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Analysis of post-mortem diagnosis of bovine cysticercosis in Kenyan cattle

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Address for correspondence: Wanzala Wycliffe, Division of Parasitology and Immunology, Department of Zoology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya. TeleFax: 254-02-445763, e-mail: wwanzala@hotmail.com

ABSTRACT

Wanzala W, Onyango-Abuje JA, Kang'ethe EK (PhD), Zessin KH, Kyule NM, Baumann MPO, Ochanda H, Harrison LJS, Analysis of post-mortem diagnosis of bovine cysticercosis in Kenyan cattle, Online Journal of Veterinary Research, 1:1-9, 2003. **A total of 55 cattle divided into two groups of experimentally (n =30) and naturally (n = 25) infected animals were used to study the reliability of meat inspection methods in Kenya. 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 artificially infected 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. The results further confirm that in spite of the time and effort taken by meat inspectors in looking for cysticerci at predilection sites, this method is very insensitive. It was therefore recommended that more parts of the carcass not currently inspected according to the Kenya Meat Control Act – 1977, for bovine cysticercosis such as hind legs, ribs, lungs and liver, need to be considered as possible and equally important predilection sites and larger areas of these predilection sites should be examined. However, other better sensitive ante-mortem diagnostic methods should be developed to assist in the integrated management of the infection.**

KEY WORDS: Meat inspection; Post-mortem diagnosis; Bovine cysticercosis; Kenyan cattle

INTRODUCTION

Taenia saginata **is a cosmopolitan zoonotic parasite whose infection interferes with the health of man, and adversely affects the production of livestock industry 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. The economic losses accruing from these infections, particularly, downgrading and condemnation of infected carcasses, are substantial (Grindle, 1978; Mann, 1983; Gracey and Collins, 1992; Fan, 1997). In addition, there is great loss of export markets in livestock products from the zoonotic endemic zones (Harrison et al.,1989) and significantly contributes to reduction in labour force. The meat inspection method is still the most important public health measure and method of choice practised worldwide in the diagnosis and control of bovine cysticercosis in slaughterhouses, albeit its failure to identify light infections (Onyango-Abuje et al. 1996). Since alternative diagnostic tests such as antibody-ELISA (Ab-ELISA), antigen-ELISA (Ag-ELISA) and other control measures such as vaccination and livestock chemotherapy that have been explored still remain a matter of speculation for a lot of laboratory and fieldwork is yet to be done before their efficacy can be established for implementation, there is need to re-evaluate and assess the reliability of meat inspection method so that appropriate suggestions to improve it can be made accordingly. Even with stringent control measures in practice such as irradiation of carcasses, observation of public hygiene, education of the public, mass chemotherapy on man, cooking of meat at 57o C, deep freezing of meat at -10o C for 10 days, pickling meat in 25% salt solution for 5 days and buying only officially inspected meat (Muller, 1975; Neva and Brown, 1994; Anon, 1997), the parasite has not been eliminated and even controlled in some places like East Africa where the infection pressure is still high. This study therefore analyses the reliability of meat inspection procedures in Kenya in diagnosing bovine cysticercosis, using total dissection as a gold standard method of validity.**

MATERIALS AND METHODS

Animal Ethics Statement: **All experimental procedures requiring animals were approved by NVRC's Institutional Animal Care and Use Committee and were performed in compliance with guidelines published by Kenya Veterinary Association and Kenya Laboratory Animal Technician Association.**

Collection of parasite eggs: ***Taenia saginata*** **eggs were collected from human faeces in the Mathare Valley slums of Nairobi, Kenya, according to Onyango-Abuje et al. (1996). The viability of the eggs were tested (Stevenson, 1983) and eggs counted to infect the calves. The infectivity indices of the eggs were determined according to Silverman PH (1954) and Silverman PH (1956).**

Naturally infected cattle: **Naturally infected Zebu herds were identified through history, reports from the District Veterinary Officer in Samburu District and an antigen-ELISA test. 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 as described by Walther M and Koske JK (1980) and Onyango-Abuje et al. (1996). As stipulated in Kenya Meat Control Act-1977, only a few predilection sites are considered for inspection (heart, shoulder muscles, tongue and masseter and pterygoid muscles). In the predilection sites, the Act, again, allows only a small area to be incised and examined. 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. The viability of the recovered cysticerci was tested by observing their evagination in cattle bile overnight.**

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. 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 - 2½ 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. They were slaughtered in the 15th week and examined for cysticerci as explained above in natural infections. The second group of 15 calves was treated similarly.**

Post - mortem examination - 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.**

Post - mortem examination **- 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.**

Statistical analysis-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 Martin et al., (1987).

Statistical analysis-Analysis of sensitivity, specificity, predictive value, accuracy and apparent prevalence of 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 (Martin 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 diagnostic test evaluation Table (Martin et al., 1987) shown below was used to evaluate sensitivity, specificity, accuracy, prevalence and predictive value of meat inspection method as outlined below.**

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

a - True Positive, c - False Negative, b - False Positive, d - True Negative , sensitivity = a/a+c, specificity = d/b+d, predictive value = a/a+b, accuracy = a+d/a*b+c+d and prevalence = a+b/a+b+c+d.

RESULTS

A measure of the infectivity of the eggs of *T. saginata:* From Table 1, it is evident that the infection rate of calves fed with various doses of eggs of T. saginata was very variable. The results indicate that the infection rate of the eggs in different groups of calves was very poor. On average, group 2 and 3, calves dosed with 2500 and 5000eggs, respectively, showed the highest infection rate with an infectivity index of 0.02 whereas calves in group 4 dosed with 10,000 eggs, showed the lowest infectivity rate with an index of 0.01.

Table 1 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 Live	Dead	Total	Mean infectivity indices*
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.

***Meat inspection findings in naturally infected cattle*: The results of meat inspection are presented in Table 2. Twelve out of twenty-five (48%) carcasses were found infected with cysticerci. Thirteen animals were found without any cysticerci. Meat inspection revealed a prevalence rate of 48% in 25 animals.**

***Total dissection findings in naturally infected animals:* The recovery of cysticerci in various sites of 25 carcasses following slicing of their masculature revealed infection in 24 out of 25 (96%) carcasses (Table 2). There was only one animal without any cysticerci.**

Meat inspection findings in experimentally infected calves: **The results of meat inspection are presented in Table 2. During meat inspection, 12 out of 24 carcasses of calves which had been exposed to T. saginata eggs were found infected with cysticerci (both live and dead) ranging from 1 to 49. Twelve calves out of the 24 infected, were without any cysticerci. The meat inspection revealed a prevalence rate of 40%. The control animals were not found with any cysticerci.**

***Total dissection findings in experimentally infected calves*: The recovery of cysticerci in 24 infected calves at slaughter is shown in Table 2. All the sites sliced including visceral organs during total dissection of carcasses of the infected calves were found with varying numbers of live and dead cysticerci. 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 except one calf. One of the control calves was found infected with one live cysticercus at autopsy.**

Table 2 A summary of comparative diagnosis of bovine cysticercosis

Treated animal groups	Positives		Negatives	
	MIM	TDM	MIM	TDM
Natural Infections (n= 25)	12 (48%)	24 (96%)	13(52%)	1(4%)
Artificial Infections(n= 30)	12(40%)	24*1(80%)	8*2(26.64%)	6(20%)

MIM = Meat Inspection Method, TDM = Total Dissection Method, *1 Includes one control calf found with live cysticercus *2 Includes the 6 control calves

Table 3 A summary of the efficacy of meat inspection method in the diagnosis of bovine cysticercosis using total dissection as the gold standard.

Parameters	Naturally infected steers	Artificially infected calves
Sensitivity	40.00	53.33
Specificity	100.00	100.00
Predictive value	100.00	100.00
Accuracy	52.00	70.83
Apparent prevalance	48.00	40.00
Kappa Statistic(k)	0.218	0.461
% of animals detected by both methods		
Positive	32.00	33.33
Negative	20.00	37.50

DISCUSSION

Meat inspection methods can only detect bovine cysticercosis after the death of the animal when it is too late to make any decisions over treatment, vaccination or improving the public hygiene of the environment and safe that particular animal from getting the infection (Onyango-Abuje et al., 1996). It is physically designed to observe and identify cysticerci at specified predilection sites, thought to have high density of the cysticerci than elsewhere in the carcass (Kyvsgaard, 1990). 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, thus maintaining the transmission cycle in that particular environment. 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).

In the experimentally infected calves, one control calf was found with one live cysticercus at autopsy (Table 2). This could have resulted from accidental contamination during oral administration of eggs to the calves. 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 (Table 1). These low recovery rates were manifested in poor infectivity indices as shown in Table 1 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.

In this study, the prevalence rates of bovine cysticercosis by meat inspection method, ranged from 40% (artificial infections in calves) to 48% (natural infections in steers), which is still low compared to 80% and 96% by total dissection, respectively (Table 2). Although meat inspection method recorded 100% specificity and field performance (Table 3), analysis of results in Tables 2 and 3 indicate that the total dissection method was twice as sensitive as meat inspection method in both natural and artificial infections. Although not as accurate as total dissection method, 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 and its general marketability should the results obtained indicate that the carcass inspected was fit for human consumption.

The efficacy of meat inspection method and Kappa statistic (k) which was used to measure the level of agreement between the two diagnostic methods are presented in Table 3. In natural infections, 32% cases were detected by both meat inspection and total dissection ($k = 0.218$; $p < 0.05$) showing poor level of agreement while 33.33% cases of artificially infected calves were detected by both meat inspection and total dissection ($k = 0.461$; $p< 0.05$) showing good level of agreement. However, the good level of agreement does not mean that the two methods were detecting the same animals as either positive or negative for bovine cysticercosis-the percentage of animals detected by both methods is stil small.

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 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 two methods. This discrepancy could be probably attributed to poor infection rate shown in Table 1. The results confirm that in spite of the time and effort taken by meat inspectors in looking for cysticerci at predilection sites, this method is very insensitive and there is therefore need to improve the method or other better alternatives be developed. For routine meat inspection method to compare favourably with total dissection, more parts of the carcass not currently inspected according to the Kenya Meat Control Act – 1977, for bovine cysticercosis such as hind legs, ribs, lungs and liver, need to be considered as possible and equally important predilection sites and larger areas of these predilection sites should be examined.

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