A comparison of meat inspection method and an Antigen-ELISA test in the diagnosis of bovine cysticercosis in Kenyan cattle

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ABSTRACT

A total of 55 cattle divided into two groups of experimentally (n =30) and naturally (n = 25) infected animals were diagnosed by Antigen-ELISA (Ag-ELISA) and later by meat inspection method. Total dissection method was used as a gold standard to indicate the absence or presence of bovine cysticercosis infection in cattle. The level of agreement between the two methods was, on average, lower in naturally infected animals than in experimental calves. This was because in natural infections, there were more light infections than in experimental infections and these could not be detected by meat inspection method but could be detected by either Ag-ELISA or total dissection. The studies show that besides other advantages, Ag-ELISA is more sensitive than meat inspection in the diagnosis of bovine cysticercosis, detecting 60-80% of cattle with bovine cysticercosis compared with 40-53.3% by meat inspection method. Ag-ELISA is therefore recommended for use, accompanied by other control measures, especially treatment of infected ones or vaccination.

Key words: Meat inspection ; Antigen-ELISA; Diagnosis; Bovine cysticercosis; Kenyan cattle.

RÉSUMÉ

Un total de 55 bœufs infectés divisés en deux groupes expérimentaux (n=30) et naturels (n=25) ont été évalués pour consommation par la méthode l'ELISA (Ag-ELISA) et puis par la méthode d'inspection de la viande. La technique de dissection totale a été utilisée comme méthode standard dans le but de mettre en évidence la présence ou l'absence de l'infection à la cysticercose bovine. Les animaux naturellement infectés ont montré un taux d'infection moins significative que ceux du groupe expérimental. Ces infections ont été mieux détectées par la méthode Ag-ELISA que par celle de l'inspection de la viande. Cette observation montre que la méthode Ag-ELISA est plus sensible que celle de l'inspection de la viande dans le diagnostique de la cysticercose bovine. La méthode Ag-ELISA est ainsi recommandée pour ce type de diagnostique et elle devrait être accompagnée d'autres mesures de contrôle, comme par exemple le traitement des animaux infectés ou de la vaccination des animaux.

Mots clés: Inspection, viande, Antigène, ELISA, Diagnostique; cysticercose, bovine; bovins du Kenya.

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1. INTRODUCTION

Taenia saginata is among the zoonotic parasites whose infection interferes with the health of man, and adversely affects the production of livestock industry world wide. The economic losses accruing from the infection are substantial (Grindle, 1978, Mann, 1983; Gracey and Collins, 1992 in addition to the loss of export markets from endemic zones (Harrison *et al.*,1989. Meat inspection which is the most important public health measure practised, identifies only heavily infected cattle but when it is too late to avoid incurring losses. An ante-mortem diagnostic test would be desirable and superior to meat inspection method currently in use and decisions regarding the management of the infection can be made before slaughter of the animal to avoid unnecessary economic losses(Onyango-Abuje *et.al.*,1996) Such an ante-mortem test (like Antigen-ELISA test currently under development) has been described by Harrison *et.al.*,(1989) but the test has not yet been evaluated of its reiability in diagnosing bovine cysticercosis in comparison with the normally used meat inspection method. The assay is a double sandwich ELISA based on a mouse monoclonal antibody (McAb) coded as HP10. The monoclonal antibody is an IgM isotope which detects antigens (glycoproteins) of viable *T. saginata* cysticerci in the cattle sera. The McAb, HP10 reacts with a repetitive carbohydrate epitope on lentil-lectin adherent glycoproteins found in the biosynthetic excretions/Secretions, on the surface and somatic of *T. saginata* cysticerci. The assay has been used in Swaziland to determine seroprevalence of the infection in different geographical zones and antigenemia levels in cattle (Hughes *et al.*,1993). In Kenya, the assay has been used at best to identify ranches and farms with herds of cattle infected with cysticerci (Onyongo-Abuje *et al.*,1996). This therefore, indicates that the assay is feasible and requires more research work in order to shade more light on its reliability. Present work was carried out so as to evaluate the reliability of the assay as a diagnostic tool for the diagnosis of bovine cysticercosis in cattle by comparing it with the routinely used meat inspection method.

2. MATERIALS & METHODS

2.1. Collection of the parasite eggs

Taenia saginata proglottids were collected from human excrement in Mathare Valley slums of Nairobi area and brought to NVRC laboratories. The proglottids were collected in physiological saline (0.15M NaCl) containing 200 units/ml of Crystapen benzylpencillin (Glaxo Laboratories, U.K.); 0.2mg/ml Streptomycin Sulphate (Glaxo Lab., U.K.) and 5ug/ml fungizone (Squibb and Sons, Inc., New Jersey), as a fungicidal drug for preservation. The eggs were teased from the proglottids and washed through a tier of three sieves with 250mm, 150mm and 30mm apertures, respectively. The 30mm aperture sieve retained the eggs which were then transferred into a universal bottle containing physiological saline and antibiotics stored at 4EC untill required.

2.2. Testing the viability of the eggs

This was done in accordance with Stevenson's method (1983) . This process was first aimed at removing the embryophore using Sodium hypochlorite. Equal volumes of egg suspension and 10% Sodium hypochlorite solution were mixed together in a 15ml graduated plastic centrifuge tube and shaken vigorously for 2 minutes. Immediately, the tube was filled to the 15ml mark with physiological (normal) saline and centrifuged for 5 minutes at 4xg on the MSE Minor Centrifuge. The supernatant was drawn off leaving approximately 0.25ml to which normal saline was added to the original mark of 15ml and centrifuged again. This was repeated before a freshly made hatching solution (1.17g Sodium hydrogen carbonate + 0.05g Trypsin in 100ml of deionised water) was added upto the 2ml mark. One ml of bovine bile was added and the tube was transferred to a 37°C water bath and incubated for 45 minutes. Every 10 minutes the tube was removed and shaken vigorously for 1 minute. After incubation of the oncospheres, a drop of their suspension was put onto a slide, and examined under the microscope using both low (x10) and high (x40) power objective lenses. The number of motile oncospheres was counted in a group of a 100 oncospheres to determine their percentage. The counting was done 2 or 3 times and an average obtained. No longer than 10 minutes was spent examining one slide, otherwise death of the oncospheres would occur due to drying of the slide.

2.3. Egg counts

A bottle containing eggs was gently shaken and 0.5ml was removed (using a 1ml syringe) and transferred to a universal bottle marked at 15ml. Normal saline was added upto 15ml 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 minutes. Eggs within the grid in both chambers of the McMaster slide were counted using a microscope with x2.5 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 0.3ml) and multiplying by the dilution factor of 50. Three counts were made and cross-checked by at least 2 other people using a tally counter. An average was then obtained to determine the number of eggs per ml of the egg suspension.

2.4. 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 caculated from the percentage of hexacanth embroys in a sample, which appeared motile after treatment with hatching solution (Silverman,1954a and Stevenson, 1983). The following infectivity formula was used:-

$$\text{Infectivity index} = \frac{\text{No. of cysts found at post-mortem}}{\text{\% motility x No. of eggs fed}}$$

Acording 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.

2.5. Naturally infected cattle

Naturally infected Zebu herds were identified through history and reports from the District Veterinary Officer in Samburu District. Ninety six animals were bled for serum. Blood samples were transported to the laboratory in a cool box, kept overnight at 4EC and centrifuged at 2500g for 30 minutes in a refrigerated centrifuge.The resultant sera were aspirated and stored in eppendorf tubes at -20EC till required.The serum samples were tested for circulating antigen by Ag-ELISA (High Ag-ELISA reading usually meant a high likelihood of positivity of bovine cysticercosis). Based on the Ag-ELISA optical density (OD) values, twenty five steers (about 1-1 1/2 years old) were selected, bought and brought to the National Veterinary Research Centre (NVRC), Muguga, of the Kenya Agricultural Research Institute (KARI). Upon their arrival at NVRC, the animals were bled again and thereafter once every month for 3 months for serum which was then tested for circulating cysticerci antigens. Sixteen cattle with high Ag-ELISA OD readings (positive cattle) and 9 others with low OD readings (negative cattle), were utilized in the experiment. They were feacal sampled for nematode and fluke infections using modified McMaster egg counting and Boray sedimentation techniques, respectively. This was done in accordance with the standard operating methods in helminthology laboratory at NVRC, Muguga. Those animals which were found infected were treated with a wormicide.

The animals were slaughtered in the 3rd month and examined for cysticerci first by routine meat inspection procedures as stipulated by Kenya Meat Control Act - 1977 and then followed by total dissection of a half of the carcass. The 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 get the total number of cysticerci in the whole animal.

2.6. Experimentally infected calves.

Thirty two neonatal calves, 3 to 34 days old, were bought from Konza Ranch at Kapiti Plains Estate, Machakos District and brought to NVRC in groups of 16 in two separate instances. Immediately, they were bled and the serum tested for circulating T. saginata antigens as explained in 2:3:1. below. The calves were kept worm/cysticercosis free in pens and fed on milk initially and later on calf weaner pellets and hay. Unfortunately, 2 calves died before infection with T. saginata eggs. The infection 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 - 22 months old.

Serial dilutions were made from the egg suspension to get the number of eggs required for infection 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 no eggs (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 administered with T. saginata eggs and thereafter they were bled every two weeks till slaughtered in the 15th week. The second group of 15 calves was treated similarly.

2.7. Post - mortem examination

2.7.1. Meat inspection

Meat inspection was done in accordance with the Kenya Meat Control Act-1977 which stipulates that the cheek muscles (masseter-external muscles and Pterygoid-internal muscles), tongue, heart and *Masculus triceps brachii* (shoulder muscles) must be incised and examined for the presence of *C. bovis*. For cheek muscles, two deep linear incisions were made parallel to the mandible from its upper muscular insertion. The tongue (also examined by palpation) was incised lengthwise on the lower surface from base to root while the heart was split from base to the apex and further incisions made into the muscles. Three deep and parallel transverse incisions were made above the point of the elbow in the shoulder muscles.

2.7.2. Total dissection

Half of each carcass of naturally infected cattle was cut and divided into the following regions:- head, tongue, neck and hump, fore legs, pelvis, hind legs, lumbar,

rumen, lungs, heart, liver, kidneys and diaphragm. The muscles of these parts were cut into very thin, almost transparent slices for recovery of cysticerci. The cysticerci encountered during slicing were counted, doubled (for the whole carcass) and recorded. It was assumed that the cysticerci were evenly distributed in the carcasses. However, visceral organs were not halved. In experimental calves, the whole carcass was examined for cysticerci because the animals were small in size.

2.8. Enzyme - linked immunosorbent assay (ELISA):Antigen detection.

The ELISA method utilized 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). The assay involved sensitization of Linbro polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Virginia) with a monoclonal antibody, (HP10) at the protein concentration of 10ug/ml in borate buffered saline (BBS), pH 8.4 , 100 ul per well. The plates were incubated at 4°C overnight or for 4 hours at room temperature. The plates were washed three times with normal saline/Tween 20 with 3 minutes interval between washes, blocked using 200 ul of phosphate buffered saline, (PBS) pH 7.4 with bovine serum albumin (BSA)/Tween 20, and incubated for 1 hour at room temperature. The plates were again washed three times, allowing to stand for 3 minutes between washes. The undiluted test serum samples were added, 100 ul per well. The plates were then incubated at 37°C for 30 minutes and thereafter, washed three times as above. Biotin - conjugated McAb HP10, diluted at 1:500 to 1:1000 (depending on the batch of the reagent) in PBS/BSA/Tween 20, was added 100 ul per well and incubated at 37°C for 30 minutes. The plates were washed three times as above and Streptavidin biotinylated horseradish peroxidase conjugate at 1:1000 in PBS/BSA/tween 20 was added 100 ul per well and the plates incubated at 37°C for 30 minutes. The plates were then washed as above and 100 ul of the substrate, 3'3'5'5'- tetramethylbenzidin (TMB),was added to each well and the incubation allowed to go on for 10-30 minutes. The reaction was stopped with 100 ul of 0.2M H_2SO_4 (ARISTAR) per well and the optical density was read at 450nm on an ELISA reader (Titertek Multiscan Plus MK11).

2.9. Statistical analysis

2.9.1. Negative cut - off point.

An optical density 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).

2.9.3. Kappa statistic.

Kappa statistic (k), which compared the measure of agreement between any given two tests or methods, was used and computed according to the methods of Martens et al., (1987).

2.9:4. Analysis of sensitivity, specificity, predictive value, accuracy and apparent pprevalence of Ag-ELISA and Meat Inspection methods under study.

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 methods used to define the true health status of the animals, should be used as a gold standard (Martens *et al.*, 1987). In this study, the true status of *C. bovis* infection was established by post-mortem examination (Total dissection) done by thinly slicing the masculature of the carcasses to recover the cysticerci. A four-fold table (Martens *et al.*, 1987) shown below was used to evaluate sensitivity, specificity, accuracy, prevalence and predictive value of Ag-ELISA and meat inspection methods.

Test under study	Gold Standard		
	Positive cases	Negative cases	Total
Positive cases	a	b	a + b
Negative cases	c	d	a + d
Total	a+c	b +d	a +b +c + d

Where, a - True Positive
c - False Negative
b - False Positive
d - True Negative.

From the table,

$$\text{Sensitivity} = \frac{a}{a+c} \qquad \text{Specificity} = \frac{d}{b+d}$$

$$\text{Predictive value} = \frac{a}{a+b}$$

$$\text{Accuracy} = \frac{a+d}{a+b+c+d} \qquad \text{Prevalence} = \frac{a+b}{a+b+c+d}$$

3. RESULTS

3:1. Antigen-ELISA findings in naturally infected animals.

The results of the Ag-ELISA test are summarized in Table 1. The Table shows post-mortem results of each of the 25 animals selected from the field together with the Antigen-ELISA optical density readings before and at slaughter. The seropositivity and negativity values were based on the cut- off points as shown in Table 1. The results below shows that the Ag-ELISA test still gives cases of false negative reactions (live cysticerci recovered by total dissection but seronegative with Ag-ELISA) and false positive reactions (no live cysticerci

recovered by total dissection but seropositive with Ag-ELISA). The lowest number of cysticerci detected by Ag-ELISA test in these animals was 2, but it consistently detected animals with 5 live cysticerci. However, other animals with 2 live cysticerci escaped detection. Antigenemia was consistently demonstrated in 16 seropositive animals but not in 9 seronegative ones (Figure 1). Also, animals habouring only dead cysticerci or those harbouring less than 2 live cysticerci, could not be detected.

Table 1: Post-mortem findings of 25 animals selected from the field together with their Antigen-ELISA readings (Optical Density values) at 450nm wavelength.

Cattle No.	Total Dissection			Meat Inspection	AG-ELISA READINGS (OD VALUES)	
	L	D	T	T D L	AT SELECTION	AT SLAUGHTER
715	2	6	8	0 0 0	0.110 (0.150)	0.060
705	37	17	54	1 0 1	0.334 (0.064)	0.116
708	5	6	11	0 0 0	0.390 (0.064)	0.283
988	31	22	53	2 0 2	0.268 (0.118)	0.094
713	25	19	44	1 1 0	0.353 (0.150)	0.172
956	2	6	8	0 0 0	0.061 (0.064)	0.013
969	8	13	21	2 1 1	0.391 (0.064)	0.429
958	7	8	15	4 0 4	0.186 (0.064)	0.108
724	7	12	19	0 0 0	0.244 (0.064)	0.171
716	24	19	43	0 0 0	0.685 (0.150)	0.565
721	22	9	31	2 2 2	0.299 (0.150)	0.166
718	55	22	77	8 1 7	0.282 (0.150)	0.285
723	0	1	1	0 0 0	0.046 (0.150)	0.014
720	0	2	2	0 0 0	0.096 (0.150)	0.005
976	0	8	8	0 0 0	0.003 (0.064)	0.090
714	0	15	15	10 10 0	0.008 (0.150)	0.001
966	5	8	13	0 0 0	0.076 (0.064)	0.145
972	2	4	6	0 0 0	0.037 (0.064)	0.018
717	1	2	3	0 0 0	0.020 (0.150)	0.010
701	55	39	94	3 1 2	0.266 (0.118)	0.311
971	2	10	12	1 1 0	0.001 (0.064)	0.026
707	5	11	16	0 0 0	0.266 (0.118)	0.663
989	42	46	88	2 0 2	0.105 (0.064)	0.220
965	24	5	29	3 3 0	0.315 (0.064)	0.484
970	0	0	0	0 0 0	0.098 (0.064)	0.237(0.043)
Total	361	310	671	39 20 19	16 +ve CATTLE	18 +ve CATTLE
%* Detection	80	96	96	48 32 28	64	72

KEY

OD - Optical density
T - Total
D - Dead
L - live
-ve - Negative
+ve - Positive

Figures in parentheses represent negative cut-off points.

• The percentages were based on 25 animals selected from the field.

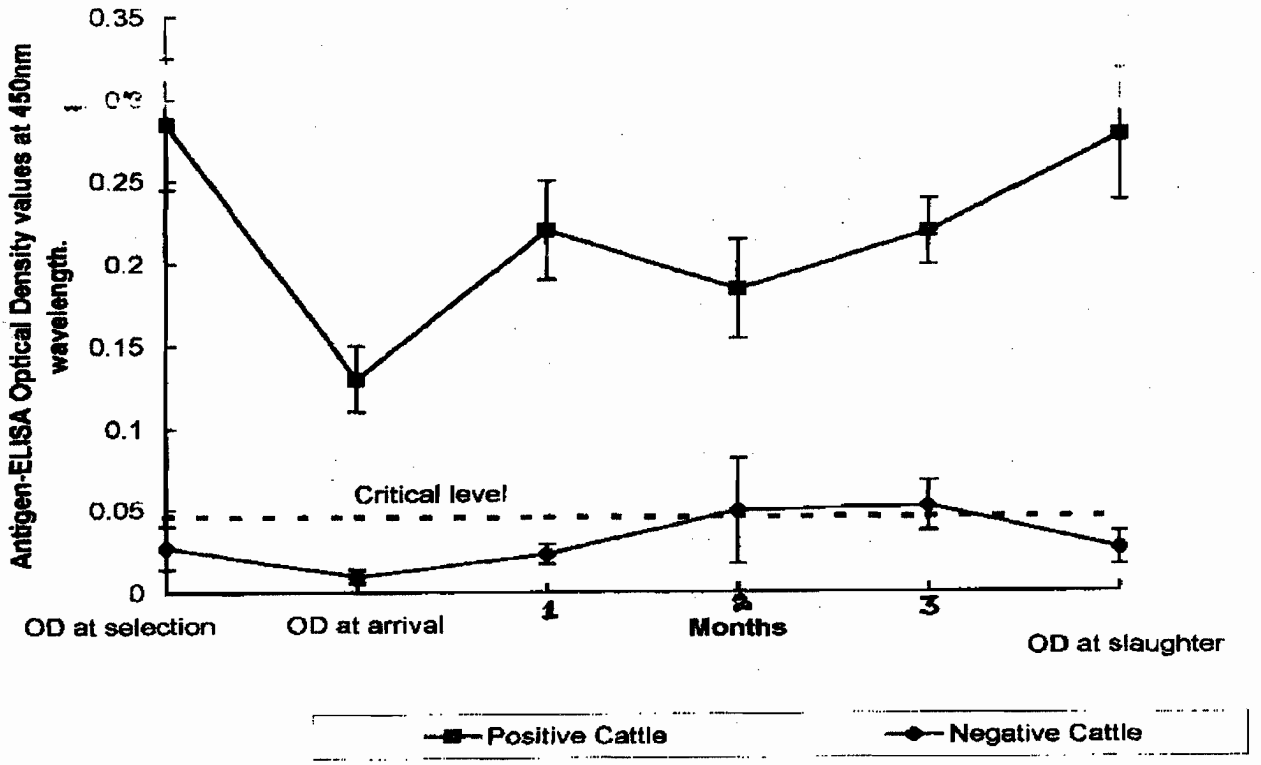

Fig.1. The average Antigen-ELISA optical density (O.D values) of 16 positive and 9 negative cattle selected from the field and monitored for three months before slaughter.

3:2. Meat inspection findings in naturally infected animals.

The results of meat inspection method from individual animals are shown in Table 1 while the frequency of cysticerci in predilection sites are shown in Table 2.

3:3.Determination of the number of animals the Ag-ELISA can detect in in Experimentally infected calves.

3:2:1. A measure of the infectivity of the eggs of Taenia saginata.

From Table 3, it is evident that the infection of calves fed with various doses of eggs of T. saginata was very variable and poor

Table 2: Frequency of cysticerci in predilection sites of naturally infected cattle during meat inspection

	CYSTS' RECORD[a]		CATTLE RECORD[b]	
Predilection sites for inspection according to Kenya Meat Control Act-1977	Total number of cysts per site for all the 25 cattle	Percentages of the number of cysts found per site during inspection	The number of animals found infected by the site inspected	Percentages of animals found infected by the site inspected
Heart	0(5)	.98(0.75)*	(4)*	36(16)
Shoulder muscles	7(2)	1.04(0.30)	(2)	6(8)
Tongue	12(12)	1.79(1.79)	3(3)	2(12)
Masseter and pterygoid muscles	0(0)	0(0)	0(0)	0(0)
Grand total	39(19)	.81(2.83)	12(7)	48(28)

* Figures in parentheses represent live cysticerci[a] or cattle[b] found with live cysticerci, respectively.

Table 3: The mean infectivity of eggs of Taenia saginata in 30 experimental calves

Group	No. of calves per group	Mean egg dose	Mean No of cysticerci recovered			Mean infectivity indices*
			Live	Dead	Total	
1	6	0000	0	0	0	0.00
2	8	2500	33	13	46	0.02
3	8	5000	44	39	83	0.02
4	8	10000	35	48	83	0.01

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

3:2:2.Recovery of Taenia saginata cysticerci in experimentally infected calves.

The recovery rate and distribution of cysticerci in various sites of the 24 infected calves at laughter, is shown in Table 4. Total dissection, like in naturally infected animals, was used as the gold standard and revealed cysticerci in all the 24 calves administered with various doses of T. saginata eggs. Twenty four of the calves administered with various doses of T. saginata eggs, were found with cysticerci (either live, dead or both) and only one calf mentioned above did not habour any cysticerci. One of the control calves was found infected with one live cysticercus at autopsy (Table 4).

3:2:3.Ag-ELISA findings in experimentally infected calves.

Table 4 shows post-mortem results of each of the 30 experimental calves used together with the Antigen-ELISA optical density readings before and at slaughter. All the 24 experimental calves were seronegative for bovine cysticercosis before administration with various doses of Taenia saginata eggs. The seropositivity and negativity values were based on the cut- off points as shown in Table 4.The controls never showed any detectable levels of antigenemia (Figure 2). Basing on the recovery of live cysticerci by total dissection at slaughter, Ag-ELISA gave 7 cases of false negative (live cysticerci recovered by total dissection but seronegative with Ag-ELISA). Unlike naturally infected animals, there was no case of false positive (no live cysticerci recovered by total dissection but seropositive with Ag-ELISA). The lowest number of cysticerci detected by Ag-ELISA in these calves was 14 live cysticerci. Antigenemia could not be demonstrated in animals habouring only dead cysticerci or those harbouring less than 14 live cysticerci. However, there was one calf (with calf code number 4153) with 17 live cysticerci was not detected by the Ag-ELISA.

Table 4: Post-mortem findings of 30 calves given various doses of Taenia saginata eggs together with their Antigen-ELISA readings at 450nm wavelength.

Calf Number	Egg No	Post-mortem finding		Ag- ELISA readings
		Total Dissection L D T	Meat Inspection L D T	Before Infection At slaughter
158	00000	0.....0.....0	0.....0.....0	-0.002 .....-0.003
4157	00000	0.....0.....0	0.....0.....0	0.001.....0.001
4151	00000	1.....0.....1	0.....0.....0	-0.004.....0.006
4170	00000	0.....0.....0	0.....0.....0	-0.024 .....-0.004
947	00000	0.....0.....0	0.....0.....0	-0.011.....-0.010
944	00000	0.....0.....0	0.....0.....0	-0.030.....0.003
4160	2500	152.....1.....153	0.....0.....0	-0.004.....0.783
4153	2500	17.....18.....35	0.....0.....0	0.007.....0.003
4154	2500	76.....12.....89	4.....0.....4	0.005.....0.387
4152	2500	3.....4.....7	0.....0.....0	0.003.....0.009
4172	2500	0.....28.....28	0.....0.....0	0.002.....-0.004
4173	2500	0.....10.....10	0.....2.....2	-0.027.....0.050
951	2500	14.....31.....45	4.....0.....4	-0.022.....0.125
945	2500	0.....0.....0	0.....0.....0	-0.023.....-0.012
4162	5000	0.....8.....8	0.....0.....0	0.006.....0.000
4161	5000	124....38.....162	2.....0.....2	0.000.....0.317
4166	5000	0.....22.....22	0.....0.....0	0.000.....-0.006
4155	5000	2.....36.....38	0.....0.....0	0.003.....-0.00
4171	5000	0.....4.....4	0.....0.....0	-0.018.....-0.004
4167	5000	1.....14.....15	0.....4.....4	-0.043.....0.024
950	5000	193.....2.....195	10.....0.....10	-0.028.....0.872
948	5000	0.....187.....87.	0.....49.....49	-0.018.....0.002
4164	10000	62.....29.....91	0.....0.....0	-0.003.....0.060
4165	10000	59.....26.....85	3.....0.....3	0.000.....0.697
4163	10000	0.....249.....249.	0.....10.....10	-0.002.....-0.006
4159	10000	12.....61.....73	1.....3.....4	0.005.....0.002
4175	10000	0.....8.....8	0.....0.....0	-0.039.....-0.007
4169	10000	55.....4.....59	17.....0.....17	-0.045.....0.879
949	10000	1.....5.....6	0.....0.....0	-0.015.....-0.010
946	10000	93.....1.....94	9.....0.....9	-0.012.....0.486
Total		864....800.....1664	72..... 68.....140	(0.032).....(0.059)
% detection*		66.67 95.83 .95.83	33.33 20.83 50	0.00 .....37.50

KEY:
L-Live
D- Dead
T-Total
*The percentages were based on 24 infected calves.
Figures in parentheses represent negative cut-off points.

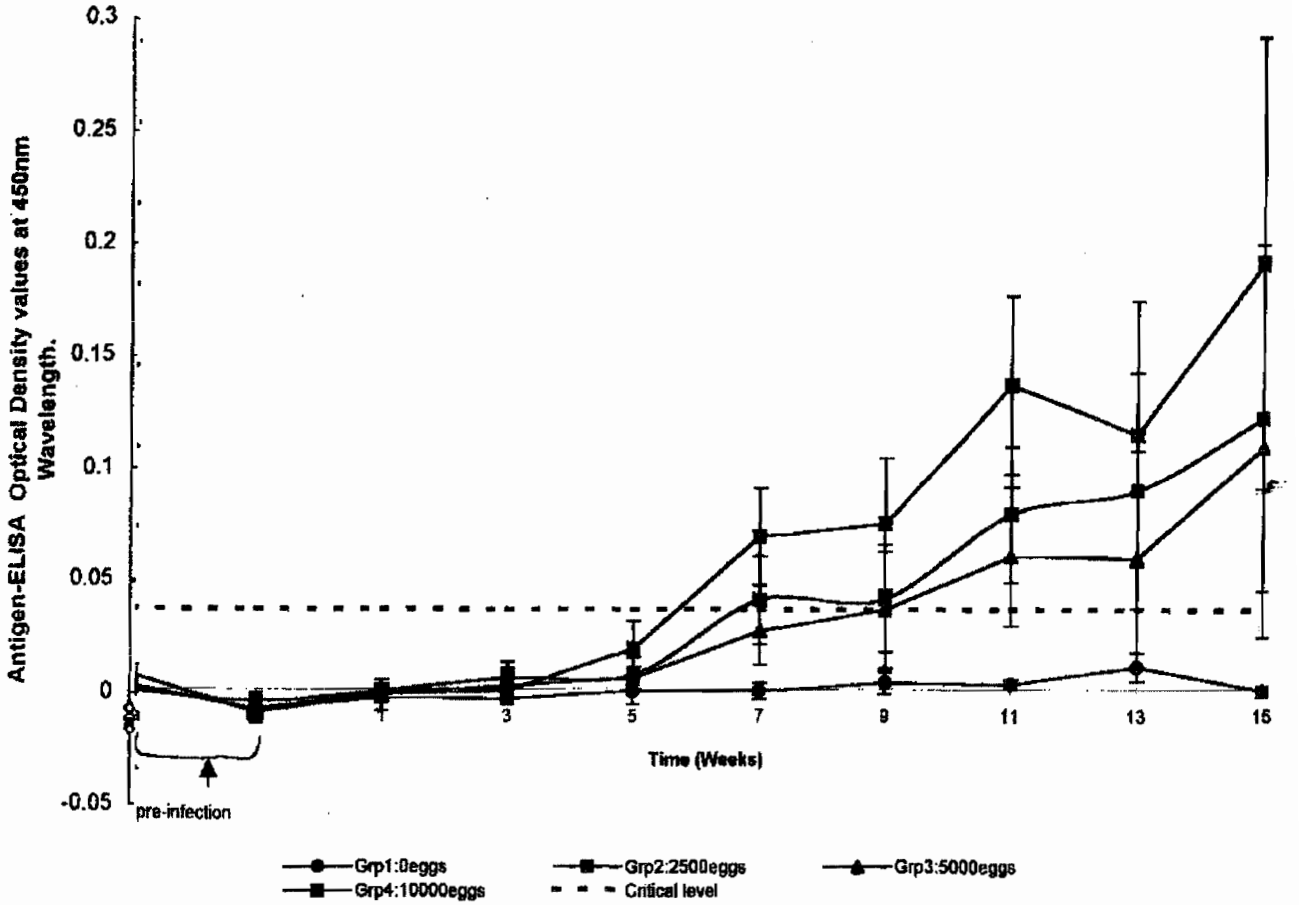

Fig. 2. The average Antigen-ELISA Optical Density values of 30 experimentally infected calves with various doses of *T. saginata* eggs and monitored every two weeks until slaughtered in the 15th. week.

3:2:4.Meat inspection findings in experimentally infected calves

The results of meat inspection method of individual animals are presented in Table 4 while the frequency of distribution of cysticerci in varoius predilection sites is shown in Table 5.

Table 5: Frequency of cysticerci in different parts of predilection sites of experimentally infected cattle during meat inspection.

Predilection sites for inspection according to Kenya Meat Control Act-1977	CYSTS= RECORD[a]		CATTLE RECORD[b]	
	Total number of cysts per site for all the 24 calves	Percentages of the number of cysts found per site during inspection	The number of animals found infected by the site inspected	Percentages of animals found infected by the site inspected
Heart	88(32)	5.29(1.92)	10(6)	41.67(25.00)
Masseter and pterygoid Muscles	11(8)	0.66(0.48)*	6(4)*	25.00(16.67)
Tongue	9(3)	0.54(0.18)	4(2)	16.67(8.33)
Shoulder muscles	30(27)	1.80(1.62)	5(3)	20.83(12.50)
Grand total	138(70)	8.30(4.21)	12(8)	50.00(33.33)

* Figures in parentheses represent live cysticerci[a] or calves[b] found with live cysticerci, respectively.

Table 6: A summary of comparison of the diagnostic methods, for both naturally and experimentally infected animals based on live cysticerci only.

Parameters		Naturally infected animals			Experimentally infected animals		
		Total dissection as the gold standard		MIM	Total dissection as the Gold standard		MIM
		MIM (%)	AET (%)	AET (%)	MIM (%)	AET (%)	AET (%)
Sensitivity of the test		40	80	-	53.33	60	-
Specificity of the test		100.00	60	-	100.00	100	-
Predictive value of the test		100.00	88.89	-	100.00	100	-
Accuracy of the test		52.00	76	-	70.83	75	-
Apparent prevalance of the the test		28	72.00	-	33.33	37.50	-
Kappa Statistic(k)		0.218	0.482	0.262	0.461	0.374	0.727
% of animals detected by both tests	Positive	32	64.00	28.00	33.33	37.5	29.17
	Negative	20	12	28.00	37.50	37.5	58.33

KEY: MIM-Meat Inspection Method AET-Antigen-ELISA Test
k-Kappa - - Not Applicable

4. DISCUSSION AND CONCLUSION

Taenia saginata infection is of concern from economic and public health point of view, as it causes downgrading and condemnation of carcasses at slaughter and ill-health in man respectively. This problem occurs worldwide and therefore solutions to the problem require strategies defined for each community and its environment (Alfonso, 1997). The results obtained by this investigation provide for ante-mortem diagnosis of bovine cysticercosis using an Antigen-ELISA. In this investigation, the efficacy of Antigen-ELISA in diagnosis of *T. saginata* cysticercosis was evaluated and compared with routine meat inspection as it is stipulated in Kenya Meat Control Act-1977, in both naturally and experimentally infected cattle.

From the public health point of view, only live cysticerci are of great importance because the transmission cycle in an environment is potentiated by man ingesting these live cysticerci. Basing on this reason, all the statistical analysis throughout the text were based on the live cysticerci. This was supported by the fact that the test being validated, that is Antigen-ELISA detects only products (antigens) of live cysticerci. Since this test was being compared with meat inspection which detects both live and dead cysticerci, it followed with logical necessity that only live cysticerci had to be considered also for the case of meat inspection in order that the two tests are compared at the same level. This factor of live cysticerci was considered in order that the Antigen-ELISA should not be under-estimated by meat inspection method.

On average, poor recovery rates of cysticerci were realized at autopsy in experimental infections. For instance, mean recovery rates from 2500, 5000 and 10,000 eggs of *T. saginata*, were 46, 83 and 83 cysticerci, respectively, which were very low. These low recovery rates were manifested in poor infectivity indices as shown in Table 3 and which resulted in very low recovery rates or complete lack of cysticerci in some carcasses. There are several possible reasons to explain this poor infectivity indices. The ability of tapeworm eggs to produce an experimental infection 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 as reported by Silverman (1956). The availability of hatching (gastric juice) and activation (bile salts) stimuli at optimal conditions in the alimentary canal of cattle determines the hatchability of oncospheres to cause the infection (Silverman, 1954). The human error which might have occurred during percentage motility determination, counting of the number of eggs for infection, affected the recovery rate of cysticerci at autopsy, and greatly contributed to the poor infectivity indices obtained (Table 3).

In contrast to post-mortem techniques, the monoclonal antibody (McAb coded as HP10), based antigen detection ELISA system was designed to detect cattle harbouring viable cysticerci, thereby indicating potential source of human infection (Harrison *et al.*, 1989). The assay, when developed was highly specific for *Taenia saginata* and *Taenia solium* infections. The results obtained in this investigation, using McAb, HP10, correspond very closely with the observation of Harrison *et al.*, (1989) and Onyango-Abuje *et al.*, (1996), using the same monoclonal antibody (McAb) HP10. McAb, HP10 reacts with a repetitive carbohydrate epitope on the glycoproteins found on the surface and in the secretions and excretions of the cysticerci in cattle. Using Antigen-ELISA, 16 out of 25 animals naturally infected were diagnosed as seropositive, at the field thereby giving a prevalence of 64%. The 16 animals which had been diagnosed as seropositive at selection, remained seropositive throughout the monitoring period of three months until slaughter (Fig.1 and Table 1). This showed a very good precision of the Antigen-ELISA in this group of animals and 15 of these were true positives at autopsy, that is, they were found with live cysticerci and only one did not habour any live cysticerci (Table 1). At slaughter, 18 of these 25 animals were diagnosed as seropositive thereby giving a prevalence of 72%. The two additional animals (715 and 976) which tested seropositive at slaughter increasing the number of seropositive animals from 16 to 18 , were from a group which had been diagnosed as seronegative at selection (Table 1). The seroconversion of these two animals from the seronegative group was not surprising because there was a corresponding rise in the level of antigenemia in these seronegative animals above the critical level two months before slaughter (Fig. 1). There are possible reasons to explain this discrepancy. The animals might have become infected between initial serum sampling and when they were purchased. Since it is well known that the larvae of taeniid tapeworms generate a strong host immunity with almost complete resistance to reinfection (Soulsby, 1972), the antibodies resulting from the mounted immune responses formed complexes with the antigens thereby removing the antigens from the circulation and therefore, making the animal test seronegative while actually they had live cysticerci. 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 increasing the sensitivity of this assay. Brandt (1992) and his co-workers 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 (pH4) and after boiling for 5 minutes and then centrifuging twice at 9000g for 3 minutes, the supernatant was recovered for use in ELISA. The cysticerci wall, separating the parasite and the host tissue, might prevent the exit of the parasite products (antigens) into circulation thereby resulting into false negative cases but later, the antigens leak through the wall and get into circulation thereby resulting into true positive cases. Surprisingly, the encysted cysticerci already present are often unaffected by the host's immune responses, in spite of evidence that globulins can diffuse through the wall around the cysticerci, indicating that they are able to resist or avoid the immunological attack (Muller, 1975).

In experimentally infected calves, the antigenemia levels were first detected at 7-11 weeks post-infection (Figure 2). From week 9, the antigen level remained above the cut-off point and kept on increasing for the entire period of 15 weeks in all the infected groups of calves. The control group of calves never showed any detectable level of cysticercal antigens throughout the period and the antigen level remained far below the cut-off point (Figure 2). Likewise, in naturally infected animals, the animals diagnosed as seropositive at selection, had antigenemia level well above the cut-off point throughout the monitoring period of three months thus indicating that the cysticerci were still alive. Between the second and third month of monitoring the naturally infected animals, the antigenemia level rose slightly above the cut-off point in the group of animals which had been serodiagnosed as negative at selection. This rise in the antigenemia level was an indication that the cysticerci antigens might have been present in the 9 animals serodiagnosed as negative at selection. The distribution of the antigen level in both seropositive and seronegative groups of animals may some times overlap as stated by Martens et al (1987). This supports the results shown in Fgure 1. This therefore showed that it is difficult to rule out absolutely the infected and the noninfected individual animals when using the Antigen-ELISA. Although with flactuations, both figure 1and 2 showed that the cysticerci antigens remain present in the circulation once produced until removed from circulation at a time which is not yet known. This is because, from figures 1 and 2, there are no animals which were serodiagnosed as positive and after sometimes, they tested seronegative. This could have been manifested in their graphs going below the cut-off point. The flactuations of the antigen level in the animals and the removal of these antigens from circulation, undoubtedly corresponds with the constantly occurring reactions between antibody and antigens. As the parasite produces metabolic products (antigens), the host re-

sponds by producing antibodies which react with the antigens forming antigen-antibody complexes. These are then removed from circulation thereby reducing the antigenemia level or completely removing the antigens from circulation. By these reactions, initially seropositive animal may eventually test seronegative and vice versa.

When using Antigen-ELISA , the cut-off point is very important in making decisions about the infected and noninfected animals. In both Figures 1 and 2, if the cut-off level was increased by moving it upwards, the chances of false-positiveness occurring increased, hence, decreasing specificity and increasing sensitivity of the test. In reality, false-positive cases which do not have cysticerci and get excluded from a feedlot, are preferred to false-negative cases which have live cysticerci and when included in a feedlot, enhance the transmission cycle between humans and bovines in the environment. It follows therefore that for the test to have favourable perfomance in the field and to serve its purpose as a test geared towards helping in the control of both human taeniasis and bovine cysticercosis, the cut-off point should be relatively very high. A high cut off point would help eliminate most if not all cases of false-negatives (Martens et al., 1987). Therefore, the Figures 1 and 2 are very important in making decisions about the cut-off point when validating the Antigen-ELISA.

There was considerable variation in the diagnosis of bovine cysticercosis when using three methods, namely, Antigen-ELISA, total dissection and routine meat inspection. In 25 naturally infected cattle, 80, 72 and 28 per cent of the animals were detected positive of bovine cysticercosis by total dissection, Antigen-ELISA and meat inspection, respectively (Table 1). In experimentally infected calves, the order of these prevalence rates were, 66.67, 37.50 and 33.33 per cent for total dissection, Antigen- ELISA and meat inspection, respectively (Table 4). Except for meat inspection, the corresponding values of prevalence rates were higher in naturally infected animals than in experimentally infected calves. These results indicated that although total dissection was used as a gold standard, in practice, it may not have been 100% efficient in the detection of bovine cysticercosis. Total dissection can not be used for detecting cysticerci in slaughterhouses during inspection of meat because it is a tedious and time consuming method. Furthermore, it would greatly lower the quality of meat should the results obtained indicate that the carcass under inspection was fit for human consumption. The antigen detection assay was more sensitive than meat inspection method for it diagnosed more animals as positive for bovine cysticercosis than the latter in both naturally and experimentally infected animals. Although the prevalence rate of Antigen-ELISA was just slightly above that of meat inspection by 4.17% in experimental calves, it was almost three times as high as that of meat inspection in natural infections. Meat inspection method can only be used to detect infection after the death of the animal when it is too late to make any decisions over treatment or improving the public hygiene of the environment. Meat inspection method was the least sensitive of the three methods. As stated before, meat inspection method is physically designed to observe, identify cysticerci at specified predilection sites, thought to have high density of the cysticerci than elsewhere in the carcass (Kyvsgaard, 1990) (Tables 2 and 5). This therefore means that an animal could be diagnosed as negative even if cysticerci were located elsewhere in the carcass being examined. During the inspection of various carcasses, it was realised that except for the dead, degenerate or calcified cysticerci which often formed spots of white and fibrotic lesions, a careless meat inspector is most likely to miss out quite a number of viable cysticerci which blend with the translucent and pinkish-red colour of the background and pass on for human consumption. This significantly lowers the sensitivity of the meat inspection method and hence its unreliability and low detection rate previously observed especially in lightly infected animals (Dewhirst et al.,1967; Walther and Koske, 1980). The Antigen-ELISA is therefore superior to meat inspection method and, becomes such an important diagnostic test for bovine cysticercosis because, it is more effective than meat inspection method in helping to break the life cycle thereby helping in the control strategies of the parasite.

From the results of diagnostic test evaluation tables summarised in Table 6, the epidemiological usage of the parameters (sensitivity, specificity, predictive value, accuracy, and prevalence) was considered when comparing the diagnostic methods. In naturally infected animals, the antigen assay displayed a sensitivity of 80% (in animals harbouring 2-55 live cysticerci) while meat inspection method had 40% (in animals harbouring 1-7 live cysticerci) when total dissection was used as a gold standard. The results indicated that the assay was twice as sensitive as meat inspection method in natural infections. These results compared favourably with those obtained previously by Onyango-Abuje et al (1996) using the same assay. In experimentally infected calves, the sensitivity of the assay was still higher (60%) than that of meat inspection (53.33%) in animals harbouring 14-193 and 1-17 live cysticerci, respectively) when total dissection was used as a gold standard. The low sensitivity of the assay in experimental calves might have been due to poor infectivity of *T. saginata* eggs in

the calves and this was the possible reason also for the low prevalence rates obtained in these calves with the Antigen-ELISA. This problem could be avoided or improved by at least trickle infections of the susceptible host species of cattle. Otherwise, in both natural and experimental infections, the prevalence rates for meat inspection method were lower than those of Antigen-ELISA because the meat inspection method identified fewer animals as positive for bovine cysticercosis at slaughter than the Antigen-ELISA. In both experimentally and naturally infected animals, specificity and predictive values were 100% for routine meat inspection method while for Antigen-ELISA, the values ranged from 60% (for the assay specificity in natural infections) to 100% for predictive value(in natural and experimental infections) and specificity (in experimental infections) (Table 6). This is because the meat inspection method does not give false-positive cases like the Antigen-ELISA. Accuracy which measures the overall preformance of a test in the laboratory or in the field, was higher in natural infections than in experimental infections for Antigen-ELISA and vice versa for meat inspection (Table 6). This indicated that the Antigen-ELISA gave a true measure of the infection in the animals more than the meat inspection method because meat inspection method leaves out many lightly infected carcasses which are detected by Antigen-ELISA. However, this was the case in natural infections but not in experimental infections (Table 6). Therefore, the Antigen-ELISA may be the most appropriate method of diagnosing bovine cysticercosis infection but on a herd basis because of its false- positive and negative reactions in light infections. However, in heavily infected herds, the assay can be used for individual diagnosis.

The Kappa statistic (k) was used to measure the level of agreement between any two diagnostic methods for bovine cysticercosis infection in cattle. Of the 25 animals selected from the field, 28% cases were detected by both meat inspection and total dissection ($k = 0.218$; $p > 0.05$), 64% cases by both Antigen-ELISA and total dissection ($k = 0.482$; $p > 0.05$) and 28% cases by both Antigen-ELISA and meat inspection ($k = 0.262$; $p > 0.05$) (Table 6). In all these three comparisons, the Kappa measure of agreement was poor except between Antigen-ELISA and total dissection where the agreement was moderate. This suggests that the methods were not detecting the same animals as either positive or negative for bovine cysticercosis infection. Of the 24 experimentally infected calves, 33.33% cases were detected by both meat inspection and total dissection ($k = 0.461$; $p > 0.05$), 33.33% cases by both Antigen-ELISA and total dissection ($k = 0.374$; $p > 0.05$) and 29.17% cases by both Antigen-ELISA and meat inspection ($k = 0.727$; $p > 0.05$) (Table 6). In all these three comparisons, the Kappa measure of agreement was good except between Antigen-ELISA and total dissection where the agreement was moderate. This therefore, suggests that the methods were in most cases, detecting the same animals as either positive or negative for bovine cysticercosis. The Kappa statistic values were on average lower in natural infections than in experimental ones. This was because in natural infections, there were more light infections which could not be detected by meat inspection method but could be detected by either Antigen-ELISA or total dissection, than in experimental infections. Generally, in both naturally and experimentally infected animals, there was little overlap between animals diagnosed positive for bovine cysticercosis by the three methods except between Antigen-ELISA and total dissection in naturally infected animals. This discrepancy was due to a variety of reasons, some of which, in the case of total dissection and meat inspection, have already been explained and discussed before in the above paragraphs. As for the Antigen-ELISA, it still gives false-positive cases owing partly to the fact that it is not yet really known when the cysticerci antigens disappear from circulation following the death of the cysticerci. Also contributing to the above discrepancy, were the antigen-antibody reactions that remove antigens from circulation by forming undissociated complexes thereby resulting into false-negative cases. These are possible reasons to help explain the above discrepancy.

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