Serodiagnosis of bovine cysticercosis by detecting live *Taenia saginata* cysts using a monoclonal antibody-based antigen-ELISA

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ABSTRACT

An *ante mortem* antigen-ELISA-based diagnosis of *Taenia saginata* cysticercosis was studied in artificially ($n = 24$) and naturally ($n = 25$) infected cattle with the objective of further validating the assay as a field diagnostic test. Based on total dissection as the definitive method of validity, the assay minimally detected 14 live cysticerci in artificially infected calves and 2 in naturally infected steers. In natural infections, the minimum number of live cysticerci consistently detected by Ag-ELISA was 5 while in artificial infections it was above 14. However, other animals with 12 and 17 live cysticerci in artificially infected calves, and 1 and 2 live cysticerci in naturally infected steers, escaped detection for unknown reasons. Animals harbouring dead cysticerci gave negative reactions in the assay as was the case in non-infected experimental control calves. There was a statistically significant positive linear correlation between Ag-ELISA optical density values and burdens of live cysticerci as obtained by total dissection of both artificially infected calves ($r = 0.798$, $n = 24$; $P < 0.05$) and naturally infected steers ($r = 0.631$, $n = 25$; $P < 0.05$). These results clearly show the potential effectiveness of *ante mortem* monoclonal antibody-based antigen detection ELISA in the diagnosis of bovine cysticercosis in cattle. Its value lies in the diagnosis of infection in cattle as a screening test in a herd, rather than as a diagnostic test at the individual level, due to false positive and negative reactions. In a herd of heavily infected cattle, the assay may, however, provide for individual diagnosis. Nevertheless, more work is recommended to increase its sensitivity so as to be able to diagnose light infections consistently in the field.

Key words: antigens, bovine cysticercosis, ELISA, sero-diagnosis, *Taenia saginata,* total dissection.

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INTRODUCTION

Taenia saginata infection significantly hampers livestock production and adversely affects human health worldwide. The adult stage of this parasite occurs in human small intestines, causing taeniasis, whereas the larval stage occurs in cattle muscles, causing bovine cysticercosis. Worldwide, economic losses due to infections are substantial[7,10,14] and the parasite therefore warrants effective control measures.

The inspection of meat, which is the most important public health control measure, identifies only a minor fraction of heavily infected animals[5], and also only when it is too late to avoid losses[18]. An *ante mortem* diagnostic test that reflects the number of live cysticerci would be desirable because as it could assist in identifying infected animals before slaughter. A reliable *ante mortem* diagnostic test has yet to be defined. However, there have been endeavours to develop an enzyme-linked immunosorbent assay (ELISA) that can be used for the diagnosis of *T. saginata* cysticercosis infection under natural and controlled conditions[1,3,4,6,8,9,11,13,18,23]. Results from antibody-ELISA were found to be unreliable and insensitive due to the tests failure to distinguish between animals harbouring live and dead cysticerci[18]. A serological test that detects live cysticerci, which are important from both a medical and a public health perspective, was described by Harrison *et al.*[11] and found to be specific, stable and resistant to degradation in serum and thus suitable for use, particularly in the tropics. The assay is a double-sandwich ELISA based on a mouse monoclonal antibody (McAb) coded as HP10. This McAb is an IgM isotope that detects antigens (glycoproteins) of viable *T. saginata* cysticerci in cattle sera. However, the assay also detects antigens of *T. solium* cysticerci[11]. HP10 reacts with a repetitive carbohydrate epitope on lentil-lectin-adherent glycoproteins in the biosynthetic excretions/secretions on the surface and somatic regions of *T. saginata* cysticerci. The assay has been used in seroepidemiological surveys in Swaziland[12] and Kenya[18]. A survey carried out in Belgium[5], using McAbs of the IgG isotope[22], provided similar results. These investigations showed that the assay has great potential as a screening test that could be incorporated in integrated quality control programmes for livestock production, and in developing certain standards of hygiene for individual farms[5].

There are , however, a number of problems associated with the assay. These include the inability of the assay to reliably detect bovine cysticercosis in infected cattle at the individual level, and in the determination of the lowest number of live cysticerci in infected cattle. While sensitivity and specificity are very important, correlation between the assay's optical density (OD) values and the number of live cysticerci detected is also important in its validation, but to date this has been evaluated only in 1 study[18]. Although not directly indicative of the number of live cysticerci in individual animals, the correlation will greatly help approximate the number of live cysticerci once OD values are obtained. Decisions can then be made about whether to reject or accept animals for human consumption before slaughter. Current losses as a result of downgrading or condemnation of infected carcasses would therefore not be incurred. The present work was carried out in order to validate the Ag-ELISA assay in the detection of live *Cysticercus bovis* both in naturally and experimentally infected animals.

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MATERIALS AND METHODS

Collection of the parasite eggs

Taenia saginata proglottids were collected from human excrement in the Mathare Valley slums of Nairobi and taken to National Veterinary Research Centre (NVRC) laboratories. The proglottids were collected in physiological saline (0.15 M NaCl) containing 200 units/mℓ Crystapen benzylpenicillin (Glaxo Laboratories, U.K.), 0.2 mg/mℓ Streptomycin sulphate (Glaxo Laboratories, U.K.) and 5 ug/mℓ Fungizone (Squibb, New Jersey, USA) for preservation. Species identification of the proglottids were done using the methods described by Silverman[20].

The eggs were teased from the proglottids and washed through a tier of 3 sieves with 250, 150 and 30 μm apertures, respectively. The 30μm aperture sieve retained the eggs which were then transferred into a universal bottle containing physiological saline and antibiotics and stored at 4 °C until required.

Testing the viability of eggs

Viability of the eggs was assessed according to the methods of Stevenson[21] by use of bile from healthy cattle through a process of egg hatching and activation, and embryophore disruption under a microscope. The number of motile oncospheres was counted in a group of 100 oncospheres to determine the percentage. The counting was done 2 or 3 times and an average obtained. No longer than 10 minutes was spent examining a slide as death of the oncospheres may occur due to drying .

Egg counts

A bottle containing eggs was gently shaken and 0.5 mℓ was removed and transferred to a universal bottle containing 15 mℓ physiological saline. After shaking the bottle well, a pasteur pipette was used to fill both chambers of a McMaster slide, which was allowed to stand for 2–3 minutes. Eggs within the grid in both chambers of the McMaster slide were counted using a microscope with ×2.5 objective. The number of eggs in the volume of egg suspension was calculated by adding the number of eggs in both chambers (a volume of 0.3 mℓ) and multiplying by the dilution factor of 50. Three counts were made and cross-checked by at least 2 other persons using a tally counter. An average was then obtained to determine the number of eggs per millilitre of the egg suspension.

Evaluation of infectivity of eggs

The infectivity of the eggs was determined according to the methods of Silverman[20] by comparing the number of larval cysticerci that developed with the number of potentially infective eggs fed. The number of potentially infective embryos in a suspension of tapeworm eggs was calculated from the percentage of hexacanth embroys in a sample that appeared motile after treatment with hatching solution[19,22]. The following infectivity formula was used:

$$\text{Infectivity index} = \frac{\text{No. of cysts found at } \textit{post mortem}}{\%\ \text{Motility} \times \text{No. of eggs fed}}$$

According to this formula, an infectivity index of 1 means perfect infectivity (*i.e.* 100 % infectivity of the number of eggs fed to the animal) while an infectivity index of 0 means that the eggs were not infective.

Naturally infected cattle

Naturally infected Zebu herds were identified using case histories and reports from the District Veterinary Officer in Samburu District. Ninety-six animals were bled and the resultant serum samples tested for circulating antigens of *Cysticercus bovis* by Ag-ELISA. Sixteen cattle with high Ag-ELISA OD values (16-seropositive group) and 9 others with low OD readings (9-seronegative group), were selected, bought and taken to NVRC, Muguga (Table 1). They were feacal sampled for nematode and fluke infections, respectively using modified McMaster egg counting and Boray sedimentation techniques established at the NVRC Laboratories by Harrison and co-workers in 1989[11]. Those animals which were found to be infected were treated. They were bled on arrival and thereafter once a month. The serum was used to test for *C. bovis* circulating antigens by Ag-ELISA. The animals were slaughtered in the 3rd month and examined for cysticerci, 1st by routine meat inspection procedures as stipulated in the Kenya Meat Control Act – 1977[16] and then by total dissection of a half of the carcass. Total dissection was done by thinly slicing the entire masculature of the carcasses in order to recover the cysticerci. The number of cysticerci obtained in one half was noted and doubled to estimate the total number of cysticerci in the whole animal. The viability of the recovered cysticerci was tested by observing their evagination in cattle bile overnight.

Experimentally infected calves

Thirty-two calves, 3 to 34 days old, were bought from Konza Ranch at Kapiti Plains Estate, Machakos District, and taken to NVRC. They were bled on arrival and the serum tested for circulating *T. saginata* antigens as explained above. The calves were kept worm/cysticercosis-free in pens and fed on milk initially and later on calf weaner pellets, hay and water *ad libitum.* Unfortunately, 2 calves died before infection with *T. saginata* eggs. The infection of the calves was staggered. The 1st 15 calves were given eggs earlier than the 2nd group but the eggs were always administered when the calves were 8–14 weeks old. Serial dilutions were made from the egg suspension to acquire the number of eggs required for infection per animal in each group. The 1st 15 calves were divided into 4 groups of 3, 4, 4 and 4 calves which were given varying doses of *T. saginata* eggs as follows: group 1 received only sterile distilled water (control), group 2 received 2500 eggs each, group 3 received 5000 eggs each and group 4 received 10 000 eggs each. The calves were bled just before being administered with *T. saginata* eggs and thereafter they were bled every 2 weeks until they were slaughtered in the 15th week post-infection and examined as above. Carcasses were thinly sliced and the cysticerci recovered. The cysticerci were tested for viability as described above. The 2nd group of 15 calves was treated similarly.

Ag-ELISA for the detection of C. bovis *antigens*

Circulating antigens were detected by Ag-ELISA using the methods described by Harrison and co-workers[11]. The absorbance was read at 450 nm on a computerised ELISA reader (Titertek Multiscan Plus MK11) which provided OD values.

Statistical analysis

Total dissection was considered to be the standard method of validity which gave the true status of infection in cattle. Since the Ag-ELISA detects antigen of live cysticerci only, analysis of the results was based only on live cysticerci so that the standard method of validity should be able to give a true measure of the Ag-ELISA test.

Negative cut-off value

An optical density cut-off value to distinguish between positive and negative results of Ag-ELISA OD values, was taken as the mean of the negative controls plus 3 standard deviations[18].

Correlation analysis

Correlation analysis was applied to compare the relationship between live cysticerci burdens and Ag-ELISA optical density values. The Spearman rank-order correlation coefficient (r), which determines the extent to which the variables,

Table 1: **Recovery of cysticerci from naturally infected cattle and the corresponding Ag-ELISA OD values at selection and slaughter at 450 nm wavelength** ($n = 25$).

Cattle code number	Total dissection method of validity			AG-ELISA OD values	
	Live	Dead	Total	At selection	At slaughter
715N[a]	2	6	8	0.110 (0.150)[b]	0.060(0.043)
705P	37	17	54	0.334 (0.064)	0.116(0.043)
708P	5	6	11	0.390 (0.064)	0.283(0.043)
988P	31	22	53	0.268 (0.118)	0.094(0.043)
713P	25	19	44	0.353 (0.150)	0.172(0.043)
956N	2	6	8	0.061 (0.064)	0.013(0.043)
969P	8	13	21	0.391 (0.064)	0.429(0.043)
958P	7	8	5	0.186 (0.064)	0.108(0.043)
724P	7	12	19	0.244 (0.064)	0.171(0.043)
716P	24	19	43	0.685 (0.150)	0.565(0.043)
721P	22	9	31	0.299 (0.150)	0.166(0.043)
718P	55	22	77	0.282 (0.150)	0.285(0.043)
723N	0	1	1	0.046 (0.150)	0.014(0.043)
720N	0	2	2	0.096 (0.150)	0.005(0.043)
976N	0	8	8	0.003 (0.064)	0.090(0.043)
714N	0	15	15	0.008 (0.150)	0.001(0.043)
966P	5	8	13	0.076 (0.064)	0.145(0.043)
972N	2	4	6	0.037 (0.064)	0.018(0.043)
717N	1	2	3	0.020 (0.150)	0.010(0.043)
701P	55	39	94	0.266 (0.118)	0.311(0.043)
971N	2	10	12	0.001 (0.064)	0.026(0.043)
707P	5	11	16	0.266 (0.118)	0.663(0.043)
989P	42	46	88	0.105 (0.064)	0.220(0.043)
965P	24	5	29	0.315 (0.064)	0.484(0.043)
970P	0	0	0	0.098 (0.064)	0.237(0.043)
Total no. animals detected	20	24	24	16 seropositive cattle	18 seropositive cattle

[a]N = seronegative at selection, P = seropositive at selection.
[b]Figures in brackets represent negative cut-off points for Ag-ELISA OD values.

that is, living cysticerci burdens and Ag-ELISA optical density values, are linearly related, was calculated using the following formula:

$$r = 1 - \frac{6(\text{sum of } d^2)}{n(n^2 - 1)},$$

where r = Spearman rank-order correlation coefficient; d = difference between independent (number of live cysticerci burdens) and dependent (Ag-ELISA OD values) variables; n = number of pairs of variables.

The interpretation of r values at the probability level of $P < 0.05$ was done in accordance with Olds[17].

RESULTS

Naturally infected cattle

Table 1 shows the recovery of live cysticerci from naturally infected cattle together with Ag-ELISA OD values at selection and slaughter. According to Ag-ELISA, all 16 seropositive cattle remained seropositive throughout the 12 weeks of monitoring, based on calculated cut-off values. Two of 9 cattle in the seronegative group were seropositive at slaughter. Of these 2 animals, in one (No. 976) no cysticerci could be recovered (a false positive result by Ag-ELISA) and the other one (No. 715) yielded 2 live cysticerci. There was another animal (No. 970) without cysticerci but was found to be seropositive at slaughter. In total, Ag-ELISA identified 72 % of the 25 infected animals as seropositive. Ag-ELISA consistently detected 3 animals with 5 live cysticerci and 1 animal with 2 live cysticerci as seropositive at slaughter. However, it failed to detect 3 other animals harbouring 2 live cysticerci on 2 different occasions (*i.e.* at selection and slaughter). In naturally infected animals the lowest number of live cysticerci consistently detected by the test was, therefore, 5. Total dissection revealed live cysticerci ranging from 5–55 in 20 of 25 animals, giving a prevalence of 80 %. Of these 20 animals, 5 were from the 9-seronegative group with live cysticerci ranging from 1 to 2. Of these 5 animals, 4 were serodiagnosed as false negative. The Spearman rank-order correlation analysis showed that there was a positive linear correlation between the live cysticerci burdens and Ag-ELISA values ($r = 0.631$, $n = 25$, $P < 0.05$).

Artificially infected calves

Table 2 shows *post mortem* results for each of the 30 artificially infected calves together with their corresponding Ag-ELISA OD readings before and at slaughter. Before infection with *T. saginata* eggs, all the calves were seronegative. Following infection, total dissection revealed live cysticerci ranging from 1 to 193 in 15 of 24 calves, giving a prevalence of 62.7 %. Only 1 calf was without cysticerci (No. 945). At autopsy, 1 of the control calves (No. 4151) was found to have 1 cysticercus. According to Ag-ELISA, 9/24 calves were seropositive (37.5 %) using serum samples collected at slaughter and based on a calculated critical value of 0.059. All control calves were seronegative. Unlike naturally infected animals, there were no false positive cases. All Ag-ELISA-positive calves were positive by total dissection as well. All calves with dead cysts detected by total dissection were seronegative. Ag-ELISA gave 29.2 % false negatives. The lowest number of live cysticerci detected by Ag-ELISA was 14 although calves with 17 (No. 4153) and 12 (No. 4159) cysticerci and all other calves with fewer than 12 cysticerci were diag-

Table 2: ***Post mortem*** **findings of 30 calves given various doses of** ***Taenia saginata*** **eggs together with their Ag-ELISA OD values at 450 nm wavelength.**

Calf no.	Egg dose	Total dissection			Ag-ELISA OD values	
		Live	Dead	Total	Before infection	At slaughter
4158	00000	0	0	0	–0.002(0.032)*	–0.003(0.059)
4157	00000	0	0	0	0.001(0.032)	0.001(0.059)
4151	00000	1	0	1	–0.004(0.032)	0.006(0.059)
4170	00000	0	0	0	–0.024(0.032)	–0.004(0.059)
947	00000	0	0	0	–0.011(0.032)	–0.010(0.059)
944	00000	0	0	0	–0.030(0.032)	0.003(0.059)
4160	2500	152	11	153	–0.004(0.032)	0.783(0.059)
4153	2500	17	18	35	0.007(0.032)	0.003(0.059)
4154	2500	76	12	89	0.005(0.032)	0.387(0.059)
4152	2500	3	4	7	0.003(0.032)	0.009(0.059)
172	2500	0	28	28	0.002(0.032)	–0.004(0.059)
4173	2500	0	10	10	–0.027(0.032)	0.050(0.059)
951	2500	14	1	45	–0.022(0.032)	0.125(0.059)
945	2500	0	0	0	–0.023(0.032)	–0.012(0.059)
4162	5000	0	8	8	0.006(0.032)	0.000(0.059)
4161	5000	124	38	162	0.000(0.032)	0.317(0.059)
4166	5000	0	22	22	0.000(0.032)	–0.006(0.059)
4155	5000	2	36	38	0.003(0.032)	–0.00(0.059)
4171	5000	0	4	4	–0.018(0.032)	–0.004(0.059)
4167	5000	1	14	15	–0.043(0.032)	0.024(0.059)
950	5000	193	2	195	–0.028(0.032)	0.872(0.059)
948	5000	0	187	187	–0.018(0.032)	0.002(0.059)
4164	10000	62	29	91	–0.003(0.032)	0.060(0.059)
4165	10000	59	26	85	0.000(0.032)	0.697(0.059)
4163	10000	0	249	249	–0.002(0.032)	–0.006(0.059)
4159	10000	12	61	73	0.005(0.032)	0.002(0.059)
4175	10000	0	8	8	–0.039(0.032)	–0.007(0.059)
4169	10000	55	4	59	–0.045(0.032)	0.879(0.059)
949	10000	1	5	6	–0.015(0.032)	–0.010(0.059)
946	10000	93	1	94	–0.012(0.032)	0.486(0.059)
Number detected		15	23	23	0	9 seropositive calves

*Figures in brackets represent negative cut-off points.

nosed as false negative. The Spearman rank-order correlation analysis showed that there was a positive linear relationship between live cysticerci burdens and Ag-ELISA readings ($r = 0.798$, $n = 24$, $P < 0.05$).

Infectivity of the eggs in artificially infected calves

The infectivity index was very poor. Calves in groups 2 (dosed with 2500 eggs each) and 3 (dosed with 5000 eggs each), had an infectivity index of 0.02 while group 4 (dosed with 10 000 eggs each) had an infectivity index of 0.01 (Table 3).

DISCUSSION AND CONCLUSIONS

In the naturally infected animals, Ag-ELISA diagnosed 16 animals at selection as seropositive and indeed, all 16 animals except 1 (No. 970) were confirmed at slaughter to be true positives by total dissection, thus confirming the reliability of Ag-ELISA test in the diagnosis of *T. saginata* cysticercosis infection in cattle. However, some animals that tested negative at selection did seroconvert (22.2 %). This seroconvertion, however, renders it difficult distinguish with certainty between infected and non-infected individual animals using the Ag-ELISA test, particularly in lightly infected cases. This may be due to the permeability of the wall around the larvae that influences the amount of excretory/secretory products (antigens detected by Ag-ELISA) released

Table 3: **Mean infectivity rate of eggs of** ***Taenia saginata*** **in 30 artificially infected calves.**

Group	No. of calves per group	Mean egg dose	Mean no. of cysticerci recovered			Mean infectivity index*
			Live	Dead	Total	
1	6	0	0	0	0	0
2	8	2 500	33	13	46	0.02
3	8	5 000	44	39	83	0.02
4	8	10 000	35	48	83	0.01

*The calculation of infectivity indices was based on the total number of cysticerci recovered from individual calves.

into circulation[2], or constantly occurring reactions between antibody and antigens that result in undissociated immunocomplexes. The lowering of antigenaemia levels or the removal of antigens from circulation by this mechanism, results in the assay producing false negative cases, which may enter the feedlot and enhance the transmission cycle of the parasite between humans and bovines in the environment. The fact that no animals serodiagnosed as positive subsequently tested seronegative, suggests that cysticerci antigens remain present until removed from circulation at a time that is currently unknown. This is partly supported by the observation that animals harbouring only dead cysticerci tested seronegative and remained seronegative throughout the monitoring period, suggesting that the antigens were no longer present and that cysticerci, once they die, either do not produce antigens or, if they do, the products are undetectable by Ag-ELISA. In naturally infected animals, the lowest number of live cysticerci that Ag-ELISA was able to detect, albeit inconsistently, was 2. However, all animals that harboured 5 or more live cysticerci were consistently diagnosed as seropositive at selection and slaughter. These results suggest that an increase in sensitivity by either freeing parasite antigens from antigen-antibody complexes[24] or by calculating a high cut-off point for Ag-ELISA readings[15], will be necessary in the development of a reliable field test. This is because the burdens of live cysticerci present in whole carcasses of lightly infected animals, which epidemiologically are the most important group, vary between 1 and 5[18], which Ag-ELISA is currently unable to detect consistently.

In experimentally infected calves, only 9 of 24 calves (37.5 %) tested seropositive at slaughter. From these, the lowest number of live cysticerci recovered that tested seropositive was 14. Lower numbers of live cysticerci than this did not result in antigenaemia levels that tested seropositive. A calf (No. 4160) with 17 live cysticerci also tested seronegative at slaughter. The level of false negatives detected in experimentally infected animals was 29.2 %. Possible reasons for all these discrepancies have been discussed above under natural infections. Accidental contamination during oral administration of eggs to the calves might have resulted in 1 of the control calves (No. 4151) being found with 1 live cysticercus (Table 2). Poor infectivity indices in the calves dosed with varing numbers of eggs was possibly due to innate resistance of the host to infection[21], lack of or inadequate hatching and activation stimuli at optimal conditions in the alimentary tract which determines the hatchability of oncospheres to cause the infection[19] and finally, due to human error during percentage motility determinations and counting *T. saginata* eggs for infection.

OD values were not indicative of the corresponding actual number of live cysticerci burdens of a seropositive animal and *vice versa* but they did indicate the presence of live cysticerci. This applied to both experimental and natural infections. A given Ag-ELISA value may not indicate a given number of live cysticerci burden because of the constantly varying antigenaemia level due to the immune response of the host that may remove the antigens from circulation.

Detection of the lowest number of live cysticerci was lower in natural infections than in experimental calves. This was possibly due to multiple infections at different times resulting in continued production of antigens whose accumulative effect allowed the detection of light infections in naturally infected animals. However, it is not currently known if any re-infection cysticerci would survive to the fully developed stage in an immunologically active environment of the animal so as to realise this accumulative effect. This discrepancy in the experimental calves may also have been due to poor infection rates of *T. saginata* eggs, as shown in Table 3.

Owing to the false negative and positive results of Ag-ELISA, the test can best be applied as a herd diagnosis to define areas where, and the time when, infections occur. This could assist the farmer to adopt control measures such as treatment with various drugs (*e.g.* praziquantel) or the reduction of field contamination by human feaces or infected humans. In heavy infections, however, the test can provide individual diagnosis.

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