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ORIGINAL PAPER

Evaluation of an antigen-ELISA in the diagnosis of bovine cysticercosis in Kenyan cattle

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Abstract A monoclonal antibody-based antigen-ELISA (Ag-ELISA) was studied in Kenyan cattle with the objective of evaluating its reliability in diagnosing bovine cysticercosis. A total of 55 cattle divided into artificially (n=30) and naturally (n=25) infested animals, were utilized. Total dissection was used as a gold standard of validity at autopsy. In natural infestations, the assay identified 16 cases as true seropositives, 2 cases as false seropositives, 3 cases as true seronegatives and 4 cases as false seronegatives. While in artificial infestations, the assay identified 9 cases as true seropositives, 14 cases as true seronegatives and 7 cases as false seronegatives. There weren't any false seropositive cases identified with artificial infestations. The assay showed good precision level and kappa level in quantifying the relative quality of the amount of agreement in natural (n=25; k=0.482; p>0.05) and artificial (n=24; k=0.374; p>0.05) infestations. The study showed that, besides other advantages, the Ag-ELISA with its sensitivity of 60.00–80.00%, specificity of 60.00–100%, predictive value of 88.89–100%, apparent prevalence of 37.50–72.00% and accuracy of 75.00–76.00% may be recommended for use in combination with other control measures, viz chemotherapy, post-mortem diagnosis and or vaccination.

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Introduction

Taenia saginata is among the zoonotic parasites that adversely affect livestock production worldwide. In addition to the loss of revenues from animals coming from endemic markets for export (Harrison et al. 1989), the economic losses accruing from infestation are substantial (Gracey et al. 1999; Fan 1997; Harrison 1996; Gracey and Collins 1992; Mann 1983; Grindle 1978). The strategic control of this parasite is therefore very necessary and crucial.

Meat inspection, which is the only public health measure practised, identifies only heavily infested cattle but only at a stage when it is too late to avoid incurring losses. An ante-mortem diagnostic test would be desirable and superior to meat inspection for decisions can be made before slaughter of the animal. Such an ante-mortem test was described by Harrison et al. (1989). The assay is a double sandwich

enzyme-linked immunosorbent assay (ELISA) based on a mouse monoclonal antibody (McAb) coded as HP10. The monoclonal antibody is an IgM isotope, which detects antigens (glycoproteins) of only viable *T. saginata* cysticerci in the cattle sera, hence the name, antigen-ELISA (Ag-ELISA) (Harrison et al. 1989; Onyango-Abuje et al. 1996). The assay was used in Swaziland to determine seroprevalence of infestation and antigenemia levels in cattle (Hughes et al. 1993). In Kenya, the assay was used at best to identify individual ranches and farms with herds of cattle infested with cysticerci (Onyango-Abuje et al. 1996). The reliability of this assay is yet to be evaluated and its performance as a diagnostic tool ascertained.

Objective

In Kenya, the routine meat inspection procedure plays a major role in the diagnosis of bovine cysticercosis (Kang'ethe 1995). The procedure solely depends on the physical incision and careful observation in the so-called predilection sites: cheek muscles of the head, tongue, heart and triceps brachii (the sites considered to have a higher density of cysts than elsewhere in the carcass in Kenya) (Kenya Meat Control Act Chapter 356 revised 1977). Wanzala et al. (2005) have shown a considerable number of cysticerci to exist in other areas in a carcass other than the predilection sites. This method again detects *Cysticercus bovis* when it is too late to avoid incurring considerable economic losses in slaughterhouses. Further, in lightly infected carcasses, the post-mortem examination procedure rarely detects *C. bovis* (Abuseir et al. 2006; Kyvsgaard et al. 1990) and in Kenya, the inspection method was shown to be unreliable (Wanzala et al. 2002, 2003; Kang'ethe 1995; Walther and Koske 1980). In addition, visual inspection method results in a lot of mistaken identity of *C. bovis* (Wanzala et al. 2003), hence the search for an alternative diagnosis method.

The objective of this study therefore was to evaluate the reliability of the developed ante-mortem Ag-ELISA in the diagnosis of bovine cysticercosis infection in Kenyan cattle.

Materials and methods

Collection of the parasite eggs

T. saginata proglottids were collected from human excrement in the Mathare Valley slums of Nairobi City and brought to the National Veterinary Research Centre (NVRC) laboratories. The proglottids were collected in physiological saline (0.15 M NaCl) containing 200 units/ml of Crystapen benzylpencillin (Glaxo Laboratories, U.K.); 0.2 mg/ml Streptomycin Sulphate (Glaxo Laboratories, U.K.) and 5 ug/ml fungizone (Squibb and Sons, Inc., NJ, USA.), as a fungicidal drug for preservation. The eggs were teased off the proglottids and washed through a tier of three sieves with 250 nm, 150 nm and 30 nm apertures, respectively. The 30 nm aperture sieve retained the eggs, which were then transferred into a universal bottle containing physiological saline and antibiotics for storage at 4°C until required.

Testing the viability of the eggs

This was done according to the methods of Stevenson 1983. Equal volumes of egg suspension and 10% sodium hypochlorite solution were mixed together in a 15 ml graduated plastic centrifuge tube and shaken vigorously for 2 min. Immediately, the tube was filled to the 15 ml mark with physiological saline and centrifuged for 5 min at 4000 ×*g* on the MSE Minor Centrifuge to remove the embryophore. The supernatant was drawn off leaving approximately 0.25 ml to which physiological saline was added to the original mark of 15 ml and centrifuged again. This was repeated twice before a freshly made hatching solution (1.17 g sodium hydrogen carbonate+0.05 g trypsin in 100 ml of deionised water) was added up to the 2 ml mark. 1 ml of bovine bile was added and the tube was transferred to a 37°C water bath and incubated for 45 min. Every 10 min the tube was removed and shaken vigorously for 1 min. After incubation of the oncospheres, a drop of their suspension was put onto a slide, and examined under the microscope using both low (×10) and high (×40) power objective lenses. The number of motile oncospheres was counted in a group of 100 oncospheres to determine their percentage. The counting was done two or three times and an average obtained. No longer than 10 min was spent examining one slide, otherwise death of the oncospheres would occur due to drying of the slide.

Egg counts

A bottle containing eggs was gently shaken and 0.5 ml was removed (using a 1 ml syringe) and transferred to a universal bottle marked at 15 ml. Physiological saline was added up to the 15 ml mark. 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 min. 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 two other people using a tally counter. An average was

then obtained to determine the number of eggs per ml of the egg suspension.

Evaluation of infectivity of the eggs

This was done according to the methods of Silverman (1956). The measure of the infectivity of the eggs was evaluated by comparing the number of larval cysticerci, which 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 embryos in a sample, which appeared motile after treatment with the hatching solution (Stevenson 1983; Silverman 1954). The following infectivity formula was used:

$$Infectivity\ index = \frac{Number\ of\ cysts\ found\ at\ post-mortem}{\%\ Mortility \times\ Number\ of\ eggs\ fed}$$

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

Naturally infested cattle

Naturally infested Zebu herds were identified through history and reports from the District Veterinary Officer in Samburu District. Ninety-six animals were bled for serum and the serum samples tested for circulating antigens of *C. bovis* by an antigen-ELISA test (Ag-ELISA). Sixteen cattle with high Ag-ELISA optical density (OD) values (values that are above Ag-ELISA OD cut-off value, which was taken as the mean of the negative controls plus three standard deviations) (16-seropositive group) and 9 others with very low Ag-ELISA OD values (values that are far much below Ag-ELISA OD cut-off value) (9-seronegative group), were selected, bought and brought to the NVRC, Muguga (Table 1). The animals' faeces were sampled and microscopically examined for nematode and fluke infestations using modified McMaster egg counting and Boray sedimentation techniques, respectively. This was done in accordance with the standard operating methods in the helminthology laboratory at NVRC, Muguga. Those animals, which were found infested, were treated with a wormicide to avoid cross-reactions from closely related parasites with *T. saginata*. The animals were then kept in a *T. saginata* cysticercosis-free environment (an environment with stringent safety measures and strict adherence to a set of operating standard rules with a view to controlling bovine cysticercosis transmission cycle: cattle (other animals)↔environment↔humans) until slaughter. They were bled on arrival and once every month. The serum samples were used to test for *C. bovis* circulating antigens by Ag-ELISA. The animals were slaughtered in the 3rd month and examined for the recovery of cysticerci, which were then classified as either live or dead using cattle bile. The recovery of cysticerci was done by total dissection of a half of the carcass of each animal. This was done by thinly slicing the entire musculature of the carcasses to recover the cysticerci. The number of cysticerci obtained in one half was noted and doubled to get the total number of cysticerci in the whole animal.

Artificially infested calves

Thirty-two neonatal calves, 3 to 34 days old, were bought from the Konza Ranch at Kapiti Plains Estate, Machakos District and brought to the NVRC. Immediately, they were bled and the serum tested for circulating *T. saginata* antigens as explained in the "Naturally infested calves" section. The calves were kept in worm/*T. saginata* cysticercosis-free pens and fed on milk initially and later on calf weaner pellets, hay and water ad libitum. Unfortunately, 2 calves died before infestation with *T. saginata* eggs. The infestation of the calves was staggered. The first 15 calves were given eggs earlier than the second lot but the eggs were always administered when the calves were 2–212 months old. Serial dilutions were made from the egg suspension to get the number of eggs required for infestation per animal in each group. The first 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 2,500 eggs each, group 3 received 5,000 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 till slaughtered in the 15th week post-infestation for post-mortem examination as in the "Naturally infested calves" section. The carcasses were thinly sliced and the cysticerci present recovered. The recovered cysticerci were tested for their viability also. The second group of 15 calves was treated similarly.

Enzyme-linked immunosorbent assay (ELISA): antigen detection

The ELISA method utilised in the study was based on screening serum samples for circulating *T. saginata* antigens using a mouse monoclonal antibody (McAb), HP10, as described by Harrison et al. 1989. Linbro polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., VA, USA.) were coated with a monoclonal antibody (McAb), 100 μl per well of HP10 an IgM isotope that detects glycoproteins as antigens of viable *C. bovis* (1) at the protein concentration of 10 ug/ml in borate buffered saline

Table 1 Recovery of cysticerci from naturally infested cattle and Ag-ELISA optical density at 450 nm wavelength

Cattle number	Total dissection			Ag-ELISA optical density (OD) readings		
	Live	Dead	Total	At selection	During monitoring	At slaughter
715N	2	6	8	0.110 (0.150)	0.091	0.060
705P	37	17	54	0.334 (0.064)	0.401	0.116
708P	5	6	11	0.390 (0.064)	0.290	0.283
988P	31	22	53	0.268 (0.118)	0.109	0.094
713P	25	19	44	0.353 (0.150)	0.415	0.172
956N	2	6	8	0.061 (0.064)	0.010	0.013
969P	8	13	21	0.391 (0.064)	0.469	0.429
958P	7	8	5	0.186 (0.064)	0.238	0.108
724P	7	12	19	0.244 (0.064)	0.316	0.171
716P	24	19	43	0.685 (0.150)	0.598	0.565
721P	22	9	31	0.299 (0.150)	0.416	0.166
718P	55	22	77	0.282 (0.150)	0.316	0.285
723N	0	1	1	0.046 (0.150)	0.001	0.014
720N	0	2	2	0.096 (0.150)	0.046	0.005
976N	0	8	8	0.003 (0.064)	0.100	0.090
714N	0	15	15	0.008 (0.150)	0.005	0.001
966P	5	8	13	0.076 (0.064)	0.110	0.145
972N	2	4	6	0.037 (0.064)	0.007	0.018
717N	1	2	3	0.020 (0.150)	0.009	0.010
701P	55	39	94	0.266 (0.118)	0.468	0.311
971N	2	10	12	0.001 (0.064)	0.015	0.026
707P	5	11	16	0.266 (0.118)	0.466	0.663
989P	42	46	88	0.105 (0.064)	0.138	0.220
965P	24	5	29	0.315 (0.064)	0.569	0.484
970P	0	0	0	0.098 (0.064)	0.187 (0.086)	0.237 (0.043)
No. detected	20	24	24	16 +ve cattle	18 +ve cattle	18 +ve cattle
Detection[a] (%)	80	96	96	64	72	72

[a] % was based on 25 animals selected from the field. Figures in parentheses represent −ve cut-off points
−ve — negative
+ve — positive
N — seronegative at selection
P — seropositive at selection

(BBS), pH 8.4. The plates were incubated at 4°C overnight or for 4 h at room temperature. The plates were washed three times with physiological saline/Tween 20 with 3 min interval between washes, blocked with 200 µl per well of phosphate buffered saline, pH 7.4 (PBS) containing bovine serum albumin (BSA) and Tween 20 and incubated for 1 h at room temperature. After washing the plates three times and allowing them to stand for 3 min between washes, undiluted test serum samples were added, 100 µl per well and incubated at 37°C for 30 min. The plates were washed three times as above and biotin-conjugated McAb HP10, diluted at 1:500 or 1:1,000 (depending on the batch of the reagent) in PBS/BSA/Tween 20, was added 100 µl per well and further incubated at 37°C for 30 min. The plates were washed three times as above and streptavidin biotinylated horseradish peroxidase conjugate diluted at 1:1,000 in PBS/BSA/Tween 20, was added 100 µl per well and the plates were further incubated at 37°C for 30 min. The plates were then washed as above and 100 µl of the substrate, 3′3′5′5′-tetramethylbenzidin (TMB), was added to each well and the incubation allowed to go on for 10–30 min, depending on the constancy of colour change in its intensity within microtiter plate wells as observed physically. The reaction was stopped with 100 µl of 0.2 M H_2SO_4 (ARISTAR) per well and the obsorbance was read at 450 nm wavelength on a computerized ELISA reader (Titertek Multiscan Plus MK11).

Statistical analysis

Negative cut-off point

The Ag-ELISA OD cut-off value, to distinguish between positive (+ve) and negative (−ve) results, was taken as the mean of the negative controls plus three standard deviations (SDs) (Onyango-Abuje et al. 1996).

Kappa statistic

The Kappa statistic (k), as a quantification of the relative quality of the amount of agreement in the results of any given two tests or methods was used and computed according to the methods of Martin et al. 1987.

Analysis of sensitivity, specificity, predictive value, accuracy and apparent prevalence of Ag-ELISA test

In evaluating the sensitivity, specificity, accuracy, prevalence and predictive value of a given test, a method or diagnostic technique, which is biologically independent of the diagnostic methods under evaluation, should be used as a gold standard to define the true health status of the animals (Martin et al. 1987). In this study, the true status of *C. bovis* infestation in cattle was established by post-mortem examination using total dissection method. Diagnostic test evaluation 2 by 2 matrix tables as described by Martin et al. (1987), was used to analyse the efficacy of Ag-ELISA in diagnosing bovine cysticercosis infestation in cattle by calculating sensitivity, specificity, accuracy, prevalence and predictive values of the Ag-ELISA in comparison with the total dissection as gold standard. Total dissection method, which gives the true infestation status of live cysticerci in cattle, was taken as a gold standard. Since the Ag-ELISA was designed to detect antigens of live cysticerci only, the evaluation was also based on live cysticerci only so that total dissection, which identifies both dead and live cysticerci, can produce a good measure of Ag-ELISA under evaluation.

Results

Antigen-ELISA and total dissection findings in naturally infested animals

The results of Ag-ELISA and total dissection in naturally infested animals are presented in Table 1. All the 16-seropositive cattle selected from the field remained seropositive throughout the 3 months of monitoring, based on calculated Ag-ELISA OD cut-off values. Two out of nine cattle in the seronegative group became seropositive during the study period and remained seropositive until slaughter in the 3rd month thereby increasing the number of seropositive cases from 16 at selection to 18 during monitoring and at slaughter (Table 1). A very good precision of the assay is therefore suggested by these results and supported by the fact that 15 of the animals were true positives at autopsy, that is, in the same 15 animals, live cysticerci were identified by the total dissection method. In summary, the assay identified 16 cases as true seropositives, 2 cases as false seropositives, 3 cases as true seronegatives and 4 cases as false seronegatives at slaughter. By the total dissection method, cysticerci were detected in 15 out of 16 and 9 out of 9 cattle in the seropositive and seronegative groups, respectively. All the 15 cattle had only both live and dead cysticerci while 4 and 5 cattle in the seronegative group had dead and both dead and live cysticerci, respectively.

Antigen-ELISA and total dissection findings in artificially infested calves

The results of Ag-ELISA and total dissection in artificially infested calves are presented in Table 2. All the 30 calves used in this experiment were confirmed seronegative for bovine cysticercosis before infestation of 24 calves with various doses of *T. saginata* eggs. The controls never showed any detectable levels of antigenemia, despite the fact that one calf was found with a live cysticercus, which was considered to have infested the calf accidentally. By the Ag-ELISA method, 9 out of 24 calves were seropositive by using serum samples collected during monitoring and at slaughter and based on calculated cut-off Ag-ELISA OD values. By the total dissection method, 23 out of 24 infested calves and 1 out of 6 control calves were found with live cysticerci. Out of the 23 calves, 8 and 15 calves had dead and both dead and live cysticerci, respectively. All the calves found seropositive by Ag-ELISA were also positive by the total dissection method. All calves found with dead cysticerci only by the total dissection method were seronegative by Ag-ELISA. Unlike in the naturally infested animals, there was no false seropositive case (there was no case with live cysticerci recovered by total dissection and seropositive by Ag-ELISA). Based on the recovery of live cysticerci by total dissection at autopsy, the assay identified 9 cases as true seropositives, 14 cases as true seronegatives and 7 cases as false seronegatives.

A measure of the infectivity of the eggs of *T. saginata* in artificially infested calves

Results of the infectivity indices are presented in Table 3. From the results, it clearly shows that the egg dose was not a factor in influencing infestation of the eggs in the calves and hence the recovery of cysticerci at autopsy. Infestation of *T. saginata* eggs in all the calves was relatively very poor with the lowest index being 0.01 and the highest 0.02.

Analysis of the efficacy of Ag-ELISA

The results of the analysis of the efficacy of Ag-ELISA are summarised in Table 4. The results give a full spectrum of the reliability of the Ag-ELISA in diagnosing bovine

Table 2 Post-mortem findings of 30 calves given various doses of *T. saginata* eggs together with their Ag-ELISA optical density (OD) readings at 450 nm wavelength

Calf no.	Egg dose	Total dissection			Ag-ELISA optical density (OD) readings		
		Live	Dead	Total	Before infestation	During monitoring	At slaughter
4158	00000	0	0	0	−0.002	−0.001	−0.003
4157	00000	0	0	0	0.001	0.002	0.001
4151	00000	1	0	1	−0.004	0.005	0.006
4170	00000	0	0	0	−0.024	−0.007	−0.004
947	00000	0	0	0	−0.011	−0.018	−0.010
944	00000	0	0	0	−0.030	0.001	0.003
4160	2,500	152	11	153	−0.004	0.362	0.783
4153	2,500	17	18	35	0.007	0.000	0.003
4154	2,500	76	12	89	0.005	0.186	0.387
4152	2,500	3	4	7	0.003	0.018	0.009
172	2,500	0	28	28	0.002	−0.002	−0.004
4173	2,500	0	10	10	−0.027	0.031	0.050
951	2,500	14	1	45	−0.022	0.096	0.125
945	2,500	0	0	0	−0.023	−0.013	−0.012
4162	5,000	0	8	8	0.006	0.017	0.000
4161	5,000	124	38	162	0.000	0.189	0.317
4166	5,000	0	22	22	0.000	−0.001	−0.006
4155	5,000	2	36	38	0.003	0.027	−0.00
4171	5,000	0	4	4	−0.018	−0.001	−0.004
4167	5,000	1	14	15	−0.043	0.051	0.024
950	5,000	193	2	195	−0.028	0.181	0.872
948	5,000	0	187	187	−0.018	0.008	0.002
4164	10,000	62	29	91	−0.003	0.076	0.060
4165	10,000	59	26	85	0.000	0.316	0.697
4163	10,000	0	249	249	−0.002	−0.003	−0.006
4159	10,000	12	61	73	0.005	0.001	0.002
4175	10,000	0	8	8	−0.039	−0.010	−0.007
4169	10,000	55	4	59	−0.045	0.682	0.879
949	10,000	1	5	6	−0.015	−0.019	−0.010
946	10,000	93	1	94	−0.012	0.292	0.486
					(0.032)	(0.074)	(0.059)
Number detected		15	23	23	00	9	9
Detection (%)		62.50	95.85	95.83	00.00	37.50	37.50

[a] The percentages were based on 24 infested calves. Figures in parentheses represent negative cut-off points

Table 3 Infectivity of the eggs of *T. saginata* in artificially infested calves

Group	No. of calves per group	Mean egg dose infestation	Mean no. of cysticerci recovered			Mean infectivity indices[a]
			Live	Dead	Total	
1	6	0000	0	0	0	0.00
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

[a] The calculation of the infectivity indices was based on the total number of cysticerci recovered in individual calves

cysticercosis infestation in cattle under both controlled and field situations.

Discussion

World survey records on bovine cysticercosis clearly show that routinely used post-mortem meat inspection method as the only public health control measure, may not help reduce the rocketing economic losses accruing from the infestation (Fan 1997; Gracey and Collins 1992; Harrison et al. 1989; Mann 1983; Grindle 1978). This problem occurs worldwide and is zoonotic in nature, being very much related to sociocultural practices of people in their respective communities and environments. Alfonso (1997) therefore

Table 4 Summary of the Ag-ELISA evaluation in both naturally and artificially infested animals, based on live cysticerci and total dissection as a gold standard

Characteristics		Naturally infested animals (n=25) (%)	Artificially infested animals (n=24) (%)
Sensitivity of the test		80.00	60.00
Specificity of the test		60.00	100
Predictive value of the test		88.89	100
Accuracy of the test		76.00	75.00
Apparent prevalence of the test		72.00	37.50
Kappa statistics (k)[a] (at p>0.05)		0.482	0.374
Percentage of animals detected by both tests	Positive	32.00	37.50
	Negative	20.00	37.50

[a]Kappa statistics values (k) are not in percentages, instead, they are proportions

advises that solutions to the problem require strategies defined for each community and its environment. The results obtained by this investigation provide for ante-mortem diagnosis of bovine cysticercosis using Ag-ELISA in Kenyan cattle. The study evaluates the efficacy of Ag-ELISA by using the total dissection method as the ultimate confirmatory test (the gold standard of validity) to indicate the presence or absence of bovine cysticercosis. However, the confirmatory test may not be 100% efficient due to human errors and other unavoidable environmental factors reported earlier (Kang'ethe 1995; Cheruiyot 1981; Walther and Koske 1980). Nevertheless, these problems affecting the gold standard of validity might have also to some extent contributed partly to the occurrence of false positive cases reported in the results of this study.

All the statistical analysis of the results were based on the live cysticerci only so that the assay and the total dissection method compare at the same level without the total dissection method underestimating the Ag-ELISA. Moreover, only live cysticerci are of great importance from public health point of view because the transmission cycle in an environment is potentiated and maintained by people ingesting these live cysticerci and contaminating the cattle grazing environment with their faeces containing *T. saginata* eggs.

There was considerable variation in the diagnosis of bovine cysticercosis using the two methods: total dissection and Ag-ELISA. In naturally infested animals, 80% and 72% of the animals were diagnosed positive for the infestation by total dissection method and Ag-ELISA, respectively (Table 1). While in the artificially infested calves, the order was 62.50% and 37.50%, respectively (Table 2). The Ag-ELISA figures confirm the apparent prevalence figures obtained with the diagnostic test evaluation tables shown in Table 4. In both population samples, the apparent prevalent proportions obtained by the assay show a relatively high incidence of the infestation, particularly in the population sample from the field. This implies that the population sample of naturally infested animals came from a zone with a very high rate of bovine cysticercosis infestation. In naturally infested animals, the assay gave a sensitivity of 80.00% (in animals harbouring 2–55 live cysticerci) while in artificially infested calves; the sensitivity of the assay was 60.00% in animals harbouring 14–193 live cysticerci. The average of these two values gives a sensitivity of 70.00%, which is very close to the sensitivity value obtained by Correa et al. (1989) (72.00%) when using the same monoclonal antibody, HP10 in human cysticercosis infestation. The low sensitivity of the assay in artificially infested calves was probably due to the occurrence of high cases of false seronegatives, due to either the calves mounting a strong immune response that might have managed to kill quite a number of cysts as evidenced in Table 2 or poor infectivity of *T. saginata* eggs administered to them as shown in Table 3. The former explanation describes the concomitant immunity phenomenon, which has long been considered a common feature of worms such as schistosome infections (Coelho et al. 1995). Antigens resulting from dead cysticerci are not detected by this assay (Harrison et al. 1989; Onyango-abuje et al. 1996) (Table 2). This probably was also the possible reason for the lower apparent prevalence figures obtained with artificially infested calves than in the natural infestations by the assay (Table 4). Poor infectivity of the eggs in calves is however due to a number of reasons. The ability of tapeworm eggs to produce an infestation in the appropriate intermediate host is dependent on: the state of maturity of the eggs, and the resistance of the host by innate and specific acquired immunity (Silverman 1956). The availability of hatching (gastric juice) and activation (bile salts) stimuli at optimal conditions in the alimentary canal of cattle determines to a great deal the hatchability of oncospheres to cause the infestation (Silverman 1954). These four factors cannot be ruled out in this case. In addition, human error, which might have probably occurred during determination of percentage motility by counting the number of eggs for infestation, greatly contributed to the poor infectivity indices obtained in Table 3 and might have also had a negative impact on the recovery rate of cysticerci at autopsy. This problem could be solved probably by using trickle infestations in susceptible intermediate hosts.

In artificially infested calves, the predictive value of the assay was 100% while in natural infestations, the value was 88.89%. The specificity of the assay in natural infestations (60.00%), was far much below that in artificially infested calves by 40.00% (Table 4). This great variation is due to a number of reasons: (1) the use of small sample size, (2) the absence of false positive cases in artificially infested calves, thus making the assay very specific and giving it a good reflection of the way its results can be used in the field and (3) the presence of a significant proportion of false positive cases in naturally infested animals, thus lowering specificity and predictive values of the assay. From predictive values obtained in both natural and artificial infestations, the likelihood of this assay diagnosing and identifying diseased animals in the field is quite good, ranging from 0.89 to 1 (Table 4).

Usually, from the epidemiological point of view, sensitivity and specificity describe the discriminatory power of the test. For most of the surrogate tests like Ag-ELISA being discussed here, there is an inverse relationship between sensitivity and specificity (Table 4). That is, if the negative cut-off OD values of the assay are altered so that the sensitivity is increased, the specificity automatically decreases. This is because, the circulating antigens of *C. bovis* being measured, may be present in non-diseased as well as diseased animals, although at different levels and with different frequencies and often their distributions overlap, producing an inverse relationship between sensitivity and specificity (Martin et al. 1987) (Table 4).

Accuracy, which measures the overall performance of a test in the laboratory or in the field, was quite good in both natural (76.00%) and artificial (75.00%) infestations (Table 4). This indicates that the assay gave a true approximation of the infestation in the animals. From the analysis of these results, it therefore follows that the assay may be the most appropriate method of diagnosing bovine cysticercosis infestation in cattle. However, because of false positive and negative reactions still associated with the assay in light infestations and even sometimes in heavy infestations (Tables 1 and 2), it cannot be confidently used to diagnose animals at individual level. In heavily infested herds, the assay may be used for individual diagnosis but accompanied by other diagnostic measures to fully confirm the disease in the animals. On the other hand, research continues to explain the occurrence of these false positive and negative reactions.

Of the 25 animals selected from the field, 32.00% cases were detected positive for bovine cysticercosis while 20.00% cases were detected negative for bovine cysticercosis by both Ag-ELISA and total dissection (k=0.482; p>0.05). In this case, the Kappa measure of agreement was moderate, thus suggesting that the two methods had about half of their results purportedly agreeing in diagnosing bovine cysticercosis infestation as either positive or negative for bovine cysticercosis. Of the 24 artificially infested calves with *T. saginata* eggs, 37.50% cases were detected positive and negative for bovine cysticercosis by both Ag-ELISA and total dissection (k=0.374; p>0.05). In this case also, the Kappa measure of agreement was more or less moderate like before. It is however worthwhile to note here that as a surrogate test, the results of the assay are directly dependent on the absence or presence of antigens released by cysticerci. It therefore, follows with logical necessity that it is actually unlikely that what the total dissection as a pathognomonic test picks as positive for bovine cysticercosis, is also picked by the assay. The animal may have the cysticerci but tests negative by the assay, for whatever happens with the antigens after being released in the bloodstream is not exactly known and is beyond the scope of this study. However, the results from Table 2 shows that it is likely that what is picked by the assay as positive for bovine cysticercosis may also be positive by the total dissection method except for two cases shown in Table 1 (970P and 976N). The Kappa statistic value was higher in natural infestations than in artificial ones. This was because in natural infestations the diagnosis of bovine cysticercosis by both total dissection and Ag-ELISA was more in agreement with one another than in artificial infestations, which is probably attributed to poor infestations of the eggs as shown in Table 3. Generally, in both naturally and artificially infested animals, there was little agreement of results between animals diagnosed either positive or negative for bovine cysticercosis by the two methods (Table 4). This discrepancy, if explained, could probably lead or direct future research whose results may be used to validate the assay further. More particularly is the issue of the false positive and negative reactions, which is still a matter of speculation, thus spearheading hot debate on this subject. The false positive cases still associated with the assay are partly due to the fact that it is not yet really known when cysticerci antigens disappear from circulation after the death of the cysticerci. Also, contributing to the above discrepancy, are the antigen–antibody reactions that remove antigens from circulation by forming undissociated complexes, thereby resulting in false negative cases. Freeing parasite antigens from antigen–antibody complexes might be the ultimate solution to, not only avoiding the occurrence of the above-mentioned discrepancy, but also to increase the sensitivity of this assay. Brandt et al. (1992) attempted to free parasite antigens from the antigen–antibody complexes according to the method of Weil and Liftis (1987), but they did not succeed. Their method involved mixing one part of serum and three parts of 0.1 M Na_2 EDTA (pH 4) and after boiling for 5 min, centrifuging twice at 9000 $\times g$ for 3 min and then the resultant supernatant is recovered for use in the ELISA.

The seroconversion of the two animals in the naturally infested group from being seronegative to seropositive during the study period in a cysticercosis-free environment remains another discrepancy yet to be understood and explained.

From a general point of view, the results show that there wasn't much difference in the performance of the assay between naturally infested animals selected from the field and artificially infested calves bought from the ranch except in the infestation procedure. This factor was relatively crucial in influencing the low figures of sensitivity and apparent prevalence obtained in artificially infested calves (Table 4). In the field, the naturally infested animals are subjected to accumulative trickle infestations producing multiple infestations on different occasions whereas the artificially infested calves were subjected to a single dose administered only once, which may or may not produce the infestation depending on the availability of optimal conditions in the susceptible host, the maturity of the eggs and the immunity of the host. This does not really assume the field situation and therefore, when used alone, it becomes very perplexing to imagine and ascertain the performance of the assay in the field, outside the controlled conditions. However, this situation does not apply to this study because the two were compared and contrasted accordingly.

From the foregoing therefore, the assay can, at best, be used as a screening test and not as a diagnostic test due to its unexplained false positive and negative reactions. However, the assay may provide for individual diagnosis when used with other test reliably known to diagnose the infestation. If this assay will be fully developed in the future as a diagnostic test, there is a need to increase its sensitivity so that it can be reliably used as a field test since the burdens of live cysticerci present in the whole carcasses of lightly infested animals, which epidemiologically are the most important group in a population, vary between 1 and 17, which the assay is currently unable to detect consistently. Should further research provide solutions to the problems currently surrounding the assay, it may be one of the most powerful diagnostic tools in the control of bovine cysticercosis infestations and drastically reduce economic losses due to downgrading and condemnation of the infested carcasses during meat inspection.

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