ELISA in detecting taeniosis

Test		Copro Ag – ELISA		
	Result	Positive	Negative	Total
rES33, EITB	Positive	16	36	52
	Negative	53	781	834
	Total	69	817	886
Kappa Value (p)		0.21 ($p < 0.001$)		

rES33, EITB-Enzyme-linked immunoelectrotransferblot.Copro-Antigen-ELISA–Enzyme-linked immunosorbent assay for the detection of *T. solium* antigens in faeces)

Discussion

This study gives the performance and agreement of diagnostic tests to detect *T. solium* infection We demonstrated that the tests under comparison differ at the basic level, that is the Antibody versus Antigen detection hence, the reason why they don't always agree. In this study we found that a comparison in performance of Copro-Ag ELISA and rES33, EITB in diagnosing taeniosis showed that Copro-Ag ELISA detected more positives. Probably, this is so because serum antibodies appear sometime after the initiation of infection and may persist after parasites/antigens have been cleared. The copro-Ag ELISA for stool samples is also known not to be species specific but genus specific, thus it is possible that it could have detected some stool samples from individuals that could have been infected with *T. saginata* [30], thus accounting for the higher prevalence when compared to the rES33, EITB results.

According to [29], copro-Ag ELISA detects immature tapeworm stages hence; this may also explain the higher number of copro-Ag ELISA positive cases compared to rES33, EITB. However, the Kappa statistics for the two diagnostic tests copro-Ag ELISA and EITB in diagnosing taeniosis was 0.2781 and according to [32], such a Kappa value indicates fair agreement.

In this study rT24, EITB detected 84 cysticercosis postives and 69 by sero-Ag ELISA. This could be to the fact that rT24, EITB test detects circulating antibodies (Ab), and hence, an apparent cysticercosis prevalence of 9.5% was found in the current study. This prevalence indicates exposure to the parasite and hence, making these assay useful for understanding the presence of the parasite in a population, but cannot identify those who are currently harbouring the infection. Because of this, it is possible that some of the cases deemed positive with this test, are either individuals who had just been exposed without the infection setting in or they were individuals in whom the infection had cleared. This makes this test a very reliable tool for epidemiological surveys in which the risk or threat of cysticercosis is to be assessed. On the other hand, the sero-Ag ELISA detected cysticercosis prevalence of 7.8% indicating the presence of viable cysts hence an active infection in such individuals. The prevalence of cysticercosis recorded in this study is comparable with that recorded in other endemic areas, based on Ag-ELISA, such as in the Andean region of Ecuador and in North Vietnam [33,34].

The two techniques were subjected to a Kappa test which revealed a value of 0.2117.

According to [32], such a value indicates a fair agreement between rT24, EITB and sero-Ag ELISA. The fact that the results for the two tests are comparable for detection of cysticercorsis means that all those detected in this survey had active, viable infections hence high lighting the need for serious intervention in this area as the rate is very high. Also that the results were comparable with the findings from previous studies [35], and therefore suggests the need for up scaling of intervention strategies in the area.

Conclusion

A serological comparison of EITB with the other diagnostic tests (Copro-Ag and Sero-Ag) in diagnosing taenosis/cysticercosis was fair as shown by Kappa. Among possible explanations could be that the diagnosis of taeniosis remains difficult because current tests are still not a gold standard, and because taeniosis causes little or no symptoms and also that, patients do not present themselves for evaluation unless they see the proglottids in their feces and recognize their significance. Similarly, data based on serological methods may overestimate infection rates because presence of antibody may be the result of exposure to eggs and early but non-persisting infection. Using the kappa statistic configuration, the agreement was not good between antibody and antigen tests and so selection of a test must also be carefully made, with consideration of what is needed.

Abbreviations

Ab: Antibody *Ag:* Antigen *CNS:* Central nervous system. *CT:* Computed tomography *CWGESA:* Cysticercosis Working Group in Eastern and Southern Africa *ELISA:* Enzyme linked immunosorbent assay. *MRI:* Magnetic resonance imaging. *NCC:* Neurocysticercosis *WHO:* World Health Organization. UNZABREC: University of Zambia Biomedical Research Ethics. SDS-PAGE: Dodecyl Sulphate sodium- polyacrylamide gel electrophoresis
ELISA: Enzyme-linked immunosorbent assay. H2SO4: Sulphuric acid. MoAb: Monoclonal antibody. MoH: Ministry of Health. MRI: Magnetic resonance imaging. PBS: Phosphate buffered saline. PBS-Tween 20: Phosphate buffered saline in 0.05% Tween 20.PCR: Polymerase chain reaction. Sero-Ag ELISA: Enzyme-linked immunosorbent assay for the detection of circulating antigens of the metacestode of *T. solium* in serum. EITB: Enzyme-Linked immunoelectrotransfer blot.

Declarations

Ethics approval and consent to participate

All clinical samples used in this study were collected in previous studies with specific permission for future use of stored samples. Samples were anonymized and the study was performed in compliance with protocols approved by the ethical review boards Committee (IRB0001131, 012-04-09) from the University of Zambia Biomedical Research Ethics (UNZABREC). Further approval was obtained from the Ministry of Health of Zambia and also from the local District health authorities of Petauke and Katete districts where the studies were conducted. Meetings were held with the community leaders (headmen) and their subjects where the purpose of the studies was explained and their permission requested to conduct the studies in their area. Informed consent was also sought from the individual subjects to participate in the study. Subjects were not forced to participate and participation was requested of individuals of all ages after written informed consent. For individuals below the age of 16, permission was sought from their parents or guardians by way of written informed consent. All activities were carried out with the highest ethical standards as guided by the appropriate guidelines and enforced by the Review Boards.

Availability of data and materials

Raw data are available on request at any time.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the study: DS, SG and KEM. performed the field survey and laboratory testing: KEM, MC, SG and DS. Analyzed the data: DS, KEM, CS, IGK. wrote the paper: DS. reviewed the paper: KEM, JN, SG, PD, IGK, and CS. All authors read and approved the final manuscript.

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