RELIABILITY OF AN ANTIGEN-ELISA IN THE DIAGNOSIS OF BOVINE CYSTICERCOSIS

BY

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TO

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SEPTEMBER, 1999.

I Wycliffe Wanzala, hereby declare that this is my own original work and has not been presented anywhere for a degree in any other university.

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DEDICATION

This thesis is dedicated to my late sister,

JANEPHER B. OSUNDWAH

WHO

Really needed my attention when she was ill,

But could not get it during the course of this study

AND TO THE

The beautifying nature of Muguga Forests whose silence kept me company as I lonely walked through without even the sympathy of the on looking beautiful birds, mammals and snakes of the forests throughout the rains' and drought seasons of the years I was under academic stress.

TO THE DEAD AND TO THE LIVING, I SAY MAY GOD BLESS YOU ABUNDANTLY.

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ABSTRACT

Taenia saginata is among the zoonotic parasites that interfere with the human health and production of livestock, worldwide. The adult worm causes taeniasis in humans while the larvae causes cysticercosis in cattle. The economic losses accruing from these infections are substantial.

Meat inspection which is the most important public health control measure practised, identifies only heavily infected animals when it is too late to avoid incurring losses. For this reason, an ante-mortem diagnostic test would be very much desirable. Currently, there is no established test for diagnoses of bovine cysticercosis in live cattle but an antigen-ELISA (Ag-ELISA) which has been developed recently, has shown to be feasible as a herd test. This study was carried out in order to determine the number of live cysticerci that the Ag-ELISA can reliably detect in infected cattle thereby validating the test. The Ag-ELISA was compared with routine meat inspection method using total dissection as a measure of the true status of infection in the animals.

Two groups of animals were used in these experiments, namely, 25 naturally infected animals from pastoralists in Samburu District and 30 neonatal calves experimentally infected with various doses of T. saginata eggs. Both groups of animals were bled immediately after arrival and before infection in the case of neonatal calves and thereafter, every two weeks (neonatal calves) and monthly (naturally infected animals) till slaughtered in the 15th week and 3rd month, respectively. All the sera from either experimentally or naturally infected animals were tested for circulating cysticerci antigens by Ag-ELISA.

The results showed that in experimentally infected calves, the parasite antigens were first detected 7-11 weeks post- infection. As in the naturally infected seropositive

animals, the antigen level fluctuated but remained above the cut-off point, until the animals were slaughtered. Although the sensitivity of the test varied from one animal to another, the minimum number of live cysticerci which was detected by the Ag-ELISA was 14 in experimental calves and 2 in naturally infected animals. However, other animals with 12 and 17 live cysticerci in experimental calves and 1 to 2 live cysticerci in naturally infected animals, escaped detection. Animals harbouring dead cysticerci gave negative reactions as in non-infected experimental control calves, indicating that the assay only detected products of viable cysticerci in cattle. There was a statistically significant positive linear correlation between Ag-ELISA optical density values and burdens of live cysticerci as obtained by total dissection in both experimentally ($r = 0.798$, $n = 24$; $p > 0.05$) and naturally ($r = 0.631$, $n = 25$; $p > 0.05$) infected animals. In naturally infected animals, the Ag-ELISA showed a good precision.

Comparison of Ag-ELISA with routine meat inspection method in naturally infected animals showed that the Ag-ELISA was more than twice as sensitive as meat inspection method, while the sensitivity of the two methods was the same in the experimental infections. This was probably due to poor infection rates in the experimental calves. However, there seemed to be very little overlap between animals diagnosed positive by the two methods. In all the cases, however, Ag-ELISA diagnosed more animals as positive for bovine cysticercosis than the routine meat inspection method whose regulations limits it to examination of very few predilection sites.

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.

From the results obtained by this investigation, it was concluded that the monoclonal antibody-based antigen detection ELISA is of value for the diagnosis of bovine cysticercosis infection in cattle as a screening test in a herd. This is because, the assay still gives false-positive and negative reactions in lightly infected cattle which, epidemiologically, form the most important group in the transmission cycle of this parasite. In a herd of heavily infected cattle, the assay can provide for individual diagnosis. Although, as a screening test, it could be adopted as a control method for the parasite, more work is still needed to increase its sensitivity in order to develop it as a field test.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1:1. INTRODUCTION:

Bovine cysticercosis is a tissue infection of cattle caused by larvae of the human intestinal cestode, Taenia saginata. The cestode belongs to the family Taeniidae of the order Cyclophyllidea.

The adult tapeworm causes taeniasis, an intestinal infection in man. Man is not normally a host for the larval stage but, there have been a few reported cases of metacestode of T. saginata occurring in man (Webbe,1967; Pawlowski and Schultz, 1972; Neva and Brown,1994).

This parasite is cosmopolitan in distribution, particularly in beef-eating countries. It is endemic in several developing countries, especially those of Asia, Africa and Latin America where the level of poverty is too high and that of hygiene too low to prevent transmission cycle of the parasite from defintive host to intermediate host and vice versa. Many authors have reported varying prevalence rates between and within continents and countries. For instance,in 1947 Stoll estimated that 39 million people had taeniasis and in 1965 Froyd estimated the figure at 40 million people. These figures drastically rose to 61 million persons almost two decades later (Peters and Gilles, 1982). In 1972, Pawlowski and Schultz, estimated a cysticercosis prevalence rate of 10% in many parts of the world including Kenya, Uganda, Nigeria, South Africa, and Chad. In 1975, Muller recorded a human prevalence rate ranging

from 20% in Africa (Kenya) to 0.03% in Europe (Poznan) for taeniasis and 2% for bovine cysticercosis. Cheruiyot,(1981),while working in Kenya, recorded a cysticercosis prevalence rate ranging from 0.74% in the Coast Province to 18% in Kisii District, Nyanza Province. In 1974 and 1991, the bovine cysticercosis prevalence rates were 8.8% and 1.1%, respectively in eight provinces of Kenya, (Kang'ethe,1995).

In Africa, T. saginata has been reported to be prevalent in eastern, southwest and southern countries whereas its larva C.bovis has been found to occur sporadically in the northeast Africa and Morocco (Onah and Chiejina, 1986; Cheesbrough, 1987). In 1989, Botero reported an overall prevalence rate ranging from 0.5% to 5% in the continents of Africa and Latin America.

Taenia saginata infection has also been reported in China, India, Cambodia, Southeast Asia, Iran and Iraq as well as in Laos where it was highly prevalent (Oryan, Moghaddar and Gaur, 1995). In United States of America and Colombia, there was a notified decrease in incidence and prevalence of both taeniasis and bovine cysticercosis since 1989 (Alfonso, 1997). In Sao Paulo Brazil, a bovine cysticercosis prevalence of 5.5% was reported from 896 654 heads in 385 municipalities during the year 1986 by Alfonso (1997). Eckert (1996) however, reported that in many parts of the world where sanitation is of little concern and beef is a major diet, these prevalence rates may reach 50%. These differences in prevalence rates are probably due to insufficient understanding and inconsistent reporting of the infection in both man and cattle, most probably resulting from sporadic and fragmented studies of certain groups of the entire population and not others (Alfonso, 1997) .

Infection in man is usually acquired by ingestion of raw or poorly cooked beef that contains viable cysticerci. The cysticerci evaginate in the human small intestine (upper jejunum mainly), attaches to the mucosa by its scolex, and grows to become an adult parasite in about 3 months, occupying and following the small intestine loops for much of its length (Neva and Brown, 1994). The parasite may survive for as long as 20 years (Botero, 1989) or upto 25 years (Neva and Brown, 1994) in man. After 3 months of post-ingestion of cysticerci, the mature worm begins shedding gravid or ripe proglottids (segments with only uterus containing eggs) which may crawl independently out of the anus or may be passed out in the feacal bolus (Neva and Brown. 1994). Each gravid proglottid is known to contain about 200,000 eggs (Anon., 1997). Almost immediately after the patient passes these proglottids they expel a milky fluid full of eggs from their anterior border by muscle contraction of the parasite,thus contaminating the environment with the eggs. In varying environments, these eggs have been known to remain viable as follows:- in liquid manure for 71 days, in untreated sewage for 16 days, on grass for 159 days or 180 days (Chandler and Read, 1961; Schmidt and Roberts, 1985) and in river water for 33 days (Soulsby, 1982). The deposited eggs may be disseminated in the environment by wind, flies, earthworms, birds, rains and even by beetles that roll away the faeces (Chandler and Read, 1961; Silverman and Griffiths, 1955). The eggs also may be disseminated by man when untreated sewage is used as fertilizer in cattle grazing fields.

Infection in cattle is by ingestion of these disseminated eggs. This occurs in a variety of ways. Direct picking of eggs from grass during grazing in the contaminated field and from

infected workers feeding neonatal calves with contaminated hands, present major routes of eggs' ingestion by cattle (Chandler and Read, 1961; Dewhirst et al., 1967; Slonka et al., 1975; Schmidt and Roberts, 1985; Neva and Brown, 1994). The contamination of food for example, hay and water given to neonatal calves also increase chances of cattle being infected (Slonka et al., 1975). In Kenya, calves have been reported to be infected in the first week of their life through contaminated food and hands of the workers (Urquhart, 1958 and 1961; Froyd, 1964 a,b; Froyd and Round, 1960). Ginsberg (1958) and Mc Manus (1960 and 1963), reported that prenatal infections can also occur although not common. It is possible that such prenatal infections are confused with early neonatal infections (Soulby, 1982).

When the eggs are ingested by cattle, the oncospheres hatch under the influence of gastric and intestinal juices and penetrate the intestinal mucosa to reach the general circulation via lymphatics or blood vessels and are carried to the intramuscular connective tissues (striated muscles) via the liver (Never and Brown, 1994). The embryos (oncospheres) are disseminated throughout the body and develop not only in skeletal and cardiac muscles, but also in fat and visceral organs (Walther and Koske, 1980; Soulsby, 1982). In the muscles, they develop into cysts (metacestodes or cysticerci) scientifically known as Cysticercus bovis, a bladderworm. There are controversies about the existence of predilection sites, that is , sites with a higher density of the cysticerci than elsewhere in the carcass. Although heart , masseter and pterygoid muscles, tongue, shoulder muscles, oesophagus and sometimes diaphragm, have been reported in literature as predilection sites, the actual distribution of cysticerci between muscle groups varies greatly (Kyvsgaard et al., 1990). By dissecting and slicing 23 experimentally infected

calves, Kyvsgaard et al , (1990) got a contrasting order of infected sites with cysticerci to that obtained by Mango and Mango (1972). Gracey and Collins (1992) who had their order almost similar to that of Kyvsgaard et al., (1990), stated that there were no particular predilection sites for cysticerci and that their distribution was purely mechanical by the volume and intensity of the arterial blood due to day-to-day activities of the animals. The existence of these predilection sites is based on the fact that cysticerci require oxygen for their respiration processes and therefore, they will always prefer to occupy the most active and highly oxygenated muscle groups and organs within the animal so that these sites act as a source of oxygen to them (Kang'ethe pers. commun.).

A part from cattle, domestic and wild ruminants have been reported worldwide as being intermediate hosts of T. saginata. In south and east Africa, cysticerci were recorded in camels and sheep (Graber, 1959) and in goats in Taiwan (Muller, 1975). Also Neva and Brown, (1994) reported the role of camels as intermediate hosts of T.saginata. However, none of these authors described the infection as being either natural or artificial thereby causing doubt as to whether these animals are true intermediate hosts of T. saginata parasite. Although Soulsby, (1982) reported cysticerci in wild ruminants such as the giraffe, the wildebeeste and the antelope, these animals are not normally hosts of T. saginata metacestodes. However, he stated,where farming of game animals was undertaken, this situation might change and, in the absence of wild game taeniids, the wild ruminants might become susceptible to T. saginata infection. Cysticerci have been recorded in an antelope, and a wildebeeste in East Africa (Nelson et al., 1965), in a reindeer in the U.S.S.R. (Muller, 1975) and in a buffalo in Africa

(Gracey and Collins, 1992). Using six experimentally confined Thomson's gazelles fed with 42,000 eggs of T. saginata, Fay, (1972) failed to produce an infection in any of them. The role of wild animal reservoirs therefore is not well understood (Nelson et al., 1965).

The need for new and effective control measures for this parasite, is partly prompted by the fact that the parasite, (both at larval and adult stages) present health hazard to man. Though the adult tapeworm does not cause serious diseases in man, its presence may result in epigastric pain, vague abdominal discomfort, nervousness, vertigo, nausea, vomiting, diarrhoea, either increased or loss of appetite, intestinal obstruction, mucosal lesions, appendicitis, pancreatic necrosis, moderate eosinophilia and increased levels of serum IgE, thus having a debilitating effect on man (Botero, 1989; Neva and Brown 1994). The resulting expenses due to medical treatment are therefore great. Economic losses to agricultural and related livestock industries are incurred through:- (1) condemnation of heavily infected carcasses (2) downgrading of lightly infected carcasses (3) losses due to costs by refrigeration (4) costs of boiling (5) costs of storage after refrigeration and boiling and (6) costs of transport of carcasses for example, to places where carcasses are processed. In Kenya, Botswana and Great Britain, annual losses have been estimated at £1.0 million, £0.5 million (Grindle, 1978) and 1.2 million (Gracey and Collins, 1992), respectively. In the African continent, Mann (1983), reported an annual loss of US$ 1.8 billion where an overall infection rate was found to be 7%. In South America, where an overall infection rate was estimated at 2.0%, bovine together with porcine cysticercosis caused an annual loss of US$ 428 million (Fan, 1997). More important also is the loss of potential export markets of beef from endemic

areas of the World (Harrison et al., 1989).

The control of this parasite is therefore necessary. However, the control measures which have been and are still in use such as the ones listed below, have never been able to eliminate the parasite and even control it in some places like East Africa where infection pressure is still high (Harrison et al., 1984). These control measures include:- meat inspection and subsequent processing of infected carcasses, irradiation of carcasses and improved sanitation (the use of lavatories and observation of hygiene of feedlots and zero grazing stalls). Other control measures are:- the use of treated human faeces to fertilize pastureland, keeping animals in fenced pastures and education of the public on the parasite life cycle , its transmissional mechanisms and its importance. Human diagnosis followed by treatment of infected persons, thorough cooking of meat at 57°C (especially if suspected to be infected) until it has lost its rich reddish tinge, pickling infected meat in 25% salt solution for 5 days and avoiding to buy meat from informal/unregistered butchers are some of the effective control measures if strictly followed. Even in countries with very strict meat inspection regulations like Kenya (Hughes, 1996) and favourable socioeconomic conditions (Walther and Koske, 1980), the parasite is still a problem. Vaccination and chemotherapy following ante-mortem diagnosis has proved feasible but is still at the experimental stage and so remains the ultimate hope for the future (Teodor et al., 1971; Rickard and Adolph, 1976; Pawlowski et al., 1978; Clegg and Smith, 1978; Gallie and Sewell, 1978,1983; Walther and Koske, 1979; Harrison et al.,1984; Babiker-Sheiba et al., 1987; Johnson et al., 1989; Marshall et al., 1996).

Of all the control measures mentioned above,meat inspection is still the most important

public health measure with respect to prevention and control of bovine cysticercosis (Muller, 1975; Kang'ethe, 1995). However, this method, is very unreliable and insensitive in the diagnosis of light infections of bovine cysticercosis (Dewhirst, et al., 1967; Walther and Koske, 1980). For instance, Walter and Koske (1980) found that meat inspection method could only detect 38.3% of infected animals while total dissection by slicing the entire masculature of the carcass detected 75.9% of the animals. Although total dissection undoubtedly proves to be quite good, it cannot be used in slaughterhouses routinely because it is tedious, time wasting and finally, it makes the meat lose its marketability as no customer would like to buy sliced meat. Since there is no any other effective control measure for this parasite so far, the continued use of meat inspection method in slaughterhouses means that the above mentioned economic losses are still being inccurred by butchers and farmers as well.

Endeavours are therefore being made to develop more reliable ante-mortem diagnostic methods to facilitate the control of this parasite. Of these methods,serological tests such as:- enzyme-linked immunosorbent assay (ELISA), immunofluorescent assay, precipitin test, immunoprecipitation assay, immunoelectrophoresis, counterimmunoelectrophoresis, radioimmunoassay, complement fixation test, duoble immunodiffusion assay, indirect haemagglutination test, latex agglutination test and intradermal test have been used in the diagnosis of bovine cysticercosis with varying success (Fife, 1971; Geerts et al., 1971). Of these, ELISA has been considered the most successful method especially in sero-epidemiological studies (Walther and Sanitz, 1979). Such ante-mortem diagnostic methods like ELISA are very important because they help in identifying infected cattle before slaughter

so that decisions can be made as whether to treat the infected animals or improve farm/ranch management practices, with a view to curbing T. saginata transmission (Harrison and Sewell, 1989). By such control strategy, the farmer does not incur losses due to downgrading or condemnation of infected carcasses. To date, there is no test which reliably diagnoses bovine cysticercosis disease in cattle.

A monoclonal antibody-based ELISA which was developed by Harrison et al., (1989) for detecting T. saginata antigens in cattle is currently under evaluation for the diagnosis of bovine cysticercosis. It is a double sandwich ELISA based on a mouse monoclonal antibody (HP10), an IgM isotope, which detects antigens (glycoproteins) of viable T. saginata cysticerci in the host serum. The monoclonal antibody (McAb, HP10), is reactive with a repetitive carbohydrate epitope on lentil-lectin adherent glycoproteins found in the biosynthetic excretions/secretions and on the surface of T.saginata cysticerci and thereby allowing the construction of a two-site ELISA capture assay. The monoclonal antibody is used as a trapping layer and the bound antigen (glycoproteins present in the sera) is revealed by the use of Biotin conjugated McAb HP10, and Streptavidin-biotinylated horseradish peroxidase complex (conjugate) as the developing system. Harrison et al.,(1989), described McAb HP10,ELISA system as being specific, stable,and resistance to degradation in serum and therefore suitable for use particularly in the tropics. The assay was used in seroepidemiology in Swaziland (Hughes et al., 1993) and in Kenya (Onyango-Abuje et al., 1996) where also it is still currently being evaluated as a screening and diagnostic test for bovine cysticercosis.

The current problem of this assay is however, the establishment of the lowest

number of the live cysticerci it can reliably detect in infected cattle following total dissection of the carcass of a seropositive animal. The decision is based on the optical density values correlated with the total number of live cysticerci in the diagnosed animal. This was the theme of the investigation.

1:2. LITERATURE REVIEW:

1:2:1. Immunodiagnosis of bovine cysticercosis.

For the last three and a half decades, serology of parasitic diseases has been expanding and many publications have appeared in the scientific literature regarding its application in the control of parasitic infections. Although highly valuable immunoassay systems have emerged, they cannot readily be applicable to the diagnosis of many tropical parasitic infections due to their short comings. Some methods for example, immunofluorescent assays, complement fixation tests and radioimmunoassays, have been reported to have high clinical value in the diagnosis of some infections such as cysticercosis, fascioliasis, filariasis, trichinosis, hydatid, schistosomiasis, malaria, amoebiasis, pneumocystis, leishmaniasis, trypanosomosis and toxoplasmosis (Voller and De Savigy, 1981; Fleck and Moody, 1993). Others such as antibody- and antigen-ELISA have been shown to be useful in seroepidemiological surveys of bovine cysticercosis (Craig and Rickard, 1981b; Geerts et al., 1981; Harrison et al., 1989; Hughes et al., 1993; Drealants et al., 1995; Bogh et al., 1996; Onyango-Abuje et al., 1996).

Due to unreliability of meat inspection techniques (Viljoen, 1937; Mann and Mann, 1947; Dewhirst et al., 1967; Walther and Koske, 1980), much effort has been put into finding a reliable immunodiagnostic method for cysticercosis and other helminth infections. Since in some parasitic infections, an immune response (whether humoral or cell-mediated) may be the only evidence of indicating an infection in a live organism, the need of developing these immunodiagnostic techniques is therefore very important. This has resulted into the development and application of quite a number of immunodiagnostic techniques as an

alternative to meat inspection techniques. These immunodiagnostic techniques have been reviewed by Geerts et al., (1977), Gathuma (1977), Onyango-Abuje (1984) and Kamanga-sollo (1981, 1984) and are now briefly mentioned below.

1:2:1:1. Intradermal test (IDT).

This test was used for the first time in 1911 (Casoni, 1911, Quoted by Gathuma, 1977). Since then, IDT has been one of the immunodiagnostic tests that has been widely used in the diagnosis of many parasitic diseases (Fife, 1971). It is a sensitive test and results are obtained within a few hours. Limitations such as non-specificity (Fife, 1971; and Froyd, 1963), frequent false positive (Bugyaki, 1961) and negative (Kagan, 1968) reactions, were reported. However, Dewhirst (1960, 1967) and his co-workers found the test to be very fast and sensitive. Later, Mechnicka-Roguska and Swierz (1970), while working on human cysticercosis found that the problems of IDT mentioned above could be attributed to the use of impure and heterologous antigens for which Enyenihi (1970) suggested that the use of purified antigens could ultimately improve on its specificity and sensitivity.

1:2:1:2. Complemen. Fixation Test (CFT).

Complement fixation test is among the oldest serological techniques to be developed for diagnosing parasitic infections. Soulsby (1963) described the sensitivity of CFT to be relatively high in diagnosing calcified and encapsulated T. saginata cysticerci. He also found that the test could diagnose prenatal infections more efficiently than neonatal infections but

did not state why. However, complement fixation test was found to be non-specific (Kagan et al., 1960) and insensitive (Kagan, 1968) in detecting hydatidosis infections. Gathuma (1977) reported the inability of CFT in detecting antibodies in cattle during chronic infections with C. bovis cysticerci. Kamanga-sollo (1984) reported CFT to be comparatively more sensitive than IHAT. Cheesbrough (1985) described the test to be complex and accompanied with frequent cross-reactions. These findings make CFT less useful than other tests in immunodiagnosis of bovine cysticercosis especially in endemic areas such as Kenya where calves are usually infected early in life and may harbour cysts for a long time (Urquhart, 1961; Froyd, 1964a).

1:2:1:3. Precipitin test (PPT).

Precipitin test is also amongst the antibody test that have been used in the serological investigation of parasitic infections. In 1958, Biagi and Tay applied a precipitation test on the diagnosis of bovine cysticercosis and found it to be feasible, although with limitations of false negative and positive reactions. Later, Dewhirst et al., (1960), Maddison et al., (1961) and Morris et al., (1968), employed the test on the detection of antibodies against cysticerci of T. saginata and T. solium and found that the test was not sensitive. This was probably due to impure antigens employed, explained Morris et al., (1968). The test was also found to be unable to detect early antibody responses to infection by Enyenihi (1970) and this was attributed to low concentrations of precipitating antibodies at early stages of infection (Enyenihi, 1970). Gallie and Sewell (1974a, 1976) found the test to be insensitive and non-

specific in light infections of bovine cysticercosis. Onyango-Abuje et al (1989) used gel double immunodiffusion to identify unique oncospheral antigen of diagnostic significance in bovine cysticercosis. However, by using 5-15% (w/v) acrylamide SDS-PAGE gradient gels run under reducing conditions in tubes, Joshua et al (1989) were able to identify potentially useful oncospheral antigens, at least 8 weeks post-infection. Immunoprecipitation analysis using monoclonal antibody directed against glycoproteins of mature T. saginata cysticerci has been used to confirm common antigens present on the surface and in the excretory/secretory products of the parasite (Harrison, et al., 1984).

1:2:1:4. Latex agglutination test (LAT).

This test has been used to diagnose hydatidosis infections (Fischman, 1960) and its sensitivity and specificity evaluation was based on hydatidosis (Szyfres and Kagan, 1963) or taeniasis (Sokolovskaya, 1969). However, these authors reported several instances where the test produced false positive and negative reactions and poor specificity. Enyenihi (1970) also reported the lack of specifity with LAT when diagnosing cattle infected with T. saginata cysticerci. Morris et al., (1968) explained that the solution to the above problems would be the use of purified and standardized antigens.

1:2:1:5. Indirect haemagglutination test (IHAT).

Indirect haemagglutination test has been extensively used for immunodiagnosis of

parasitic diseases and its literature has been extensively reviewed by Gathuma (1977) and later, by Kamanga-Sollo (1981 and 1984) for bovine cysticercosis. By use of antigens prepared from proglottids, scolices and cysticerci membranes of T. saginata by different workers, IHAT has given favourably promising results (Dewhirst et al., 1967, Enyenihi, 1970, Gallie and Sewell, 1974a,b and Onyango-Abuje, 1984). Gallie and Sewell (1974a,b) successfully detected antibodies in sera of experimental calves using IHAT. Gathuma (1977), reported sensitivity and specificity of IHAT being higher than those of indirect fluorescent antibody test (IFAT). He obtained these results when screening the sera from naturally infected animals using crude antigens of mature tapeworm segments of T. saginata . He also found that specificity and sensitivity of IHAT increased with purification of these crude antigens. However, further development and practical use of this test has been adversely affected by considerable false positive reactions resulting from cross-reactions (Enyenihi, 1970 and Cheesebrough, 1987). It was suggested that this problem could be solved by purification of antigens (Morris et al., (1968).

1:2:1:6. Immunoelectrophoresis (IEP) and Counter-Immunoelectrophoresis (CIEP).

Immunoelectrophoresis has been the focus of intensive immunochemical analysis especially in hydatidosis infections (William and Sanderman, 1981). Chordi and Kagan (1965) used IEP to identify and characterize antigenic components of sheep hydatid fluid. Like IEP, CIEP was also found to be a highly sensitive test and has been widely used

in various laboratories, basically in the diagnosis of hydatidosis infections (Yarzabal et al., 1974 and Varela-Diaz et al.,1975). When evaluating CIEP for the diagnosis of cysticercosis, Geerts et al., (1980) found that CIEP was somewhat more senitive than IEP but less specific. By using IEP, Geerts et al., (1979) defined antigenic components of T. saginata and host origin. Although IEP was found to be stage-specific under experimental conditions, it occasionally lacked the desired level of sensitivity. False positive and negative reactions were generally observed and its procedures were lengthy and demanding, thereby becoming unsuitable for routine laboratory use (Geerts et al., 1979; Geerts et al., 1981 and Cheesebrough 1987). Geerts et al., (1981) also found CIEP to be a more rapid, more simple and more sensitive test than IEP. The same authors found that, at times, CIEP failed to provide the desired level of specificity and showed limited possibilities for sero-epizootiological studies which could provide basic data about the frequency of cysticercosis on a herd basis. This, coupled with false positive and negative reactions as reported by Kamanga-Sollo (1984) and Geerts et al., (1981), makes the tests quite unreliable and therefore not suitable to be used in the laboratory for bovine cysticercosis.

1:2:1:7. Indirect fluorescent antibody test (IFAT).

Since early 1960s, IFAT has been used in the diagnosis of a variety of parasitic diseases ranging from those caused by helminths (Sadun et al., 1962; Anderson et al., 1962; Sadun, 1963; Kagan et al., 1965; Bisseru and Woodruff, 1968; Beggs and Fischman, 1970; Gathuma, 1977) to those caused by protozoa (Boonpucknavig and Nairn, 1967; Fisher et al.,

1970; Fleck and Moody , 1993). Although IFAT has been widely recognised as a diagnostic tool for parasitic infections, it shows a lot of cross-reactions in the diagnosis of cestode infections (Gathuma, 1977). Rydzewski et al., (1975) reported IFAT to be as sensitive as IHAT while studying human cysticercosis.

1:2:1:8. Radioimmunoassay (RIA).

Radioimmunoassay has been described as a technique that has a high clinical value in the diagnosis of many tropical parasitic diseases (Voller and De Savigy, 1981).Like ELISA, RIA has an advantage over other serological tests in that it is an antigen detecting test as well (Cheesebrough, 1987). Assaying the antibodies with an isolated antigen (Antigen II) from C. bovis cysticerci, Kamanga-Sollo (1981) used solid-phase radioimmunoassay to detect antibody response in calves experimentally infected with C. bovis. He was also able to distinguish experimentally infected animals from non-infected controls. Radioimmunoassay has also been found to diagnose cattle 4 weeks post-infection by determining changes in proteins and glycoproteins of viable T. saginata larvae during their development from the oncospheres to the mature cysticerci (Joshua et al., 1988 and Joshua et al., 1989). Radioimmunoassay has also been used by Harrison (1989) and her co-workers to detect antigens of viable cysticerci that would be of value in the diagnosis of bovine cysticercosis. Although RIA is used to detect antibodies in specimens, it has short comings of cross-reactions like any other antibody detecting serologic test (Cheesebrough, 1987). Use of radioisotopes limits its use in many laboratories especially in developing countries.

1:2:1:9. Enzyme - linked immunosorbent assay (ELISA).

This test was first described and used by Engvall and Perlmann in 1971 for detecting antibody. Amongst the serological tests, ELISA has been preferred to others as it is exquisitely sensitive in detecting antigens (Ags) and antibodies (Abs) and yields, at least, to some extent, the desired levels of sensitivity and specificity in the diagnosis of various parasitic infections (Voller et al., 1974, 1976; Geerts et al., 1981; Gallie and Sewell, 1981; Steward and Male, 1989; Onyango-Abuje, 1984)). Recently, ELISA has been found to be the test of choice over other serological tests (Fleck and Moody, 1993) because it has several advantages. The test was found to be objective, to use small amounts of reagents and less expensive apparatus, to require only one dilution and to be adaptable to mass screening. However, antibody - ELISA was found to be unreliable in screening for bovine cysticercosis (Geerts et al., 1981; Harrison and Parkhouse, 1986). This unreliability was due to cross-reactions resulting from common antigens amongst the parasite community (mainly helminths) and to removal of antibodies from circulation caused by an excess of antigen in antibody-antigen reactions. These problems therefore meant that it was impossible to provide a species specific-diagnosis (Cheesebrough, 1987). Antibody - ELISA also does not differentiate between present and past infections and between live and dead or degenerate cysticerci (Drealants et al., 1995). As stated by Voller and De Savigny (1981), immunoassays designed to detect antibody assays, may provide at best only a presumptive diagnosis while those designed to detect antigens provide definitive serological diagnosis. Immunodiagnosis of

bovine cysticercosis based on the antigen detection is preferred, for it has been shown to statistically correlate with the presence of viable infective cysticerci (Onyango-Abuje et al., 1996).

The reliable diagnosis of cestode parasitic infections may depend on immunoassays of specific circulating excreted/secreted (ES) parasite antigens in clinical specimens like serum or urine (Morris et al., 1968; Voller and De Savigny, 1981b). Such serological assays (ELISA), which use mouse monoclonal antibodies (McAb) to detect antigens produced by viable cysticerci of T. saginata in sera of cattle, have been described by Harrison et al., (1989), Brandt et al., (1992) and Draelands et al., (1995). The monoclonal antibody based ELISA developed by Harrison et al., (1989) has been ued by Onyango-Abuje et al., (1996) to detect the presence of circulating antigens in Kenyan cattle. The assay was not reliable in diagnosing bovine cysticercosis in individual animals. This is because, the assay still produces false positive and negative reactions (Onyango-Abuje et al.,1996).

Antigen-ELISA was able to distinguish animals harbouring living cysts from those carrying dead ones (Joshua et al., 1988; Harrison et al., (1989; Hughes, 1996 and Onyango-Abuje et al.,1996). The McAb ELISA was specific for T. solium and T. saginata and did not react with other helminths or protozoa (Harrison et al., 1989). Parkhouse and Harrison (1987) also reported that glycoprotein fractions of the surface labelled material of cysticerci when used as antigens, were species specific, and did not give cross - reactions observed when crude parasite extracts were used. Of particular interest, is the fact that the developed assay was shown to indicate current infections by diagnosing only live cysticerci which are actively

producing surface turn-over, excretory and secretory products as antigens in infected cattle (Joshua et al., 1988; Harrison et al., 1989 and Onyango-Abuje et al., 1996).

Based on the Ag-ELISA which uses McAb HP10, Harrison, et al.,(1989) showed the minimum detection level to be approximately 200 live cysticerci. They also found that the antigens appeared in circulation, 4-5 weeks post-infection. Brandt et al., (1992) also working with surface/excreted/secreted glycoproteins but using a different monoclonal antibody, came up with a minimum number of living cysticerci that could be detected by their Ag - ELISA system to be 88. In a different experiment, Bogh et al., (1996) using a different monoclonal antibody, detected between 2 and 22 live cysts but the same test was unable to detect several other animals which haboured between 2 and 41 viable cysts at slaughter. In naturally infected cattle, Onyango-Abuje et al.,(1996) found the sensitivity of the McAb HP10 Ag - ELISA test to be 83% of those with 30 or more live cysts and 22% of those with 1-29 live cysts but failed to detect cattle harbouring less than 4 cysticerci. Therefore, as reported by Onyango-Abuje et al., (in press, c) the assay presently may be used in the field to identify herds with high or no infection with bovine cysticercosis but with low infections there are either false positive or false negative results. At its present state therefore, antigen-ELISA is not specific and sensitive enough to diagnose cysticercosis in individual animals. From the above literature,the exact number of live cysticerci that can be reliably detected by Ag-ELISA using McAb HP10 is therefore not yet known. Therefore, further work is necessary to evaluate on the exact number of live cysticerci that the assay can reliably detect in the infected animal and correlate this with the OD values obtained by the assay in order to make the assay powerful

and validate it for field use in controling bovine cysticercosis.

1:3. OBJECTIVES OF THE STUDY:

The aim of the study was to evaluate the reliability of an antigen - ELISA using a monoclonal antibody (McAb), HP10 developed by Harrison et al. (1989), as a diagnostic tool for bovine cysticercosis in cattle.

1:3:1. Specific objectives were:-

1. **To determine the number of live T. saginata cysticerci (C. bovis) the Antigen - ELISA can detect in naturally infected cattle.**
2. **To determine the number of live T. saginata cysticerci (C. bovis) the antigen-ELISA can detect in experimentally infected cattle.**
3. **To evaluate the efficacy of Ag - ELISA by comparing it with conventional routine meat inspection and total dissection procedures.**

1:4. HYPOTHESIS:

Detection of circulating cysticercal antigens in the serum using McAb HP10 in an antigen-ELISA can provide a rapid and reliable test for the diagnosis and screening of bovine cysticercosis in naturally infected cattle.

1:5. SIGNIFICANCE OF THE STUDY:

While control of the parasite, T. saginata, can be achieved to some extent through improvements in hygiene, adequate sanitary installations, proper animal management and husbandry practices, the development of specific and sensitive diagnostic procedures such as the antigen-ELISA would facilitate the execution of reliable epidemiological surveys which form the basis for pinpointing target control areas and evaluating control measures. Such surveys, are also essential for the design of environmentally appropriate control strategies, including the introduction of recombinant vaccines.

CHAPTER TWO

MATERIALS AND METHODS

2:1. THE PARASITE EGGS:

2:1:1. Collection of the 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 250nm, 150nm and 30nm apertures, respectively. The 30nm aperture sieve retained the eggs which were then transferred into a universal bottle containing physiological saline and antibiotics stored at 4°C untill required.

2:1:2. 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.

2:1:3. 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.

2:1:4. 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 embryos 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.

2:2. THE ANIMALS:

2:2:1. 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 4°C and

centrifuged at 2500g for 30 minutes in a refrigerated centrifuge.The resultant sera were aspirated and stored in eppendorf tubes at -20°C 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 1-1½ 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 following symmetrical dissection of the entire carcass, was noted and doubled to get the total number of cysticerci in the whole animal.

2:2:2. 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 - 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. 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.

2:2:3. Post - mortem examination:

2:2:3:1. 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.

2:2:3:2. 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 transparent slices of about 1mm, 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.

2:3. SEROLOGICAL TESTS:

2:3:1. 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).

2:4. STATISTICAL ANALYSIS:

2:4:1. 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).

2:4:2. Correlation Analysis:

Correlation st..tistical analysis was applied to compare the relationship between live cysticerci burdens and Antigen-ELISA optical density values. The Spearman rank-order correlation coefficient (r) which determined the extent to which the variables, that is, living cysticerci burdens and Antigen-ELISA optical density values were linearly related, was used and calculated using the following formula:-

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

where r = Spearman rank-order correlation coefficient

d = the difference between independent (number of live cysticerci burdens) and dependent (Antigen-ELISA optical density values) variables

n = the number of pairs of the variables.

The interpretation of the values of r at the significance level of 0.05 was done in accordance with the methods of Olds (1938).

2:4:3. 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). The interpretation of this Kappa statistics (k) was as follows:-

0----------> no agreement beyond chance level,

0.1 - 0.3----------> poor agreement,

0.4 - 0.5----------> moderate agreement,

0.6 - 0.9----------> good agreement and,

1----------> perfect agreement.

2:4:4. Analysis of sensitivity, specificity, predictive value, accuracy and apparent prevalence of 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

method.

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

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

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

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

cysticerci recovered from their carcasses at slaughter (Table 2). Five (animals with the following code numbers:- 715, 956, 972, 717 and 971) out of nine seronegative animals (at selection) were found with live cysticerci during slicing of the masculature at slaughter (Appendix 5). Of these five animals, one had 1 live cysticercus and the rest 2 live cysticerci each (Table 2). Only one seropositive animal at selection was found without any cysticerci at slaughter. Otherwise the rest were found with varying numbers of living and dead cysticerci.

Table 1(a). Frequency, recovery and distribution of cysticerci in different parts of the carcasses of the 25 animals selected from the field and examined by total dissection at slaughter.

Sites of the 25 carcasses	CYSTS' RECORD[a]		CATTLE RECORD[b]	
	No. of cysts per site.	% of No. of cysts found per site[1]	No. of animals infected by the site	% of No. of animals found infected[2]
Head	10(5)	1.49(0.75)[3]	5(4)[3]	20(16)
Tongue	22(20)	3.28(2.98)	7(6)	28(24)
Fore legs	166(104)	24.74(15.50)	18(11)	72(44)
Neck & hump	16(2)	2.38(0.30)	6(2)	24(8)
Pelvis	24(10)	3.58(1.49)	4(3)	16(12)
Hind legs	160(108)	23.85(16.10)	15(13)	60(52)
Ribs	62(30)	9.24(4.47)	5(5)	20(20)
Lumbar	44(25)	6.56(3.73)	9(5)	36(20)
Rumen	0(0)	0(0)	0(0)	0(0)
Lungs	20(14)	2.98(2.09)	11(8)	44(32)
Heart	59(24)	8.79(3.58)	15(8)	60(32)
Liver	82(14)	12.22(2.09)	19(3)	76(12)
Kidneys	1(1)	0.15(0.15)	1(1)	4(4)
Diaphragm	5(4)	0.75(0.60)	2(2)	8(8)
Grand Total	671(361)	100.00(53.80)	24(20)	96(80)

Mean of cysticerci per site of 25 animals = 47.93±14.62 (25.79±9.44)
Mean of cysticerci per site per animal = 1.92±0.58 (1.03±0.38)
Range of cysticerci in the sites of of 25 carcasses = 0-166 (0-108)
Range of cysticerci in a site of a carcass = 0-6.64 (0-4.32)

[1]The percentages were based on the grand total of cysticerci obtained from 25 carcasses.
[2]The percentages were based on 25 animals selected from the field.
[3]Figures in parentheses represent live cysticerci[a] or cattle[b] found with live cysticerci during slicing at slaughter, respectively.

Table 1(b). The number of cysticerci recovered from carcasses of the 25 animals selected from the field and examined by slicing the masculature at slaughter.

No. code of Cattle	Total number of cysticerci recovered by total dissection.		
	Live	Dead	Total
715	2	6	8
705	37	17	54
708	5	6	11
988	31	22	53
713	25	19	44
956	2	6	8
969	8	13	21
958	7	8	15
724	7	12	19
716	24	19	43
721	22	9	31
718	55	22	77
723	0	1	1
720	0	2	2
976	0	8	8
714	0	15	15
966	5	8	13
972	2	4	6
717	1	2	3
701	55	39	94
971	2	10	12
707	5	11	16
989	42	46	88
965	24	5	29
970	0	0	0
Grand total	361	310	671

The mean of cysticerci per animal ---- 26.84±27.38 (14.44±17.61)

The range of cysticerci in the group --- 0-94 (0-55)

3:1:2. Antigen-ELISA findings in naturally infected animals.

The results of the Antigen-Enzyme-linked Immunosorbent Assay (Ag-ELISA) are summarized in Table 2. Out of 25 animals selected from the field, 16 animals were detected seropositive for bovine cysticercosis while nine animals were seronegative. The seropositivity and negativity of the animals were based on the negative cut off points as shown in Table 2. Based on negative cut off point at slaughter, the Ag-ELISA detected 18 animals as seropositive for bovine cysticercosis. There were 2 animals which were seronegative at selection but later converted to be seropositive. There wasn't any animal which was seropositive at selection and later found to be seronegative at slaughter.

Out of 9 seronegative animals at selection from the field, only four (animals with the following code numbers:- 723, 720, 976 and 714) were without any cysticerci and one (976) out of these four was found to be seropositive at slaughter (Table 2). The remaining five animals (animals with the following code numbers:- 715, 956, 972, 717 and 971), were all found with one or more live and dead cysticerci at slaughter.

Basing on total dissection findings at slaughter, Ag-ELISA gave 3 cases of false seronegative (live cysticerci were recovered by total dissection but tested seronegative with Ag-ELISA) and 2 other cases of false seropositive (no live cysticerci were recovered by total dissection but tested seropositive with Ag-ELISA). The Ag-ELISA was able to detect seropositive cases for bovine cysticercosis in animals harbouring 2 and 5 living cysticerci upto 55 cysticerci. However, there were other 3 cases (animals with the following code numbers:- 956, 972 and 971) with 2 live cysticerci each that were not detected as seropositive (Table 2).

During the three months monitoring period at the NVRC, the seronegative animals (animals without detectable circulating cysticerci antigens at selection), initially did not have detectable antigen levels above the cut off point but between the second and third months the antigen level rose slightly above the cut off point (Figure 1). On the other hand, animals that were seropositive at selection had their antigens' level well above the cut off point throughout the monitoring period (Figure 1).

True seropositive and seronegative cases correlated well with live cysticerci burdens, both at selection and at slaughter. By using Spearman rank - order correlation coefficient (r), there was a positive linear correlation between live cysticerci burdens and Ag-ELISA optical density values ($r = 0.631$; $n = 25$) at $P = 0.05$. However, OD values were not indicative of the corresponding actual number of live cysticerci present in a seropositive animal and vice versa but they indicated the presence of live cysticerci.

Table 2. 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	0.091 (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.

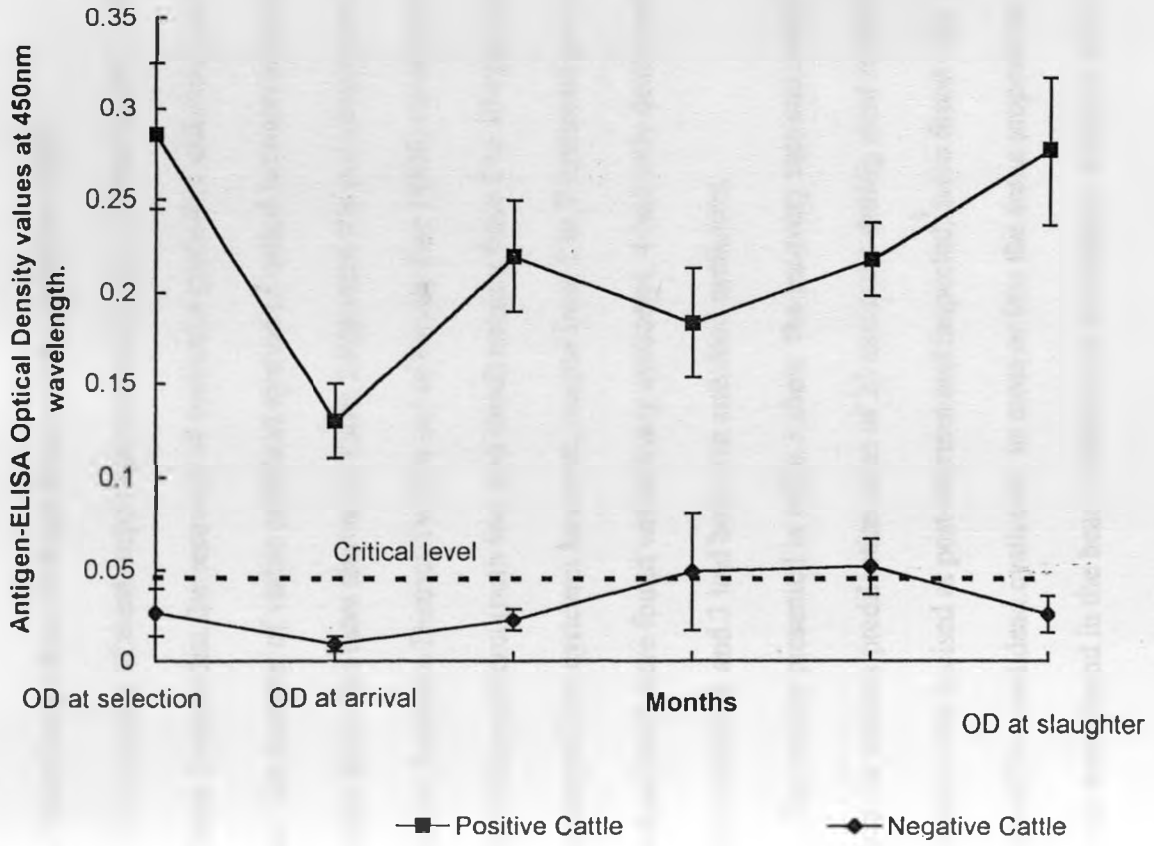

Fig.1. The average Antigen-ELISA optical density (O.D values) of 16 positive and 9 negative cattle selected from the field and monitored for three months before slaughter.

3:1:3. Meat inspection findings in naturally imfected cattle.

The results of meat inspection are presented in Tables 2 and 3. The results from Table 2 show that the recovery of cysticerci (both live and dead) in the individual animals. The number of viable and dead cysticerci varied between animals. The percentage detection rates shown in Table 2 represent the prevalence rates of this infection in Samburu District.Twelve out of twenty-five (48%) carcasses were found infected with cysticerci (both live and dead) ranging from 1 to 10 cysticerci per carcass. The number of live cystcerci however, ranged from 1 to 7 cysticerci per carcass. Thirteen animals were found without any cysticerci, 4 had only dead cysticerci, 4 had only live cysticerci and 3 had both live and dead cysticerci.

The results presented in table 3 show the recovery rate and distribution of cysticerci in various predilection sites of 25 carcasses during meat inspection. All the predilection sites incised at post-mortem and inspected, were found with varying numbers of live and dead cysticerci in more or less the same proportions. Most cysticerci were found in the heart followed by the tongue and then the triceps muscle.

Table 3: Frequency of cysticerci in predilection sites of naturally 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 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	20(5)	2.98(0.75)*	9(4)*	36(16)
Shoulder muscles	7(2)	1.04(0.30)	4(2)	16(8)
Tongue	12(12)	1.79(1.79)	3(3)	12(12)
Masseter and pterygoid muscles	0(0)	0(0)	0(0)	0(0)
Grand total	39(19)	5.81(2.83)	12(7)	48(28)

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

3:2. DETERMINATION OF THE NUMBER OF CYSTICERCI THE ANTIGEN-ELISA CAN DETECT IN EXPERIMENTALLY INFECTED CALVES:

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

From table 4 and Appendix 6, it is evident that the infection 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 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 4. 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	10,000	35	48	83	0.01

*The calculation of the infectivity indices was based on the total number of cysticerci recovered in individual calves as shown in Appendix 5 using the following formula.

$$\text{Infectivity index} = \frac{\text{Number of cysticerci recovered at autopsy}}{\text{\% motility x No. of eggs fed}}$$

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 slaughter, is shown in table 5 (a). 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, except in the kidneys. 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. Total dissection revealed live cysticerci in 16 out of 24 (66.67%) calves. Both visceral organs and skeletal muscles harboured considerable number of live and dead cysticerci. There were more dead cysticerci in the visceral organs (liver, heart, rumen, diaphragm and lungs) than there were in the skeletal muscles (hind legs, fore legs, trunk and ribs). Generally, more live cysticerci were recovered than the dead ones.

The number of cysticerci recovered from the carcasses of the individual calves is shown in table 5 (b). The number of viable and dead cysticerci also varied between carcasses of individual calves. 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 did not habour any cysticerci. One of the control calves was found infected with one live cysticercus at autopsy (Table5 (b)).

Table 5(a). Frequency, recovery and distribution of cysticerci in various parts of the carcasses of 24 experimental calves administered with various doses of Taenia saginata eggs and examined by total dissection at slaughter.

SITE	CYSTICERCI RECORD[a]		CALVES' RECORD[b]	
	No. of cysts per site in all the calves	% of No. of cysts found per site[1]	No. of calves infected by the site	% of number of animals found infected by that site[2]
Head	40(11)[3]	2.40 (0.66)[3]	9 (5)[3]	37.5(20.83)
Tongue	42(27)	2.52(1.62)	10 (7)	41.67(29.17)
Fore legs	222(163)	13.35(9.80)	15 (10)	62.50(41.67)
Neck & Hump	100(59)	6.01(3.55)	14 (10)	58.33(41.67)
Pelvis	73(55)	4.39(3.31)	13 (9)	54.17(37.50)
Hind legs	250(168)	15.02(10.10)	14 (10)	58.33(41.67)
Ribs	108 (75)	6.49(4.51)	13 (10)	54.17(41.67)
Lumbar	68 (37)	4.09(2.22)	11 (8)	45.83(33.33)
Rumen	9 (3)	0.54 (0.18)	6 (2)	25.00(8.33)
Lungs	65 (37)	3.91 (2.22)	12 (8)	50.00(33.33)
Heart	235(116)	17.13(6.97)	12 (8)	50.00(33.33)
Liver	366(93)	22.00(5.59)	21(10)	87.50(41.67)
Kidneys	0(0)	0.00(0.00)	0(0)	0.00(0.00)
Diaphragm	35 (20)	2.10 (1.20)	8 (5)	33.33(20.83)
Grand total	1663(864)	100.00(51.95)	24(16)	100.00(66.67)

Mean of cysticerci per site of 24 calves = 118.79±30.56(61.71±14.77)[3]

Mean of cysticerci per site per calf = 4.95±1.27(2.57±0.62)[3]

Range of cysticerci in the sites of 24 calves = 0-366(0-168)[3]

Range of cysticerci in a site of a calf = 0-15.25(0-7)[3]

[1]The percentages were based on the grand total of cysticerci obtained in all the calves

[2] The percentages were based on the 24 experimentally infected calves.

[3]The figures in parentheses represent live cysticerci[a] or calves[b] found with live cysticerci during total dissection at slaughter, respectively.

Table 5(b). The number of cysticerci recovered from carcasses of 24 experimental calves, administered with various doses of Taenia saginata eggs and examined by total dissection at slaughter.

Calf Code No.	Egg dose	Number of cysticerci recovered at autopsy		
		Live	Dead	Total
4158	0000	0	0	0
4157	0000	0	0	0
4151	0000	1	0	1
4170	0000	0	0	0
947	0000	0	0	0
944	0000	0	0	0
4160	2500	152	1	153
4153	2500	17	18	35
4154	2500	76	12	89
4152	2500	3	4	7
4172	2500	0	28	28
4173	2500	0	10	10
951	2500	14	31	45
945	2500	0	0	0
4162	5000	0	8	8
4161	5000	124	38	162
4166	5000	0	22	22
4155	5000	2	36	38
4171	5000	0	4	4
4167	5000	1	14	15
950	5000	193	2	195
948	5000	0	187	187
4164	10,000	62	29	91
4165	10,000	59	26	85
4163	10,000	0	249	249
4159	10,000	12	61	73
4175	10,000	0	8	8
4169	10,000	55	4	59
949	10,000	1	5	6
946	10,000	93	1	94
Grand total	140,000	864	800	1664

The mean of cysticerci per animal - 69.29±14.56(36.00±11.26)

The overall percentage mean recovery rate of cysticerci - 4.16±0.88(2.16±0.68)

The range of cysticerci in an animal - 0-249(0-193)

The percentage recovery range in an animal - 0.00-14.96(0-11.60)

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

Table 6 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 seronegative cut off points as shown in table 6. At slaughter, with an OD negative cut-off point of 0.059, the Ag-ELISA detected 9 animals (37.50%) as seropositive for bovine cysticercosis.The controls never showed any detectable levels of antigenemia.

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.

From Figure 2, the circulating cysticercal antigens could be demonstrated from 7-11 weeks post-infection and the antigenemia could not be detected in the control calves. The levels of the parasite antigens were apparently still increasing by the time the experiment was terminated at 15 weeks post-infection (Figure 2).

True seropositive and seronegative cases correlated well with live cysticerci burdens, both at selection and at slaughter. By using Spearman rank - order correlation

coefficient (r), there was a positive linear correlation between live cysticerci burdens and Ag-ELISA optical density values ($r = 0.798$; $n = 24$) at $P = 0.05$. However, OD values were not indicative of the corresponding actual number of live cysticerci present in a seropositive animal and vice versa but they indicated the presence of live cysticerci.

Table 6. Post-mortem findings of 30 calves given various doses of Taenia saginata eggs together with their Antigen-ELISA readings at 450nm wavelength.

	Egg No	Post-mortem finding		Ag- ELISA readings
Calf Number		Total Dissection L D T	Meat Inspection L D T	Before Infection At slaughter
4158	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.....0.025
951	2500	14.....31.....45	4.....0.....4	-0.022.....0.050
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.....187.	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 .....37.5

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

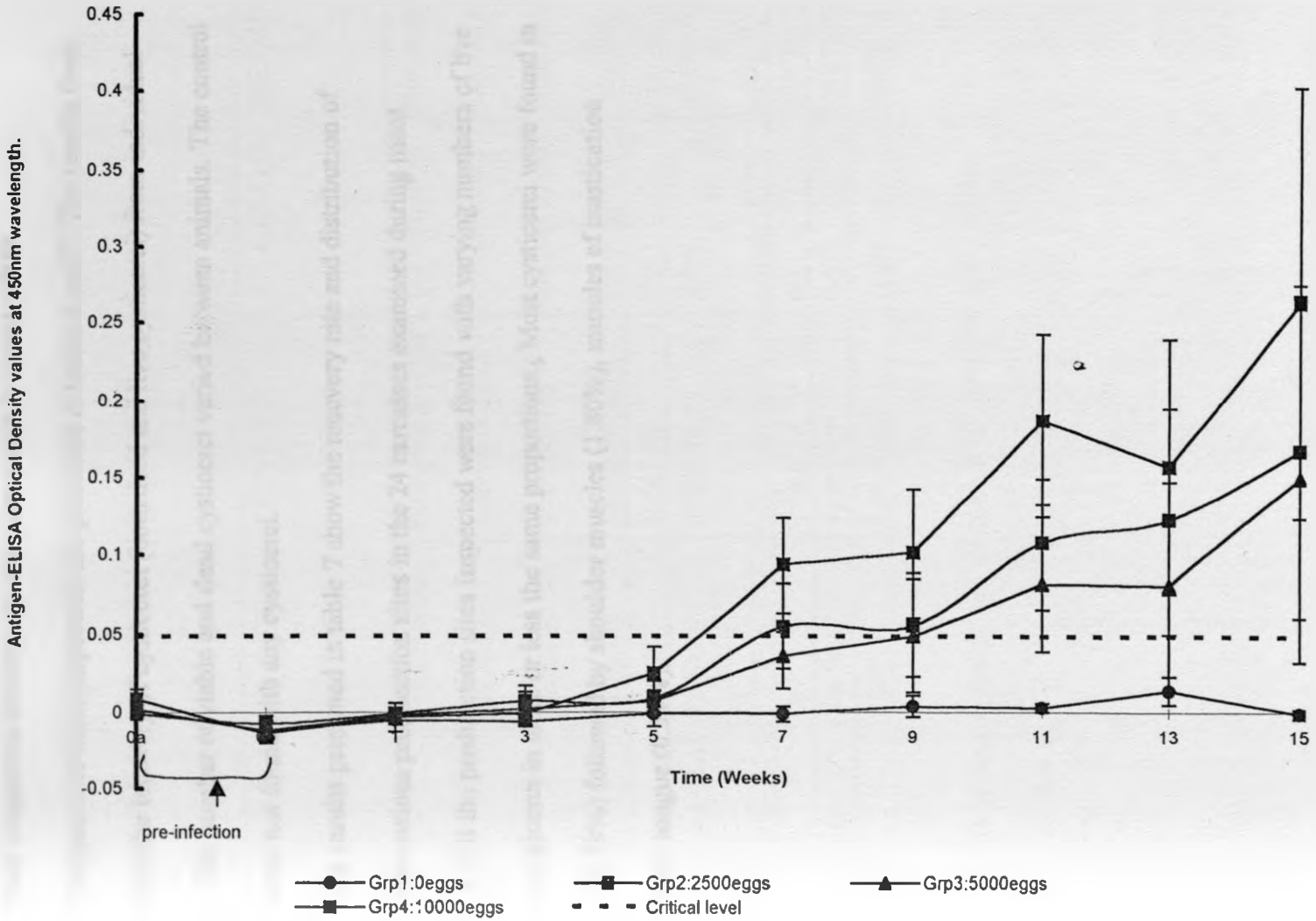

Fig. 2. The average Antigen-ELISA Optical Density values of 30 experimentally infected calves with various doses of *T. saginata* eggs and monitored every two weeks until slaughtered in the 15th. week.

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

The results of meat inspection are presented in tables 6 and 7. The results from table 6 show the recovery of cysticerci (both dead and live cysticerci) from individual animals. The number of viable and dead cysticerci varied between animals. The control animals were not found with any cysticerci.

The results presented in table 7 show the recovery rate and distribution of cysticerci in various predilection sites in the 24 carcasses examined during meat inspection. All the predilection sites inspected were found with varying numbers of live and dead cysticerci in more or less the same proportions. Most cysticerci were found in the heart (5.29%) followed by shoulder muscles (1.80%), muscles of mastication (0.66%) and tongue (0.54%).

Table 7. **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 d 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.

3:3. EFFICACY OF ANTIGEN-ELISA:

Antigen-ELISA and meat inspection were compared using diagnostic test evaluation tables. The results are shown below.

3:3:1. Diagnostic test evaluation tables for naturally infected animals.

Total dissection is taken as the gold standard which gives the "true" status of the infection in the cattle unless otherwise stated. Total dissection even though taken as the gold standard has fundamental flaws. Since the Antigen-ELISA detects antigens of live cysticerci, every test was based on live cysticerci so that all tests in considerations were at the same level when compared.

1. **Evaluation of meat inspection in naturally infected animals.**

Meat Inspection Method	Total Dissection (Gold Standard)		
	+ve	-ve	Total
+ve	7	0	7
-ve	12	6	18
Total	19	6	25

From the table, False +ve = 0

False -ve = 12

True +ve = 7

True -ve = 6 and therefore,

1. Sensitivity of the test = 7/19 x 100=36.84%
2. Specificity of the test = 6/6 x 100=100.00%
3. Predictive value of the test = 7/7 x 100=100.00%
4. Accuracy of the test = 13/25 x 100=52.00%
5. Apparent prevalence of the infection

= 7/25 x 100 = 28.00%

The level of agreement between the two methods of diagnosis (that is, between meat inspection and total dissection), Kappa (k) was = 0.218. Total dissection gave an apparent prevalence of 76%, meat inspection method gave, 28%, and both methods were positive and negative in 7 (28%) and 6 (24%) of the 25 animals selected from the field, respectively.

II. Evaluation of an Antigen-ELISA in naturally infected animals.

Antigen-ELISA	Total Dissection	(Gold Standard)	
	+ve	-ve	Total
+ve	16	2	18
-ve	3	4	7
Total	19	6	25

From the table, False +ve = 2

False -ve = 3

True +ve = 16

True -ve = 4 and therefore,

1. Sensitivity of the test = 16/19 x 100 = 84.21%
2. Specificity of the test = 4/6 x 100 = 66.67%
3. Predictive value of the test = 16/18 x 100 = 88.89%
4. Accuracy of the test = 20/25 x 100 = 80.00%
5. Apparent prevalence of the infection

   = 18/25 x 100 = 72%

The level of agreement between the two methods (that is, between Ag-ELISA and total dissection), Kappa (k) was = 0.482. Total dissection gave an apparent prevalence of 76%, theAg-ELISA gave72% and both methods were positive and negative in 16 (64%) and 4 (16%) of the 25 animals selected from the field, respectively.

III. **A comparison between an Antigen-ELISA and meat inspection in naturally infected animals.**

Antigen - ELISA	Meat Inspection Method		
	+ve	-ve	Total
+ve	7	11	18
-ve	0	7	7
Total	7	18	25

The level of agreement between the two methods (that is, between Antigen-ELISA and meat inspection), Kappa (k) was = 0.262. The meat inspection method gave an apparent prevalence of 28%, the Antigen-ELISA gave 72%, and both methods were both positive and negative in 7 (28%) of the 25 animals selected from the field.

3:3:2. Diagnostic test evaluation tables for experimentally infected calves.

In this case again, total dissection was considered as the standard of validity which gave the true status of the infection in the calves unless otherwise stated. In this section also, the comparison was based on the live cysticerci only.

1. **Evaluation of meat inspection in experimentally infected calves.**

Meat Inspection	Total Dissection (Gold Standard)		
	+ve	-ve	Total
+ve	8	0	8
-ve	7	9	16
Total	15	9	24

From the table, False +ve = 0

False -ve = 7

True +ve = 8

True +ve = 9 and therefore,

1. Sensitivity of the test = 8/15 x 100 = 53.33%
2. Specificity of the test = 9/9 x 100 = 100.00%
3. Predictive value of the test = 8/8 x 100 =100.00%
4. Accuracy of the test = 17/24 x 100 = 70.83%
5. Apparent preva;ence of the infection

= 8/24 x 100 = 33.33%

The level of agreement between the two methods (that is, between Meat inspection and total dissection), Kappa (k) was = 0.461. Total dissection gave an apparent prevalence of 62.50%, the meat inspection method gave 33.33%, and both methods were positive and negative in 8 (33.33%) and 9 (37.50%) of the 24 calves exposed to T. saginata eggs, respectively.

II. Evaluation of an Antigen-ELISA in experimentally infected calves.

Antigen-ELISA	Total Dissection (Gold Standard)		
	+ve	-ve	Total
+ve	8	1	9
-ve	7	8	15
Total	15	9	24

From the table, False +ve = 1

False -ve = 7

True +ve = 8

True -ve = 8

1. Sensitivity of the test = 8/15 x 100 = 53.33%
2. Specificity of the test = 8/9 x 100 = 88.89%
3. Predictive value of the test = 8/9 x 100 = 88.89%
4. Accuracy of the test = 16/24 x 100 = 66.67%
5. Apparent prevalence of the infection

   = 9/24 x 100 = 37.5%

The level of agreement between the two methods (that is, between Antigen-ELISA and total dissection), Kappa (k) was = 0.374. Total dissection gave an apparent prevalence of 62.50%, the Antigen-ELISA gave 37.50%, and both methods were both positive and negative in 8 (33.33%) of the 24 calves exposed to T. saginata eggs.

III. **A comparison between an Antigen-ELISA and meat inspection in experimentally infected calves.**

Antigen-ELISA	Meat Inspection Method		
	+ve	-ve	Total
+ve	7	2	9
-ve	1	14	15
Total	8	16	24

The level of agreement between the two methods (that is, between Antigen-ELISA and meat inspection), Kappa (k) was = 0.727. The usual meat inspection method gave an apparent prevalence of 33.33%, the Antigen-ELISA gave 37.50% and both methods were positive and negative in 7 (29.17%) and 14 (58.33%) of the 24 calves exposed to T. saginata eggs, respectively.

Table 8. **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		36.84	84.21	-	53.33	53.33	-
Specificity of the test		100.00	66.67	-	100.00	88.89	-
Predictive value of the test		100.00	88.89	-	100.00	88.89	-
Accuracy of the test		52.00	80.00	-	70.83	66.67	-
Apparent prevalance of the the test		28.00	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	28.00	64.00	28.00	33.33	33.33	29.17
	negative	24.00	16.00	28.00	37.50	33.33	58.33

KEY:

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

CHAPTER FOUR

DISCUSSION, CONCLUSIONS, OBSERVATIONS AND RECOMMENDATIONS

4:1. DISCUSSION

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. 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.

In naturally infected cattle, total dissection was used as the ultimate confirmatory test (the gold standard of validity) to indicate the presence or the absence of bovine cysticercosis infection in the animals investigated. According to total dissection, 25 cattle were found to have the infection and only one animal was negative. Total dissection method might not have been 100% efficient due to human error resulting from status of human eye sight. This factor is very important in explaining the absence of cysticerci or low recovery rate of cysticerci in all or some parts of the carcasses sliced as shown in Tables 1 (a) and (b). This factor (poor human eye sight), has also been reported to be among the factors that affect the efficiency of meat inspection (Walther and Koske, 1980; Cheruiyot, 1981; Kang'ethe, 1995). The above factor may have also contributed to the occurrence of false-positive cases found in diagnostic test evaluation tables in section

3:3:3. and Table 2.

The results in Table 1(a) showed that nearly all the sites except rumen were equally important sites worth being examined during routine meat inspection. However, as stipulated in Meat Control Act-1977, only a few predilection sites (heart, shoulder muscles, tongue and masseter and pterygoid muscles) are inspected. In the predilection sites, the Act allows only a small area to be incised and examined.

The results obtained by thinly slicing the carcasses, were in accordance with the previous studies conducted by Mitchell (1978), Walther and Koske (1980), Gallie and Sewell (1983), Harrison et al., (1984), Kyvsgaard et al.,(1990) and Gracey and Collins (1992) in terms of distribution of the cysticerci in various parts of animals. However, there are variations with regard to the existence and ranking of sites occupied by cysticerci in cattle. These variations depend on a number of factors. For instance, Gracey and Collins (1992) stated that the distribution of cysticerci is purely mechanical by the volume and intensity of the arterial blood owing to day-to-day activities of the animals in consideration. On the other hand, the results obtained by this investigation were in contrast to the views of Gracey and Collins (1992) with regard to predilection sites for cysticerci. This is because, Gracey and Collins (1992) stated that there are no predilection sites for cysticerci in the animals, while the results shown in Tables 1(a) and 5(a) clearly indicated that different organs and muscle groups of a given carcass did not have the same number of cysticerci and therefore, they could be ranked according to the number of cysticerci recovered from each one of them. For instance, there was a decreasing number of both live and dead cysticerci recovered from the fore legs, hind legs, liver, ribs, heart, lumbar, pelvis, tongue, lungs, neck and hump, head and diaphragm in that order which was in agreement to that of Mango and Mango (1972). In naturally infected animals, no

cysticercus was found in the rumen, fat layers, spleen and skin . The spleen is the most immunologically active part in the animal and that was the reason why probably the cysticerci could not get established in it. The skin, rumen and fat layers on the other hand, are not preferred sites for cysticerci occupation because they are not, probably, sufficiently supplied with arterial blood which is an important route of dissemination of hatched oncospheres to various sites of the animal.

Since the distribution of the cysticerci is purely mechanical by the volume and circulation of the arterial blood (Gracey and Collins, 1992), any factor affecting them determines the distribution and location of the cysticerci in the animal. For instance, blood flowing through a capillary network is partly controlled by the action of sphincter muscles in the arterioles and its tension is regulated by nerves and hormones. By dilation (expansion) of the arterioles in one part of the organism's body and their constriction in another, the flow of blood in which cysticerci are carried is adjusted and distributed according to the needs of various regions or parts of the organism (Pures and Orians, 1987). Due to this internal physiological mechanisms in the cattle, more cysticerci antigens will be available in the serum if during sampling more blood was from muscles heavily infested with live cysticerci which were actively secreting and excreting their metabolic products into blood circulation. These physiological mechanisms coupled with blood kinetics and external environmental conditions of the cattle, may help explain why some infected animals tested negative with the Antigen-ELISA while their counterparts (with similar number of live cysticerci) tested positive.

Dead cysticerci were more in the visceral organs e.g. liver and heart than in the skeletal muscles e.g. those of the hind and fore legs. This could be due to limited amount of blood carrying insufficient amount of oxygen to these organs. Although the heart

receives high volumes of oxygenated blood, the heart muscles do not receive this blood directly. Instead, this blood has to go through the general blood circulation before getting back to the heart via the blood vessel that supplies it. Alternatively, since the organs perform varying physiological activities compared to the skeletal muscles, the resultant end- and by-products may have lethal effects to the established live cysticerci. This might have resulted into the occurrence of varying proportions of both live and dead cysticerci (Tables 1(a) and 5(b)). The death of cysticerci could also be due to immune responses mounted against frequent reinfection resulting from hatching oncospheres, in both naturally and experimentally infected cattle. For the case of naturally infected animals, this kind of immunity can be maintained by the animals through picking up eggs on different occasions when grazing in fields of high infection pressure zones as reported by Penfold and Penfold, 1937 and Urquhart, 1961. The immune response mounted against the infecting oncospheres or lodged cysticerci, may also depend on the age of the animals. Usually young animals in particular, become more susceptible to reinfection than the older ones and as they become older, they develop an increasingly stronger immunity (Gallie and Sewell, 1983). In which case, the proportion of dead cysticerci would have been greater in the older animals than in the young ones but this was not supported by evidence from this study (in the naturally infected older animals - 46.20% of the cysticerci were dead and in the experimentally infected younger animals, the percentage was - 48.05%) (Tables 1(a) and 5(a), respectively). However, it is not currently known if any reinfection cysticerci would survive to the fully developed stage in an immunologically active environment of the animal. Immunity might have also contributed to the variation that existed in the recovery rates of cysticerci between carcasses and between parts of carcasses (Tables 1(a,b) and 5(a, b)).

The recovery and distribution of cysticerci in experimentally infected calves followed a slightly different pattern from that of naturally infected cattle already described above. The intensity of cysticerci (both live and dead), was higher in the visceral organs than in the skeletal muscles (Table 5 (a)). This pattern reversed in naturally infected animals which had the skeletal muscles harbouring a higher number of cysticerci (both live and dead) than the visceral organs (Table 1(a)). This was probably because of the differences in the activities of these two groups of cattle. The most active organs or muscles during the period of infection, seems to have been infested with the highest number of cysticerci. For instance, the naturally infected cattle had been moving for long distances in search of water and green pastures with pastoralists, thereby maximally making use of their skeletal muscles which received more blood than any other parts of the body thereby increasing the chances of the oncospheres in blood circulation reaching the muscles and getting established there. This resulted in the high intensity of cysticerci in these muscles (Table 1(a)). While the neonatal calves were only confined in the pens where they were reared throughout the experimental period and hence, high intensity of cysticerci in the visceral organs which were more active than the skeletal muscles. Since visceral organs harboured more dead cysticerci than the live ones, these patterns of distribution of cysticerci in these sites may also help further to explain why the calves had a higher number of dead than live cysticerci, while in the naturally infected animals, it was the reverse (Tables 1(a,b) and 5(a,b)).

In the experimentally infected calves, one control calf was found with one live cysticercus in the hind leg at autopsy (Table 6). 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. These low recovery rates were manifested in poor infectivity indices as shown in Table 4 and which resulted in very low recovery rates or complete lack of cysticerci in some carcasses (Table 6). 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 4).

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 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 (Figure 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 2). 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 2). 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 (Figure 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 0.1M-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 int. 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).

It was apparent from Tables 2 and 6 that the sensitivity of the Antigen-ELISA showed variations from one animal to another. The lowest number of live cysticerci which could be detected by the test was 2. Some animals harbouring 2 live cysticerci escaped detection while others tested seropositive. In the experimentally infected calves, animals with 14 or more live cysticerci had detectable levels of antigenemia in their sera at slaughter , while those with less number of live cysticerci were seronegative for bovine cysticercosis (Table 6). Like the naturally infected animals, other calves (calves with code numbers 4153 and 4159) habouring 17 and 12 live cysticerci respectively, at slaughter, also escaped detection by Antigen-ELISA. As explained before, these differences in sensitivity of the test might be related to the permeability of the wall around the larvae which influences the amount of excretory/secretory products (antigens) being released into the circulation (Brandt et al., 1992). The least number of living cysticerci this Antigen-ELISA was able to detect, reduced tremendously from 200 as reported by Harrison et al., (1989) to 2 and 14 in naturally and experimentally infected animals, respectively. In naturally infected

animals, the antigen assay displayed a sensitivity of 84.21% which was almost the same as that obtained by Correa et al., (1989) (72%) when using the same monoclonal antibody, HP10, in human cysticercosis infection. In experimentally infected calves, the assay displayed a sensitivity of 53.33%, which was of course very low when compared to that of naturally infected animals stated above. The poor sensitivity of the assay in experimentally infected calves was attributed to the poor infection rates of the calves with T. saginata eggs. This problem could be avoided or improved by either using trickle infections or a susceptible host (susceptible species of cattle like the Kenyan local Borans).

Despite the fact that the Antigen-ELISA did not detect other animals harbouring considerable number of live cysticerci (that is, 12 and 17) , statistical analysis revealed that the final Antigen-ELISA readings, at slaughter, correlated positively with the live cysticerci burdens in both naturally and experimentally infected animals. Using Spearman rank-order correlation coefficient (r), there was a statistically significant positive linear association between the number of live cysticerci recovered at autopsy and the Antigen-ELISA optical density values ($r = 0.631$ and $r = 0.798$, $n = 25$ and $n = 24$, for naturally and experimentally infected animals, respectively; $p < 0.05$ in each case). More specifically, this correlation analysis showed that the animals with high number of live cysticerci recovered also had high optical density values. This kind of correlation was also found by Onyango-Abuje et al (1996). These results, support the findings of Harrison et al (1989) and Onyango-Abuje et al (1996), that the test only detects viable cysticerci in cattle. It is important to note that in spite of the above positive correlation, the optical density values could not be used directly to indicate a corresponding figure of the number of live cysticerci and vice versa. For instance,

from Table 2, an animal with code number 716 had 24 live cysticerci and was seropositve with O.D. value of 0.565 while another one (705) which had a higher number of live cysticerci (37) was also seropositive but with a lower O.D. value of 0.116. Similar examples also do exist from Table 6 with calves bearing the following pairs of code numbers 4164 ; 4165 and 4161 ; 4169.

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 follows 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.

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 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 (Figure 1). 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 (Appendix 5). 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 Figures 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 responds 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. 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. 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 cannot 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-ELLISA 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 (Table 8). 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). 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 which was designed to detect products of viable cysticerci in live animals, 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 8, the epidemiological usage of the parameters (sensitivity, specificity, predictive value, accuracy, and prevalence) was considered when comparing the diagnostic methods. The meaning of these parameters have been defined in Appendix 1. In naturally infected animals, the antigen assay displayed a sensitivity of 84.21% (in animals harbouring 2-55 live cysticerci) while meat inspection method had 36.84% (in animals harbouring 1-7 live cysticerci) when total dissection was used as a gold standard. The results indicated that the assay was at least more than twice (that is, 2.3 times) 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 and meat inspection method was the same (53.33%) (in animals harbouring 14-193 and 1-17 live cysticerci, respectively) (Tables 6 and 8) when total dissection was used as a gold standard. This discrepancy might have been due to poor infectivity of the eggs in the calves and this was the possible reason also for the low prevalence rates obtained in these calves with the Antigen-ELISA. Although the Antigen-ELISA had the same sensitivity as meat inspection method in experimentally infected calves, it is advantageous to use the antigen detection assay to avoid incurring economic losses. 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 66.67% (for the assay specificity in natural infections) to 88.89% for predictive value(in natural and experimental infections) and specificity (in experimental infections) (Table 8). 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 8). 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 8). 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 8). 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 8). 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.

4:2. CONCLUSIONS.

From the preceeding discussion, the following conclusions, have been made:-

1. The Antigen-ELISA can, at best, be used as a screening test and not as a diagnostic test because it still gives false- positive and negative cases whose occurrence has not yet been clearly explained. However, the assay can provide for individual diagnosis only in heavily infected cattle.
2. An increase in sensitivity will be necessary, in order to develop a useful field test since the burdens of live cysticerci present in whole carcasses of lightly infected animals, which epidemiologically are the most important group, vary between 1 and 17, which the Antigen-ELISA is currently unable to detect consistently.

4:3. OBSERVATIONS AND RECOMMENDATIONS.

1. In this study, the lowest number of living cysticerci that could be detected by Antigen-ELISA in both naturally and experimentally infected animals was 2 and 14 , respectively.
2. In natural infections, the Antigen-ELISA was more than twice as sensitive as meat inspection method although the sensitivity of the test was the same in experimental infections.
3. There was a good correlation between the burdens of live cysticerci and the Antigen-ELISA Optical Density values in both naturally and experimentally infected animals.
4. The Antigen-ELISA was able to detect antigens of only viable cysticerci in both naturally and experimentally infected animals.

5. The Antigen-ELISA showed a good precision particularly in natural infections.

6. In spite of promising results, it is not yet economical to apply the Antigen-ELISA in the field as a screening test because some of the reagents used such Streptavidin biotinylated horseradish peroxidase conjugate/complex, are very expensive and others such as the monoclonal antibody, are not produced locally and therefore, importation process , may not be an easy job for a poor pastoralist who is the major livestock producer particularly in developing countries.

7. Should further research provide solutions to the problems currently surrounding the Antigen-ELISA as discussed above, then it may be one of the most powerful diagnostic tool in the control of bovine cysticercosis infections in the animals because the test helps the farmer in decision making with respect to beef animals (that is, whether to take the animal in the slaughterhouse for human consumption, subject it to chemotherapeutic agents or improve public hygiene of the feeding areas of the animals).

8. For routine meat inspection method to compare favourably with Antigen-ELISA more parts of the carcass not currently inspected for bovine cysticercosis such as hind legs, ribs, lungs and liver, need to be considered as predilection sites and larger areas of these predilection sites should be examined.

REFERENCES.

Alfonso, R. (1997). Prevalence and control of taeniasis and cysticercosis: International Workshop on Cysticercosis, 18 and 19 August,1997. A global perspective. Paper presented at the workshop on cysticercosis. Onderstepoort, South Africa.

Anderson, R. I., Sudan, E.H. and Williams, J.S. (1961). A technique for the use of minute amounts of dried blood in antibody test for schistosomiasis. Experimental Parasitology, **11,** 8-10.

Anonymous (1997). International Workshop on Cysticercosis, 18 and 19 August, 1997. ONDERSTEPOORT VETERINARY INSTITUTE, ONDERSTEPOORT, SOUTH AFRICA.

Babiker - Sheiba, H.A. and Zein Eldin, EL.S.A. (1987). Preliminary observations on vaccination against Bovine cysticercosis in the Sudan.Veterinary Parasitology, **24,** 297 - 300.

Beggs, W.A. and Fischman, A. (1970). A preserved antigen for the hydatid fluorescent antibody and other tests utilizing scolices. Bulletin of World Health Organization. **42,** 331 - 332.

Biagi, F.F. and Tay, J. (1958). A precipitation test for the diagnosis of cysticercosis. American Journal of Tropical Medicine and Hygiene, **1,** 63 - 65.

Bisseru, B. and Woodruff, A.W. (1968). The detection of circulating antibody in human *Toxocara* infections using indirect fluorescent antibody test. Journal of Clinical Pathology, **21,** 449 - 455.

Bogh,H.O., Grovold, J., Maeda,G.E., Nansen, P., Lind, P. Ilsoe,B., Henriksen, S.A., Geerts, S. (1996). Experimental single and trickle injections of cattle with Taenia saginata:studies of immunodiagnosis. Research in Veterinary Science, **60 (1),** 64-68.

Botero, D. (1989). Cysticercosis. Tropical Medicine and Parasitology. Eds. Robert, G. and Heyneman, D. PP., 497-502. Prentice-Hall International Editions.East Norwalk, Conn:Appleton and Lange.

Brandt, A.R.J., Geerts, S., De Deken, R., Kumar, V., Ceulemans, F., Brijs, L. amd Falla, N., (1992). A monoclonal antibody-based ELISA for the detection of circulating excretory - secretory antigens in Taenia saginata cysticercosis. International Journal of Parasitology **22,** 471-477.

Boonpucknavig, S. and Nairn, R.C. (1967). Serological diagnosis of amoebiasis by immunofluorescence. Journal of Clinical Pathology, **20,** 875 - 878.

Bugyaki, L. (1961). Diagnostic de la cystcercose a l' aide de l'intradermoreaction. Bulletin of Epizootic Diseases in Africa, **9,** 15-23.

Chandler, A.C. and Read, P.C. (1961). Introduction to Parasitology. pp.320-381 ed. **10** John Wiley and Sons, Inc., New York.

Cheesebrough, M.(1992). Medical Laboratory Manual for Tropical Countries. **Vol.1** 2nd ed. PP. 178-197. University Press,Cambridge, Butterworth - Heinemann Ltd.

Cheruiyot, H.K., (1981). The prevalence of Cysticercosis bovis in Kenya's abattoirs between 1975-1979. Bulletin of Animal Health in Africa, **29,** 135-141.

Chordi, A. and Kagan, I.G. (1965). Identification and characterization of antigenic components of sheep hydatid fluid by immunoelectrophoresis. Journal of Parasitology **51,** 63.

Clegg, J.A. and Smith, M.A. (1978). Prospects for the development of dead vaccines against helminths. Advances in Parasitology, **16,** 165 - 218.

Correa, D., Sandovaı,M.A., Harrison, L.J.S., Parkhouse, R.M.E., Plancarte, A., Meza-Lucas, A. and Flisser, A. (1989). Human neurocysticercosis comparison of enzyme immunoassay capture techniques based on monoclonal and polyclonal antibodies for the detection of parasite products in cerebrospinal fluid.Transactions of Royal Society of Tropical Medicine and Hygiene, **83,** 814 - 816.

Craig, P.S. and Rickard, M.D. (1980). Evaluation of "crude" antigen prepared from Taenia saginata for the serological diagnosis of Taenia saginata cysticercosis in cattle using the enzyme-linked immunosorbent assay,ELISA. Zeitschrift für Parasitenkunde **61,** 287-297.

Craig, P.S. and Rickard, M.D. (1981b). Studies on the specific immunodiagnosis of larval

cestode infections of cattle and sheep using antigens purified by affinity chromatography in an enzyme-linked immunosorbent assay (ELISA). International Journal of Parasitology, **11,** 441-449.

Dewhirst, L.W., Trautman, R.J., Pistor, W.J. and Reed, R.E. (1960). Studies on ante-mortem diagnostic procedures in bovine cysticercosis infection. Journal of Parasitology **46 (5 suppl.),** 10-11.

Dewhirst, W.L., Cramer, D.J., and Pistor,J.W. (1963). Bovine cysticercosis. I. Longevity of cysticercosis of Taenia saginata. Journal Parasitology, **49,** 297-300.

Dewhirst, L.W., Cramer, J.D. and Sheldon, J.J. (1967). An analysis of current inspection procedures for detecting bovine cysticercosis. Journal of American Veterinary Medical Association, **150,** 412 - 417.

Draelants, E., Hojkens, E., Harding, E., Brandt, J. and Geerts, S. (1995). Development of a dot-enzyme immunoassay for the detection of circulating antigen in cattle infected with Taenia saginata cysticerci. Research in Veterinary Science, **58,** 99-100.

Draelants, E. Brandt, A.R.J, Kumar, V. and Geerts, S.(1995). Characterization of epitopes on excretory-secretory antigens of Taenia saginata metacestodes recognized by monoclonal antibodies with immundiagnosis potential. Parasite Immunology, **17,** 143 - 147.

Eckert, E.K. (1996). Workshop Summary: Food Safety; Meat - and fish - borne Zoonoses.

Veterinary Parasitology, **64,** 143 - 147.

Engvall, E.and Permann, P. (1971). Enzyme-linked immunosorbent assay (ELISA) I. Quantitative assay of Immunoglobulin G.Immunochemistry **8,** 871-874.

Enyenihi, U.K. (1970). Analysis, purification and serologic evaluation of antigens for diagnosis of bovine cysticercosis and human taeniasis. Journal of Parasitology, **56, 2,** 91 - 92.

Fan, P.C. (1997). Annual Economic Loss caused by Taenia saginata asiatica Taeniasis in East Asia. Parasitology Today, **13,** 194 - 196.

Fay, L.D. (1972). Exposure of Thomson's Gazelle to Experimental Infection with Cysticercus bovis. Veterinary Record, **90,** 34 - 35.

Fife,E.H.J. (1971). Advances in methodology for immunodiagnosis of parasitic diseases. Experimental Parasitology, **30,** 132-163.

Fischman, A. (1960). Flocculation tests in Hydatid Diseae. Journal of Clinical Pathology, **13,** 72 - 75.

Fisher, G.U., Sulzer, A.J., Wilson, M. and Runcik, K. (1970). Fluorescent - antibody patterns in naturally acquired vivax malaria. American Journal of Tropical Medicine and Hygiene, **19,** 209 - 214.

Fleck, L.S. and Moody, H.A. (1993). Diagnostic Techniques in Medical Parasitology. 11th.

edn. ELBS edns. Great Britain,University Press, Cambridge.

Froyd, G. (1960). Cysticercosis and hydatid disease of cattle in Kenya. Journal of Parasitology, **46,** 491- 496.

Froyd, G. (1963). Intradermal tests in the diagnosis of bovine cysticercosis. Bulletin of Epizootic Diseases in Africa, **11,** 303 - 306.

Froyd, G. (1964a). The longevity of Cysticercus bovis in bovine tissues. British Veterinary Journal, **120,** 205 - 211.

Froyd, G. (1964b). The artificial oral infection of cattle with Taenia saginata eggs. Research in Veterinary Science, **5,** 434 - 440.

Froyd, G. (1965). Bovine Cysticercosis and human taeniasis in Kenya. Annals of Tropical Medicine and Parasitology, **59(2),** 169-180.

Froyd, G. and Round, M.C. (1960). The artificial infection of adult cattle with Cysticercus bovis. Research in Veterinary Science, **1,** 275-282.

Gallie, J.G. and Sewell, H. M. M. (1972). The survival of Cystcercus bovis in resistant calves. Veterinary Records, **19,** 481 - 482.

Gallie, G.J. and Sewell, M.M.H. (1974a). The serological response of three month old calves to infection with Taenia saginata (Cysticercus bovis) and their resistance to reinfection.Tropical Animal Health and Production, **6,** 163 - 171.

Gallie, G.J. and Sewell, M.M.H. (1974b). The Serological response of calves infected neonatally with Taenia saginata (Cysticercus bovis). Tropical Animal Health and Production, **6,** 173 - 177.

Gallie, G.J. and Sewell, M.M.H. (1976). Experimental immunizatiom of 6-month old calves against infection with the cysticercus stage of Taenia saginata. Tropical Animal Health production, **8**, 233-242.

Gallie, J.G. and Sewell, H. M. M. (1977). The effect of mebendazole on the cysticerci of Taenia saginata in calves.Tropical Animal Health and Production, **9**., 24.

Gallie, J. G. and Sewell, H. M. M. (1978). The efficacy of praziquantel against the cysticerci of Taenia saginata in calves. Tropical Animal Health and Production, **10** 36-38.

Gallie, J.G. and Sewell, H.M.M. (1981). Inoculation of calves and adult cattle with oncospheres of Taenia saginata and their resistance to challenge infection Tropical Animal Health and Production, **13,** 147 - 154.

Gallie, J. G. and Sewell, H. M. M. (1983). Duration of immunity and absorption of cysticerci in calves after treatment of Taenia saginata cysticercosis with praziquantel. Research in Veterinary Science, **34**, 127-130.

Gathuma, M. J. (1977). Immunodiagnosis and seroepidemiology of Cysticercus bovis infection in cattle in Kenya. Ph.D. Thesis.The University of Nairobi, Kenya.

Geerts, S., Kumar, V. and Vercrusee, J. (1977). In vivo diagnosis of bovine cysticercosis. Veterinary Bulletin, **47(9),** 653 - 664.

Geerts, S., Kumar, V. and Aerts, N. (1979). Antigenic components of Taenia saginata and their relevance to the diagnosis of bovine cysticercosis by immunoelectrophoresis. Journal of Helminthology, **53,** 293 - 299.

Geerts, S. Kumar, V. Ceulemans, F and Mortelmans, J.(1981). Serodiagnosis of Taenia saginata cysticercosis in experimentally and naturally infected cattle by enzyme-linked immunosorbent assay. Research in Veterinary Science, **30,** 288-293.

Geerts,S.,Kumar,V.,Aerts, N.and Ceulemans, F.(1981). Comparative evaluation of immunoelectrophoresis, counterimmunoelectrophoresis, and enzyme-linked immunosorbent assay for the diagnosis of Taenia saginata cysticercosis. Veterinary Parasitology, **8,** 299-307.

Ginsberg, A., Cameron, J., Goddard, W. B., and Grieve, J.M. (1956). Bovine cysticercosis, with particular reference to East Africa. Bulletin of Epizootic Diseases in Africa, **4,** 27-39.

Ginsberg, A. (1958). Helminthic zoonoses in meat inspection. Bulletin of Epizootic Diseases in Africa, **6,** 141-149.

Graber, M.par. (1959). La Cysticercose bovine Son importance dans les zones saheliennes d'elevage de la Repulique du Tchad. Rev. Elev. Med. Vet. Pays. Trop. **12,** 121- 143.

Gracey, F.J. and Collins, S.D. (1992). Meat Hygiene. 5th edn. Bailliere Tindall, 24-28 Oval Road, London NW17DX PP. 413-420.

Grindle, R. J. (1978). Economic losses resulting from bovine cysticercosis with special reference to Botswana and Kenya.Tropical Animal Heath and Production, **10 ,** 127-140.

Harrison, L.J.S. and Sewell, M.M.H. (1981). Antibody levels in cattle naturally infected with Taenia saginata metacestodes in Britain. Research in Veterinary Science, **31,** 62-64.

Harrison, S.J.L. (1984). Surface and secreted antigens of Taenia saginata. Annals in Tropical Medical Parasitology, **78,** 235.

Harrison, S.J.L.,Gallie, G.J.,and Sewell, M. M. H. (1984). Absorption of cysticerci in cattle after treatment of Taenia saginata cysticercosis with praziquantel. Research in Veterinary Science, **37,** 378-380.

Harrison, S.J.L. and Parkhouse, E.M.R. (1985). Antigens of Taeniid Cestodes in protection, diagnosis and Escape. Current Tropical Microbiology and Immunology, **120,** 159-172.

Harrison, S.J.L. and Parkhouse, E.M.R. (1986). Passive protection against Taenia saginata infection in cattle by a mouse monoclonal antibody reactive with the surface of the invasive oncosphere. Parasite Immunology, **8,** 319-332.

Harrison, S.J.L. and Parkhouse, E.M.R. (1989). Taenia saginata and Taenia solium Reciprocal models. Acta Leidensia, 142 - 152.

Harrison, S.J.L., Joshua, P.W.G., Wright, H.S. and Parkhouse,E.M.R. (1989). Specific detection of circulating surface/secreted glycoproteins of viable cysticerci in Taenia saginata cysticercosis. Parasite Immunology, **11,** 351-370.

Hughes, G., Hoque, M. , Tewes, M. S., Wright, S. H. andHarrison,S. J. L. (1993). Seroepidemiological study of Taenia saginata cysticercosis in Swaziland. Research in veterinary Science, **51,**287-291.

Hughes, G.(1996). Diagnosis and treatment of Taenia saginata (Bovine) cysticercosis as a control method . First Drug Trial Report. NVRC. Muguga.

Johnson, S.K., Harrson, L.B.G., Lightowlers, W.M., O'Hoy,L.K., Cougle, G.W., Dempster, P.R., Lawrence,B.S., Vinton, G.W., Heath, D.D. and Rickard, D.M.(1989). Vaccination against ovine cysticercosis using a defined recombinantantigen. Nature., **338,** 585 - 587.

Joshua, P.W.G., Harrison, L.J.S. and Sewell, M.M.H., (1988). Excreted/secreted products of developing Taenia saginata metacestodes. Parasitology, **97,** 477-487.

Joshua, P.W.G., Harrison, L.J.S. and Sewell. H.M.M. (1989). Development changes in proteins and glycoproteins revealed by direct radio-iodination of viable Taenia saginata larvae. Parasitology, **99,**265 - 274.

Kagan, I.G., Norman, L. and Allain, D.S. (1960). Studies on echinococcosis. Serology of crude and fractionated antigens prepared from Echinococcus granulosus and Echinococcus multilocularis. American Journal of Tropical Medicine and Hygiene, **9,** 248 - 261.

Kagan, I.G., Sulzer, A.J. and Carver, K. (1965). An evaluation of the fluorescence antibody test for the diagnosis of schistosomiasis. American Journal of Epidemiology, **81,** 63 - 70.

Kagan, I.G. (1968). A review of serological tests for diagnosis of hydatid diseases. Bulletin of World Health Organization, **39,** 25 - 37.

Kagan, I.G. (1974). Advances in the immunodiagnosis of parasitic infections. Z. Parasitenk. **45,** 163 - 195.

Kamanga-Sollo, P.I.E. (1981). Partial characterization of the antigens of Cysticercus bovis. MSc. Thesis. TheUniversity of Nairobi, Kenya.

Kamanga-Sollo, P.I.E. (1984). Immunodiagnosis of Bovine cysticercosis using selected antigens. Ph.D. Thesis. The University of Nairobi, Kenya.

Kang'ethe, K. E.,(1995). The impact of meat inspection on the control of bovine cysticercosis in Kenya. Bulletin of Animal Health and Production in Africa, **43,** 261-267.

Kyvsgaard, C.N., Henriksen, A.S., and Nansen, P., (1990). Distribution of Taenia saginata cysts in carcasses of experimentally infected calves and its significance for routine

meat inspection. Research in Veterinary Science, **49**, 29-33.

Lloyd, S. and Soulsby, L.J.E. (1976). Passive transfer of immunity to neonatal calves against the metacestodes of Taenia saginata. Veterinary Parasitology, **2,** 355 - 362.

Maddison, S.E., Whittle, H. and ELsdon-Dew, R. (1961). The antigens of tapeworms. Preliminary Note. South African Journal Science, **57,** 273 - 277.

Mango, M.A. and Mango, A.K. (1972). Recovery rate and distribution of Cysticercus bovis in calves artificially infected with Taenia saginata eggs. Research in Veterinary Science, **13 (3),** 284-286.

Mann, l. and Mann, E. (1947). The distribution of measles (Cysticercus bovis) in Africa bovine carcasses. Veterinary Journal, **103,**239-251.

Mann, I. (1983). Environmental hygiene and sanitation based on the conept of primary health care as a tool for surveillance, prevention and control of taeniasis / cysticercosis. Current Publication in Tropical Health Research, **36,** 127 - 140.

Marshall, W.L., Rolfe, R. and Gauci, G. (1996). Taenia saginata:Vaccination against Cysticercosis in cattle with Recombinant Oncosphere Antigens. Experimental Parasitology, **84**. 330-338.

Martens, W.S., Meak, H.A. and Willeberg, P. (1987). Veterinary Epidemiology - PRINCIPLES AND METHODS. 2nd. ed. Press/Ames.pp. 59-76.

McIntosh, A. and Miller, D. (1960). Bovine Cysticercosis, with special reference to the early developmental stages of Taenia saginata. American Journal of Veterinary Research **21**, 169-177.

Mc Manus, D. (1960). Prenatal infections of calves with Cysticercus bovis Veterinary Record, **72 (41),** 847-848.

Mc Manus, D. (1963). Prenatal infection of calves with Cysticercus bovis. Veterinary Record, **75 (27),** 697.

Meat Control Act (1977). Kenya Government Printers.

Mechnicka-Roguska, B. and Swierz, C. (1970). Intradermal test with antigenic fractions in Taenia saginata infection. Acta Parasitology Pol., **17,** 293 - 299.

Mitchell, R. J. (1978). Bovine Cysticercosis. Veterinary Record, **102,** 469.

Morris, N. Proctor, E. M. and ELsdon-Dew, R. (1968). A physio - chemical approch to the serodiagnosis of cysticercosis. Journal of South African Veterinary Association, **39,** 41-43.

Muller, R. (1975). WORMS AND DISEASES. A Manual of Medical Helminthology. pp. 43 - 49. William Heinemann Medical Books Limited, London.

Nelson, S.G., Pester, N.R.F. and Rickman, R. (1965). The significance of wild animals in the transmission of cestodes of medical importance in Kenya. Transactions Royal

Tropical Medicine and Hygiene, **59,** 507 - 512.

Neva., A.F. and Brown, W.H. (1994). Basic Clinical PARASITOLOGY 6th edn. PP.181-200 Prentice - Hall International Inc.

Olds, G.E.(1938). Tabulated values for Rank Correlation. Annals of Mathematical Statistics. **Vol. IX.**

Onah, D.N. and Chiejina, S.N.(1986). Taenia saginata cysticercosis in slaughter cattle in Anambra State, Nigeria. International Zoonosis. **13(1),** 32 - 39.

Onyango - Abuje, J.A. (1984). The separation of Taenia saginata Oncosphere from Feacal Debris. Journal of Parasitology, **70 (1),** 146-148.

Onyango - Abuje, J.A.(1984). Antigenic components of Taenia saginata oncospheres and their use in immunodiagnosis. Ph.D. Thesis. The University of Nairobi.

Onyango - Abuje, J.A. Waiyaki, G.P and Gathuma, M.J. (1989). Specific and shared antigenic components of Taenia saginata oncospheres. Research in Veterinary Science, **47,** 236-240.

Onyango - Abuje, J.A., Hughes, G., Opicha, M., Nginyi, M.K., Rugutt, K.M., Wright, H.S. and Harrison, S.J.L.(1996). Diagnosis of Taenia saginata cysticercosis in Kenyan cattle by antibody and antigen ELISA. Veterinary Parasitology, **61,** 221-230.

Onyango - Abuje, J.A. Rugutt, M.K. Nginyi, J.M., Adala, P.L. Olum, J.A. and Odera,

G.O. (a). On-farm diagnosis and treatment of Taenia saginata (bovine) cysticercosis. East Africa Agriculture and Forestry Journal . In press.

Onyango - Abuje, J.A., Nginyi, J.M., Rugutt, M.K., Wright, S.H.,Adala, p. L., Hughes, G. and Harrison,L.J.S. (b). Seroepidemiological survey of Taenia saginata cysticercosis in Kenya. Veterinay Parasitology. In press.

Onyango-Abuje, J. A., Rugutt, K.M., Nginyi, M.J., Adala,P.L., Olum,A.J., Odera, O.G. and Omondi, G. (C). On farm diagnosis and treatment of Taenia saginata (bovine) cysticercosis. In press.

Oryan, A., Moghaddar, N. and Gaur, S.N. (1995). Taenia saginata cysticercosis in cattle with special reference to its prevalence, pathogenesis and economic implications in Fars Province of Iran. Veterinary Parasitology, **57(4),** 319 - 327.

PAHO/WHO. (1995). Informal Consultation on the Taeniosis/cystcercosis complex. August, 23 - 25, 1995, Brasilia, Brazil.

Parkhouse, E.M.R. and Harrison, S.J.L (1987). Cyst fluid and surface associated glycoprotein antigens of Taenia sp.metacestodes. Parasitology Immunology, **9,** 263-268.

Pawlowski, Z. and Schultz, M.G. (1972). Taeniasis and cysticercosis (Taenia saginata). Advances in Parasitology, **10,** 269 - 343.,

Pawlowski, Z., Kozakiewicz, B. and Wroblewski, H. (1976). Effect of albendazole on the

metacestodes of Taenia saginata in calves. Experimentia, 34 723.

Pawlowski, Z. Kozakeiwicz, B. and Wroblewski, H. (1978). The effect of mebendazole and praziquantel against Taenia saginata cysticercosis in cattle. Veterinary Science Communications, 2, 137 - 139.

Penfold, H.J., Penfold, H.B. and Phillips, M. (1936 a). A Survey of the Incidence of Taenia saginata infestation in the Population of the State of Victoria from January, 1934, to July, 1935. Medical Journal of Australia, **1 (9)** 283-285 (W.L.13034).

Penfold, H.J., Penfold, H.B.and Phillips, M. (1936 b). Acquired Active Immunity on the Ox to Cysticercus bovis. Medical Journal of Australia, **1(13),** 417-423 (W.L.13034).

Penfold, H.B.(1937). The life history of Cysticercus bovis in the tissues of the ox. Medical Journal of Australia, **1,** 579-583.

Penfold, W. J. and Penfold, H. B. (1937). Cysticercus bovis and its prevention. Journal of Helminthology, **15,** 37 - 40.

Peters, W. and Gilles, H.M. (1982). A colour Atlas of Tropical Medicine and Parasitology. Wolfe Medical Publications, 2nd. edn.

Pugh, K. E. and Chambers, P.G. (1989). Observations on Cysticercus bovis in slaughter cattle in the Matebeleland province of Zimbabwe. Veterinary Record, **125,** 480-484.

Rickard, M. D. and Adolph, A. J. (1976). Vaccination of calves against Taenia saginata infection using a parasite-free vaccine. Veterinary Parasitology, **1,** 389-392.

Roberts, T. (1985). Microbial pathogens in raw pork, chicken and beef: benefit estimates for control using irradiation. American Journal of Agricultuiral Economics, **67,** 965 - 975.

Rydzewski, A.K., Chischolm, E.S. and Kagan, I.G. (1975). Comparison of serologic tests for human cysticercosis by indirect haemagglutination, indirect immunofluorescent antibody and agar gel precipitation tests. Journal of Parasitology, **61(1),** 154 - 155.

Sadun, E.H., Anderson, R.I. and Williams, J.S. (1962). Fluorescent antibody test for the serological diagnosis of trichinosis. Experimental Parasitology, **12,** 423 - 433.

Sadun, E.H. (1963). Seminar on immunity to parasitic helminths. V11. Fluorescent antibody technique for helminth infections. Experimental Parasitology, **13,** 72 - 82.

Sewell, M.M.H. and Gallie, G.J. (1974). Immunological studies on experimental infections with the larval stage of Taenia saginata.In: "Parasitic zoonoses" (Ed. by E.J.L. Soulsby).Academic Press, New York, 1974. pp.187 - 193.

Schmidt, D.G. and Robets, S.L.(1985). FOUNDATIONS OF PARASITOLOGY. 3rd edn. P.P 336-408. Times Mirror/Mos by college Publishing.

Silverman, P.H. (1954a). Studies on the biology of some tapeworms of the genus Taenia. I.Factors affecting hatching and activation of taeniid ova, and some criteria of their viability. Annals of Tropical Medicine and Parasitology, **48,** 207-215.

Silverman, P.H. and Griffiths, R.B. (1955). A review of methods of sewage disposal in

Great Britain, with special reference to the epizootiology of Cysticercus bovis. Annals of Tropical Medicine and Parasitology, **49,** 436 - 450.

Silverman, P.H. (1956). The infectivity of the hexacanth embryo of Taenia pisiformis. Transactions of Royal Society of Tropical Medicine and Hygiene, **50,** 7 - 8.

Slonka, G.F., Moulthrop, J.I Dewhirst, L.W. Hotchkiss, P. M., Vallaza, B. and Schultz, M.G. (1975). An epizootic of bovine cysticercosis. Journal of American Veterinary Medical Association, **166,** 678 - 681.

Skolovskaya, O.M. (1969). Kvoproci ob immunodiagnostike aeniarnkhoza. Medskaya Parazit. **38(3),** 321 - 325.

Soulsby, L.J.E. (1963). Proceedings of the 17th. World Veterinary Congress, Hanover, pp. 761-767.

Soulsby, L.J.E.(ed.) (1972). Immunity to animal parasite. Academic Press: New York and London.

Soulsby, L.J.E. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. 7th edn, PP. 106-118. BAILLIERE TINDALL, a division of cassell Ltd.

Stevenson, P., Holmes, W.P. and Muturi, M.J. (1981). Effect of albendazole on Taenia saginata cysticerci in naturally infected cattle. Veterinary Record, **109,** 82.

Stevenson, P. (1983). Observation on the hatching and activation of fresh Taenia saginata

eggs. Annals of Tropical Medicine and Parasitology, **77(4)**, 399 - 404.

Steward, M. and Male, K.D. (1989). Immunological Techniques: Immunology. Ed.By, Roitt., M., Brostoff,J and Male, K.D.

Stoll, N.R. (1947). This wormy world. Journal of Parasitology, **33,** 1-18.

Szyfres, B. and Kagan, I.G. (1963). A modified slide latex screening test for hydatid disease. Journal of Parasitology, **49(1),** 69-72.

Teodor, W., Marijan, Z. and Nikola, D.,(1971). Taenia saginata and Taenia hydatigena: Lntramuscular Vaccination of Calves with Oncospheres. Experimental Parasitology **30**, 36-40.

Urquhart, G.M. (1958). The production of experimental cysticercosis in calves in Kenya. Bulletin of Epizootic Diseases of Africa, **6,** 381-388.

Urquhart, G.M. (1961). Epizootiological and experimental studies on bovine cysticercosis in East Africa. Journal of Parasitology, **47 (6),** 857-869.

Varela - Diaz, V.M., Coltorti, E.A.,Prezioso, V., Lopez - Lemes, M.H., Guisantes, J.A. and Yarzabal, L.A. (1975). Evaluation of three immundiagnostic tests for human hydatid disease. American Journal of Tropical Medicine and Hygiene, **24,** 312 - 319.

Viljoen, N.F. (1937). Cysticercosis in swines and bovines, with special reference to South African conditions. Onderstepoort. Journal of Veterinary Research, **9,** 337 - 370.

Voller, A., Bidwell, D.E., Huldt, G. and Engvall, E. (1974). A microplate method of enzyme - linked immunosorbent assay and its application of Malaria. Bulletin of World Health Organization, **51,** 209-211.

Voller, A., Bidwell, D.E. and Bartletta, A. (1976). Enzyme immunoassays in diagnostic medicine. Theory and Practice. Bulletin of World Health Organization, **53,** 55-65.

Voller, A. and De Savigny, D (1981). Diagnostic serology of Tropical Parasitic Diseases. Journal of Immumological Methods, **46,** 1 - 29.

Walther, M. and Sanitz, W.(1970). Serodiagnosis of bovine cysticercosis by enzyme - linked immunosorbent assay (ELISA). Berl. Munch. Tierarztl. Wschr. **92,** 131-135.

Walther, M. and Koske, J. K. (1979). The efficacy of praziquantel against Taenia saginata cysticercosis in naturally infected calves. Tropenmed. Parasitol. **30,** 401-403.

Walther, M. and Sanitz, W.(1979). Quoted by Parkhouse, E.M.R. and Harrison, S.J.L. (1987). Cyst fluid and surface associated glycoprotein antigens of Taenia spp. metacestodes. Parasite Immunology, 9, 263-268.

Walther, M. and Koske, J.K. (1980). Taenia saginata cysticercosis: A comparison of routine meat inspection and carcass dissection results in calves. Veterinary Record, **106,** 401 - 402.

Webbe, G. (1967): The Hatching and Activation of Taenii Ova in Relation to the Development of cysticercosis in man. JournalTropenmed. Parasit. **18 (3),** 354-369.

Weil, G.J.F. and Liftis, F. (1987). Identification and partial characterization of a parasite antigen in sera from humans infected with Wuchereria bancrofti. Journal of Immunology, **138,** 3035 - 3041.

Williams, F.J. and Sandeman, M.R.(1981). Antigens of Taeniid cestodes. Proceedings of an International Workshop on Cysticerocsis held in San Miguel de Allende, Guanajuato,Mexico on November, 16 - 18, 1981.

Yarzabal, L.A., Leiton, J. and Lopez - Lemes, M.H.(1974). The diagnosis of pulmonary human hydatidosis by immunoelectrophoresis test. American Journal of Tropical Medicine and Hygiene , **23,** 662 - 666.

APPENDIX 1. GLOSSARY OF HELMINTHOLOGICAL AND IMMUNOLOGICAL TERMS USED:

Accuracy (= Validity) : Is the proportion of all the tests, both negative and positive that are correct and it is used to express the overall performance of a diagnostic test.

Concordance : Is the proportion of all test results on which two or more different tests agree.

Diagnostic test : Is a test evaluating diseased individuals and is used to distinguish between animals that have the disease in question and those that have other diseases on the differential list.

Gold standard : Is a quality-control device that provides the basis for determining the value of diagnostic tests, treatment strategies and prognoses.

Immunogen : A foreign molecule that induces an adaptive immune response.

Immunogenicity : The ability of a foreign molecule to induce an adaptive immune response.

Incidence : The number of new cases of an infection reported in an area in a unit of time.

Infestation : Existence of parasitic organisms on the outside of the body of the host or in the superficial tissues.

Precision (= Reproducibility) : Is the ability of the test to give a consistent measure upon repeated testing of the sample.

Predictive value : Is the probability of the infection or disease in an animal.

Positive predictive value: Is the probability of disease in an animal with a positive (abnormal) test result.

Negative predictive value: Is the probability that the animal does not have the disease when test result is negative (normal).

N/B: Unless otherwise stated, the discussion in this text was restricted to the predictive value of a positive test result.

Prevalence : The number of cases of an infection present in an area (actually per unit of population) at a fixed point in time. Prevalence = incidence x average duration.

Screening test : Is a test used for the presumptive identification of unrecognized disease or defect in apparently healthy populations.

Sensitivity : The probability that the procedure will diagnose a real positive of infected or diseased individuals (i.e. number of real positives diagnosed divided by number of real positives + number of false negatives).

Specificity : The probability that a real negative host is recognized by the test to be negative (i.e. number of real negatives divided by number of real negatives + number of false positives).

Worm burden : The number of worms present in the host (this is often estimated from the egg count).

Zoonosis : Infection or disease naturally transmitted between man and other vertebrates

PPENDIX 2. REAGENTS USED FOR ELISA.

Borate buffered saline (BBS) pH 8.2

H_3B0_3	6.18 g	
$Na_2B_4O_7.10H_20$		9.54 g
NaCI	4.38 g	

Distilled H_20 to 1 litre, adjust with HCI if necessary.

Phosphate buffered saline (PBS) pH 7.3

NaCI	10.11 g	
KCI	0.362 g	
KH_2P04		0.362 g
Na_2HP0_4		1.449 g

Distilled H_20 to 1 litre.

Tris buffered saline (TBS) pH 7.5

Trizma base	2.42 g
NaCI	29.22 g

Distilled H_20 to 1 litre, adjust with HCI.

0.9% NaCI - Tween 20

NaCI	9.0 g	
Tween 20 (Sigma)		0.5 g

Distilled H_20 to 1 litre.

PBS/Tween/BSA

Bovine serum albumin (BSA)	10.0 g (Sigma A4503)
Tween 20	0.5 g

PBS to 1 litre. Store at 4°C to avoid contamination or a liquoted at - 20°C.

0.2M H_2S0_4: 2 ml concentrated H_2S0_4 (Aristar 11M) to 100 ml distilled H_20.

0.1M Sodium acetate/Citric buffer pH 6.0.

Consists of Stock 1, 0.6M sodium acetate 49.2 g made up to 1 litre with distilled water and Stock 2, 0.5M citric acid 52.5 g made up to 500 ml with distilled water. Stock x 5 is made up by adding 0.5M citric acid to 500ml of 0.6M sodium acetate until pH 6.0 adjust volume to 600 ml. For use dilute 1:5.

Peroxide substrate.

Dissolve 5mg of 3,3, 5, 5. Tetramethylbenzidine (TMB) in 0.5 dimethl sulfoxide (Sigma). Make up to 50 ml with 0.1M sodium acetate/citric acid buffer pH 6.0, add 7.5µl of H_20_2 30% w/v.

APPENDIX 3.

MEAT INSPECTION RESULTS OF NATURALLY INFECTED ANIMALS.

The table indicates the number of cysticerci found in each predilection site of the 25 carcasses inspected at slaughter according to the Kenya Meat Control Act - 1977.

Predilection Sites for Inspection according to the Kenya Meat Control Act - 1977	CODE NUMBERS OF CARCASSES: 715	705	708	988	713	956	969	958	724	716	721	718	723	720	976	714	966	972	717	701	971	707	989	965	970	Total no. of cysts
1. Masseter Muscles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0(0)
2. Tongue	0	0	0	2(2)	0	0	0	3(3)	0	0	0	7(7)	0	0	0	0	0	0	0	0	0	0	0	0	0	12(12)
3. Heart	0	1(1)	0	0	1(0)	0	1(1)	0	0	0	2(0)	1(0)	0	0	0	IO(0)	0	0	0	1(1)	1(0)	0	2(2)	0	0	20(5)
4. Shoulder muscles	0	0	0	0	0	0	1(0)	1(1)	0	0	0	0	0	0	0	0	0	0	0	2(1)	0	0	0	3(0)	0	7(2)
TOTAL	0	1(1)	0	2(2)	1(0)	0	2(1)	4 (4)	0	0	2(0)	8(7)	0	0	0	10(0)	0	0	0	3(2)	1 (0)	0	2(2)	3(0)	0	39(19)

Recommendations:

Carcasses with : - 0 cysts are passed on directly for human consumption.

1-5 cysts are retained, frozen at -10°C for at least 10 days and released "unconditionally"

6-20 cysts are similarly treated as above but released conditionally to schools/ institutions where proper cooking is expected to be done.

Over 20 cysts are totally condemned.

In places where electricity is unavailable, the carcasses are sliced and boiled for 2 hrs at 77°C under the supervision of the inspecting officer.

N/B. Figures in parentheses represent live cysticerci.

APPENDIX 4.

THE NUMBER AND DISTRIBUTION OF CYSTICERCI IN VARIOUS SITES OF 25 CARCASSES DURING TOTAL DISSECTION OF NATURALLY INFECTED 1-1½ YEARS OLD STEERS SELECTED FROM THE FIELD:

SITE:	CODE NUMBERS OF CARCASSES:																											
	715	705	708	988	713	956	969	958	724	716	721	718	723	720	976	714	966	972	717	701	971	707	989	965	970	Total	% of Total
Head	0	2(2)	1(0)	0	0	0	0	0	0	0	2(1)	0	0	0	0	0	0	0	0	2(1)	0	0	3(1)	0	0	10(5)	1.49(0.75)
Tongue	0	4(3)	1(1)	2(2)	0	0	0	3(3)	0	0	7(7)	0	0	0	0	1(1)	0	0	4(3)	0	0	0	0	0	0	22(20)	3.28(2.98)
Fore Leg	4(2)	2(0)	0	10(8)	12(12)	2(0)	4(0)	10(4)	4(2)	10(10)	10(6)	34(22)	0	0	0	2(0)	2(0)	0	0	30(22)	2(0)	2(0)	12(4)	14(12)	0	166(104)	24.74(15.50)
Neck /H	0	0	0	0	0	0	2(0)	0	2(1)	2(1)	0	0	0	0	0	2(0)	0	0	0	2(0)	6(0)	0	0	0	0	16(2)	2.38(0.30)
Pelvis	0	14(2)	0	2(0)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6(6)	2(2)	0	24(10)	3.58(1.49)
Hind leg	4(0)	18(18)	6(4)	22(16)	8(4)	0	6(4)	0	4(0)	16(12)	8(8)	16(16)	0	0	0	0	0	4(2)	0	12(6)	0	12(4)	22(12)	2(2)	0	160(108)	23.85(16.1)
Ribs	0	0	0	14(4)	0	0	0	0	8(4)	0	0	0	0	0	0	0	0	0	0	14(10)	0	0	18(6)	8(6)	0	62(30)	9.24(4.47)
Lumbar	0	8(8)	2(0)	0	4(0)	0	6(4)	0	0	0	6(6)	0	0	0	0	0	2(0)	0	0	6(0)	0	0	8(5)	2(2)	0	44(25)	6.56(3.73)
Rumen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0(0)	0.00(0.00)
Lungs	0	0	0	1(0)	4(3)	0	0	0	0	1(1)	0	1(1)	1(0)	1(0)	0	0	3(3)	0	1(1)	3(3)	0	1(1)	3(1)	0	0	20(14)	2.98(2.09)
Heart	0	2(2)	0	1(1)	7(6)	0	3(0)	1(0)	0	1(0)	2(1)	2(1)	0	1(0)	6(0)	10(0)	1(1)	0	0	16(10)	1(0)	0	5(2)	0	0	59(24)	8.79(3.58)
Liver	0	1(0)	2(0)	0	9(0)	5(1)	0	1(0)	1(0)	13(0)	3(0)	17(8)	0	0	2(0)	1(0)	4(0)	2(0)	2(0)	5(0)	1(0)	1(0)	11(5)	1(0)	0	82(14)	12.22(2.09)
Kidneys	0	0	0	0	0	1(1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1(1)	0.15(0.15)
Diap	0	3(2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2(2)	0	0	0	0	5(4)	0.75(0.60)
TOTAL	8(2)	54(37)	11(5)	53(31)	44(25)	8(2)	21(8)	5(7)	19(7)	43(24)	31(22)	77(55)	1(0)	2(0)	8(0)	15(0)	13(5)	6(2)	3(1)	94(55)	12(2)	16(5)	88(42)	29(24)	0	671(361)	100(53.80)

Figures in parentheses represent live cysticerci.

KEY: Diap - Diaphragm

Neck/H - Neck and Hump

APPENDIX 5.

POST-MORTEM FINDINGS IN NATURALLY INFECTED CATTLE TOGETHER WITH THEIR MEAN ANTIGEN-ELISA READINGS AT SELECTION AND SLAUGHTER.

Cattle No.	No. of Cysticerci Recovered			Meat Inspection	AG-ELISA READINGS (OD VALUES)	
	L	D	T	T D L	AT SELECTION	AT SLAUGHTER
715	2	6	8	0 0 0	(-ve)-0.11 (0.150)	0.060
705	37	17	54	1 0 1	(+ve)0.334H (0.064)	0.116
708	5	6	11	0 0 0	(+ve)0.390H (0.064)	0.283
988	31	22	53	2 0 2	(+ve)0.268M (0.118)	0.094
713	25	19	44	1 1 0	(+ve)0.353H (0.150)	0.172
956	2	6	8	0 0 0	(-ve) 0.061 (0.064)	0.013
969	8	13	21	2 1 1	(+ve) 0.391 H(0.064)	0.429
958	7	8	15	4 0 4	(+ve) 0.186 M (0.064)	0.108
724	7	12	19	0 0 0	(+ve) 0.244 H (0.064)	0.171
716	24	19	43	0 0 0	(+ve) 0.685 H (0.150)	0.565
721	22	9	31	2 2 0	(+ve) 0.299 H (0.150)	0.166
718	55	22	77	8 1 7	(+ve) 0.282 H (0.150)	0.285
723	0	1	1	0 0 0	(-ve) 0.046 (0.150)	0.014
720	0	2	2	0 0 0	(-ve) 0.096 (0.150)	0.005
976	0	8	8	0 0 0	(-ve)- 0.003 (0.064)	0.090
714	0	15	15	10 10 0	(-ve)-0.008 (0.150)	0.001
966	5	8	13	0 0 0	(+ve) 0.076 M(0.064)	0.145
972	2	4	6	0 0 0	(-ve) 0.037 (0.064)	0.018
717	1	2	3	0 0 0	(-ve) 0.020 (0.150)	0.010
701	55	39	94	3 1 2	(+ve) 0.266 H (0.118)	0.311
971	2	10	12	1 1 0	(-ve) 0.001 (0.064)	0.026
707	5	11	16	0 0 0	(+ve) 0.266 H (0.118)	0.663
989	42	46	88	2 0 2	(+ve) 0.105 M (0.064)	0.220
965	24	5	29	3 3 0	(+ve) 0.315 H (0.064)	0.484
970	0	0	0	0 0 0	(+ve) 0.098 M (0.064)	0.237 (0.043)

KEY:

OD - Optical density
T - Total
D - Dead
L - Live
+ ve - positive
- ve - negative
H - High
M - Moderate
(Figures) - represent negative cut - off points.

APPENDIX 6.

THE INFECTIVITYOF THE TAENIA SAGINATA EGGS IN INDIVIDUAL CALVES

CALF NUMBER	EGG DOSE	NUMBER OF CYSTICERCI RECOVERED			INFECTIVITY INDICES*
		LIVE	DEAD	TOTAL	
4158	0000	0	0	0	0.00
4157	0000	0	0	0	0.00
4151	0000	1	0	1	0.00
4170	0000	0	0	0	0.00
947	0000	0	0	0	0.00
944	0000	0	0	0	0.00
4153	2500	17	1	35	0.02
4160	2500	151	2	153	0.07
4154	2500	76	23	99	0.05
4152	2500	3	4	7	0.00
4173	2500	0	10	10	0.01
951	2500	14	31	45	0.02
945	2500	0	0	0	0.00
4172	2500	0	28	28	0.01
4162	5000	0	8	8	0.00
4161	5000	124	38	162	0.04
4166	5000	0	22	22	0.01
4155	5000	2	36	38	0.01
4171	5000	0	4	4	0.00
4167	5000	1	13	14	0.00
950	5000	193	2	195	0.05
948	5000	0	187	187	0.05
4164	10000	62	29	91	0.01
4165	10000	59	25	84	0.01
4163	10000	0	249	249	0.03
4159	10000	12	61	73	0.01
4175	10000	0	8	8	0.00
4169	10000	55	4	59	0.01
949	10000	1	5	6	0.00
946	10000	93	1	94	0.01

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

The formular was:-

$$\text{Infectivity index} = \frac{\text{Number of cysticerci recovered during post-mortem}}{\%\text{motility x number of eggs fed}}$$

APPENDIX 7(a).

MEAT INSPECTION RESULTS OF EXPERIMENTALLY INFECTED ANIMALS IN THE 1ST GROUP OF 15 CALVES.

Predilection sites for Inspection according to the Kenya Meat Control Act 1977	The number of cysticerci found in each predilection site of the 15 carcasses examined.														
	0 eggs			2,500 eggs					5,000 eggs				10,000 eggs		
	4158	4157	4151	4153	4160	4154	4152	4162	4161	4166	4155	4164	4165	4163	4159
Masseter muscles	0	0	0	0	0	0	0	0	0	0	0	0	2(2)	1(0)	1(1)
Tongue	0	0	0	0	0	0	0	0	0	0	0	0	1(1)	4(0)	0
Heart	0	0	0	0	0	3(3)	0	0	2(2)	0	0	0	0	5(0)	2(0)
Musculaus triceps brachii	0	0	0	0	0	1(1)	0	0	0	0	0	0	0	0	1(0)
TOTAL	**0**	**0**	**0**	**0**	**0**	**4(4)**	**0**	**0**	**2(2)**	**0**	**0**	**0**	**3(3)**	**10(0)**	**4(1)**

Recommendations:

Carcasses with:- 0 cysticerci - passed on directly for human consumption,
1-5 „ - are retained, frozen at -10°C for at least 10 days and released "unconditionally",
6-20 „ - are similarly treated as above but released conditionally to institutions where proper cooking is expected to be done, and
21 and more cysticerci - are totally condemned.

Where electricity is unavailabe, the carcasses are sliced and boiled for 2 hours at 77°C under the supervision of the Inspection officer.

N/B. Figures in parentheses represent living cysticerci.

Calves with code numbers:-

4158, 4157 and 4151, were given 0 eggs of T. saginata
4153, 4160, 4154 and 4152, „ „ 2500 „ „ „ „
4162, 4161, 4166 and 4155, „ „ 5000 „ „ „ „
4164, 4165, 4163 and 4159, „ „ 10000 „ „ „ „

APPENDIX 7(b).

MEAT INSPECTION RESULTS OF EXPERIMENTALLY INFECTED ANIMALS IN THE 2nd GROUP OF 15 CALVES.

Predilection sites for Inspection according to the Kenya Meat Control Act 1977

The number of cysticerci found in each predilection site of the 15 carcasses examined.

	0 eggs			2,500 eggs					5,000 eggs				10,000 eggs		
	4170	947	944	4173	951	945	4172	4171	4167	950	948	4175	4169	949	946
Masseter muscles	0	0	0	0	0	0	0	0	2(0)	3(3)	0	0	2(2)	0	0
Tongue	0	0	0	0	0	0	0	0	2(0)	2(2)	0	0	2(2)	0	0
Heart	0	0	0	2(0)	4(4)	0	0	0	0	5(5)	47(0)	0	7(7)	0	9(9)
Musculaus triceps brachii	0	0	0	0	0	0	0	0	0	0(0)	2(0)	0	6(6)	0	0
TOTAL	**0**	**0**	**0**	**2(0)**	**4(4)**	**0**	**0**	**0**	**4(0)**	**10(10)**	**49(0)**	**0**	**17(17)**	**0**	**9(9)**

Recommendations:

Carcasses with:- 0 cysticerci - passed on directly for human consumption

1-5 „ - are retained, frozen at -10°C for at least 10 days and released "unconditionally"

6-20 „ - are similarly treated as above but released conditionally to institutions where proper cooking is expected to be done.

21 and over - are totally condemned.

Where electricity is unavailable, the carcasses are sliced and boiled for 2 hours at 77°C under supervision of the Inspection Officer.

N/B. Figures in parentheses represent live cysts.

Calves with the code numbers:-

4170, 947 and 944 were given 0 eggs of T. saginata

4173, 951, 945 and 4172 „ „ 2500 „ „ „ „

4171, 4167, 950 and 948 „ „ 5000 „ „ „ „

4175, 4169, 949 and 946 „ „ 10000 „ „ „ „

APPENDIX 8(a).

SUMMARY OF RESULTS OF THE 1ST GROUP OF 15 CALVES ON THE DISTRIBUTION OF CYSTICERCI IN BOVINE CARCASSES DURING DISSECTION/SLICING

	GROUP 1: 0 EGGS Number of cysticerci found in carcass code No.			GROUP 2:2500 EGGS Number of cysticerci found in carcass code No.				GROUP 3: 5,000 EGGS Number of cysticerci found in carcass code No.				GROUP 4: 10,000 EGGS Number of cysticerci found in carcass code No.			
SITE	**4157**	**4158**	**4151**	**4160**	**4153**	**4154**	**4152**	**4162**	**4161**	**4166**	**4155**	**4164**	**4165**	**4163**	**4159**
Head	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)	0(0)	0(0)	4(4)	0(0)	0(0)	5(4)	3(1)	20(0)	1(0)
Tongue	0(0)	0(0)	0(0)	0(0)	10(10)	5(5)	0(0)	0(0)	1(1)	0(0)	0(0)	0(0)	2(2)	10(0)	1(0)
Right front leg	0(0)	0(0)	1(1)	0(0)	16(16)	1(01)	0(0)	0(0)	01(1)	0(0)	0(0)	7(4)	0(0)	18(0)	3(3)
Left front leg	0(0)	0(0)	0(0)	0(0)	0(0)	11(11)	0(0)	0(0)	18(18)	0(0)	1(0)	8(8)	11(11)	7(0)	2(2)
Neck & hump	0(0)	0(0)	0(0)	0(0)	8(8)	3(3)	0(0)	0(0)	1(1)	0(0)	0(0)	9(8)	7(7)	30(0)	6(3)
Pelvis	0(0)	0(0)	0(0)	0(0)	10(10)	2(1)	2(2)	0(0)	4(4)	0(0)	0(0)	4(4)	10(10)	10(0)	4(0)
Right hind leg	0(0)	0(0)	0(0)	0(0)	9(9)	9(9)	0(0)	0(0)	1(1)	0(0)	0(0)	5(5)	9(9)	23(0)	3(1)
Left hind leg	0(0)	0(0)	0(0)	0(0)	17(17)	7(6)	0(0)	0(0)	17(17)	0(0)	0(0)	5(4)	8(6)	18(0)	3(2)
Right ribs	0(0)	0(0)	0(0)	0(0)	10(10)	7(7)	0(0)	0(0)	4(4)	0(0)	0(0)	7(5)	3(3)	11(0)	5(1)
Left ribs	0(0)	0(0)	0(0)	0(0)	3(3)	3(3)	0(0)	0(0)	0(0)	0(0)	0(0)	5(3)	7(5)	7(0)	0(0)
Lumbar	0(0)	0(0)	0(0)	0(0)	10(10)	0(0)	0(0)	0(0)	1(1)	0(0)	0(0)	6(5)	1(1)	9(0)	0(0)
Rumen	0(0)	0(0)	0(0)	0(0)	2(2)	0(0)	0(0)	0(0)	2(1)	0(0)	1(0)	0(0)	0(0)	3(0)	0(0)
Lungs	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)	0(0)	0(0)	20(19)	0(0)	3(0)	13(3)	3(1)	3(0)	2(0)
Heart	0(0)	0(0)	0(0)	0(0)	35(34)	30(21)	0(0)	0(0)	17(17)	0(0)	0(0)	13(5)	0(0)	39(0)	8(0)
Liver	0(0)	0(0)	0(0)	35(17)	15(15)	4(4)	5(1)	8(0)	71(35)	22(0)	34(2)	0(0)	19(2)	33(0)	35(0)
Kidneys	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Diaphragm	0(0)	0(0)	0(0)	0(0)	8(8)	5(3)	0(0)	0(0)	0(0)	0(0)	0(0)	4(4)	2(1)	8(0)	0(0)
TOTAL	**0(0)**	**0(0)**	**1(1)**	**35(17)**	**153(152)**	**89(76)**	**7(3)**	**8(0)**	**162(124)**	**22(0)**	**38(2)**	**91(62)**	**85(59)**	**249(0)**	**73(12)**

Note: Figures in parentheses represent live cysticerci.

APPENDIX 8(b).

SUMMARY OF RESULTS OF THE 2ND GROUP OF 15 CALVES ON THE DISTRIBUTION OF CYSTICERCI IN BOVINE CARCASSES DURING DISSECTION/SLICING

	GROUP 1: 0 EGGS Number of cysticerci found in carcass code No.			GROUP 2:2500 EGGS Number of cysticerci found in carcass code No.				GROUP 3: 5,000 EGGS Number of cysticerci found in carcass code No.				GROUP 4: 10,000 EGGS Number of cysticerci found in carcass code No.			
SITE	**4170**	**947**	**944**	**4172**	**4173**	**951**	**945**	**4171**	**4167**	**950**	**948**	**4175**	**4169**	**949**	**946**
Head	0(0)	0(0)	0(0)	3(0)	0(0)	0(0)	0(0)	0(0)	2(0)	0(0)	0(0)	0(0)	1(1)	0(0)	0(0)
Tongue	0(0)	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)	4(1)	6(6)	0(0)	0(0)	2(2)	0(0)	0(0)
Right front leg	0(0)	0(0)	0(0)	0(0)	0(0)	2(1)	0(0)	0(0)	0(0)	19(19)	18(0)	0(0)	6(6)	0(0)	13(13)
Left front leg	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)	1(0)	42(42)	9(0)	0(0)	3(3)	0(0)	4(4)
Neck & hump	0(0)	0(0)	0(0)	0(0)	1(0)	4(3)	0(0)	0(0)	1(0)	9(9)	4(0)	0(0)	5(5)	0(0)	12(12)
Pelvis	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)	13(13)	2(0)	0(0)	2(2)	0(0)	9(9)
Right hind leg	0(0)	0(0)	0(0)	1(0)	1(0)	1(1)	0(0)	0(0)	0(0)	23(23)	13(0)	0(0)	0(0)	0(0)	10(10)
Left hind leg	0(0)	0(0)	0(0)	2(0)	0(0)	2(0)	0(0)	0(0)	0(0)	43(42)	14(0)	0(0)	4(4)	0(0)	2(2)
Right ribs	0(0)	0(0)	0(0)	1(0)	0(0)	2(2)	0(0)	0(0)	0(0)	14(14)	2(0)	0(0)	2(2)	0(0)	4(4)
Left ribs	0(0)	0(0)	0(0)	2(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)	0(0)	8(8)
Lumbar	0(0)	0(0)	0(0)	3(0)	0(0)	1(1)	0(0)	0(0)	0(0)	17(17)	18(0)	0(0)	1(1)	0(0)	1(1)
Rumen	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)	0(0)	0(0)	0(0)	1(0)
Lungs	0(0)	0(0)	0(0)	7(0)	0(0)	2(2)	0(0)	0(0)	0(0)	2(2)	0(0)	0(0)	1(1)	0(0)	8(8)
Heart	0(0)	0(0)	0(0)	3(0)	0(0)	10(4)	0(0)	0(0)	0(0)	6(6)	95(0)	0(0)	11(11)	0(0)	18(18)
Liver	0(0)	0(0)	0(0)	5(0)	7(0)	21(0)	0(0)	3(0)	7(0)	0(0)	8(0)	8(0)	16(12)	6(0)	4(4)
Kidneys	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Diaphragm	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0)	3(0)	0(0)	4(4)	0(0)	0(0)
TOTAL	**0(0)**	**0(0)**	**0(0)**	**28(0)**	**10(0)**	**45(14)**	**0(0)**	**4(0)**	**15(1)**	**195(193)**	**187(0)**	**8(0)**	**59(55)**	**6(1)**	**94(93)**

Note: Figures in parentheses represent the number of live cysticerci.

APPENDIX 9(a).

POST-MORTEM FINDINGS OF CALVES GIVEN VARIOUS DOSES OF *TAENIA SAGINATA* EGGS TOGETHER WITH THEIR ANITIGEN-ELISA READINGS (i.e. OPTICAL DENSITIES (OD) AT 450 nm WAVE LENGTH): 1ST GROUP OF 15 CALVES)

Calf No.	Egg dose	No. of cysticerci Recovered during total dissection			Meat Inspection Findings at slaughter			Antigen-ELISA Readings		
		Live(L)	Dead(D)	Total(T)	Live(L)	Dead(D)	Total(T)	At Arrival	Just before Infection	At slaughter*
4158	0000	0	0	0	0	0	0	0.009	-0.002	-0.003
4157	0000	0	0	0	0	0	0	-0.004	0.001	0.001
4151	0000	1	0	1	0	0	0	-0.006	-0.004	0.006
4153	2500	17	18	35	0	0	0	-0.009	0.007	0.003
4160	2500	152	1	153	0	0	0	-0.004	-0.004	0.783
4154	2500	76	12	89	4	0	4	-0.009	0.005	0.387
4152	2500	3	4	7	0	0	0	0.000	0.003	0.009
4162	5000	0	8	8	0	0	0	-0.004	0.006	0.000
4161	5000	124	38	162	2	0	2	-0.004	0.000	0.317
4166	5000	0	22	22	0	0	0	-0.004	0.000	-0.006
4155	5000	2	36	38	0	0	0	0.008	0.003	-0.002
4164	10000	62	29	91	0	0	0	0.003	-0.003	0.060
4165	10000	59	26	85	3	0	3	-0.006	0.000	0.697
4163	10000	0	249	249	0	10	10	0.027	-0.002	-0.006
4159	10000	12	61	73	1	3	4	-0.011	0.005	0.002
								(0.053)	**(0.017)**	**(0.088)**

***Calves slaughtered 15 weeks post-infection. Figures in parentheses represent negative cut-off points.**

APPENDIX 9(b).

NUMBERS OF CYSTICERCI RECOVERED FROM CALVES GIVEN VARIOUS DOSES OF *TAENIA SAGINATA* EGGS TOGETHER WITH THEIR MEAT INSPECTION FINDINGS AND INITIAL; JUST BEFORE INFECTION AND FINAL ANTIGEN-ELISA READINGS AT SLAUGHTER
(2ND GROUP OF 15 CALVES)

Calf No.	Egg dose	No. of cysticerci Recovered during Total dissection			Meat Inspection Findings at slaughter			Antigen-ELISA Readings		
		Live(L)	Dead(D)	Total(T)	Live(L)	Dead(D)	Total(T)	At Arrival	Just before Infection	At Slaughter*
4170	0000	0	0	0	0	0	0	0.001	-0.024	-0.004
947	0000	0	0	0	0	0	0	0.001	-0.011	-0.010
944	0000	0	0	0	0	0	0	0.009	-0.030	0.003
4173	2500	0	10	10	0	2	2	-0.001	-0.027	0.050
951	2500	14	31	45	4	0	4	0.003	-0.022	0.125
945	2500	0	0	0	0	0	0	0.006	-0.023	-0.012
4172	2500	0	28	28	0	0	0	0.005	-0.002	-0.004
4171	5000	0	4	4	0	0	0	0.001	-0.018	-0.004
4167	5000	1	13	14	0	4	4	0.000	-0.043	0.024
950	5000	193	12	195	30	0	10	0.006	-0.028	0.872
948	5000	0	187	187	0	49	49	0.014	-0.018	0.002
4175	10000	0	8	8	0	0	0	0.039	-0.039	-0.007
4169	10000	55	4	59	19	0	17	0.000	-0.045	0.879
949	10000	1	5	6	0	0	0	0.008	-0.015	-0.010
946	10000	93	1	94	9	0	9	0.012	-0.012	0.486
								(0.017)	**(0.046)**	**(0.030)**

F'gures in parentheses represent negative cut-off points. *Calves slaughtered 15 weeks post-infection.

Appendix 10 (a).

The Average Antigen- ELISA Optical Density (O.D) Vaues of First 15 experimentally infected calves given various doses of T. saginata eggs , Monitored for 14 weeks and slaughtered in the 15th. Week.

		The number of weeks							
Calf code No	Egg dose	1	3	5	7	9	11	13	15
4157	0	-0.017	-0.009	-0.005	0.008	0.019	0.004	-0.012	-0.003
4158	0	-0.023	-0.017	-0.017	0.016	0.002	0.005	0.042	0.001
4151	0	-0.023	-0.01	-0.005	0.003	0.006	-0.003	0.005	0.006
Average	0	-0.021	-0.012	-0.009	0.009	0.009	0.002	0.012	0
4160	2500	-0.016	-0.008	-0.005	0.007	-0.011	-0.005	-0.053	0.003
4153	2500	-0.023	-0.006	-0.041	0.165	0.228	0.299	0.241	0.783
4154	2500	-0.016	-0.023	-0.017	0.144	0.131	0.174	0.533	0.387
4152	2500	-0.018	-0.016	-0.006	0.011	0.004	0.005	0.059	0.009
Average	2500	-0.018	-0.013	-0.012	0.082	0.088	0.118	0.195	0.296
4162	5000	-0.02	-0.017	0	0.006	-0.006	-0.003	-0.007	0
4161	5000	-0.023	-0.014	0	0.081	0.218	0.241	0.445	0.317
4166	5000	-0.023	-0.017	-0.009	0.006	-0.001	-0.025	-0.052	0.006
4155	5000	-0.008	-0.017	-0.005	0.009	0.001	-0.003	-0.012	0.002
Average	5000	-0.019	-0.0167	-0.004	0.026	0.053	0.053	0.094	0.077
4164	10,000	-0.023	-0.004	0.017	0.095	0.019	0.083	0.077	0.06
4165	10,000	-0.011	-0.01	0.128	0.192	0.179	0.352	0.655	0.697
4163	10,000	-0.023	-0.006	-0.003	0.11	0.022	-0.009	0.085	0.006
4159	10,000	-0.016	-0.023	-0.017	0.037	0.15	0.224	0.187	0.002
Average	10,000	-0.018	-0.011	0.031	0.109	0.093	0.163	0.252	0.188
Negative Cut-off points of O.Ds.		0.072	0.068	0.064	0.011	0.014	0.045	0.075	0.088

APPENDIX 10(b).

The average Antigen - ELISA results of the Second group of 15 calves monitored for 14 weeks of post infection with various doses of T. saginata eggs until slaughtered in the 15th. Week

Calf Code No..	Egg Dose	The number of weeks								
		1	3	5	7	9	11	13	14	15
944	0	0.013	0.008	-0.005	-0.006	0.002	-0.008	-0.002	-0.004	0.003
947	0	0.005	-0.004	0.035	-0.011	-0.023	-0.005	-0.015	-0.007	-0.1
4170	0	0.019	-0.005	-0.008	-0.013	-0.016	0.005	-0.003	-0.002	-0.004
Average	0	0.012	0	0.007	-0.01	-0.002	-0.003	-0.007	-0.004	-0.004
4172	2500	0.025	0.014	-0.004	-0.011	-0.018	-0.009	-0.003	-0.007	-0.004
		0.022	-0.004	0.008	0.058	0.032	0.037	0.044	0.055	0.05
4173	2500	-0.002	0.065	0.078	0.082	0.085	0.107	0.047	0.121	0.125
951	2500	0.017	0.003	0.034	-0.019	-0.005	-0.003	-0.005	-0.001	-0.012
945	2500	0.016	0.026	0.029	0.028	0.024	0.033	0.021	0.042	0.04
Average	2500	-0.004	0.066	0.047	-0.007	-0.014	0.007	-0.004	-0.004	-0.004
4171	5000	0.004	-0.007	0.002	0.035	0.021	0.018	0.022	0.084	0.024
4167	5000	0.047	0.01	0.005	0.156	0.184	0.24	0.263	0.271	0.872
950	5000	0.017	0.009	0.016	-0.003	-0.01	-0.009	-0.002	0	0.002
948	5000	0.016	0.02	0.018	0.045	0.045	0.064	0.07	0.088	0.224
Average	5000	0.002	-0.007	0.027	-0.008	-0.02	-0.013	-0.0.5	-0.007	-0.007
4175	10000	0.026	0.009	0.017	0.158	0.262	0.264	0.262	0.265	0.879
4169	10000	-0.003	-0.006	-0.006	-0.009	-0.002	-0.006	-0.012	-0.003	-0.01
949	10000	0.026	0.041	0.039	0.172	0.198	0.254	0.26	0.22	0.486
946	10000	0.003	0.009	0.019	0.078	0.11	0.125	0.124	0.119	0.337
Average	10000	-0.028	0.013	0.009	0.019	0.078	0.110	0.125	0.124	0.337
Negative cut-off points of O.Ds.		0.054	0.054	0.047	0.039	0.039	0.035	0.03	0.03	0.03

APPENDIX 11. 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.

Group 1	Cattle No.	O.D at selection	O.D at arrival	May O.D	June O.D	July O.D	O.D at slaughter
Cattle sected as positive	716	0.685	0.208	0.492	0.544	0.332	0.565
	969	0.391	0.21	0.254	0.254	283	0.429
	708	0.39	0.108	0.284	0.268	0.237	0.283
	713	0.353	0.191	0.145	0.15	0.166	0.172
	705	0.334	0.261	0.187	0.145	0.132	0.116
	965	0.315	0.198	0.378	0.297	0.275	0.484
	721	0.299	0.108	0.184	0.142	0.116	0.166
	718	0.282	0.087	0.275	0.325	0.26	0.285
	988	0.268	0.098	0.208	0.182	0.156	0.094
	707	0.266	0.108	0.173	0.021	0.326	0.663
	701	0.266	0.093	0.025	0.145	0.227	0.311
	724	-0.244	0.098	0.187	0.087	0.161	0.171
	958	0.186	0.097	0.15	0.114	0.173	0.108
	989	0.105	0.076	0.228	0.014	0.233	0.22
	970	0.098	0.105	0.262	0.122	0.253	0.237
	966	0.076	0.036	0.085	0.128	0.172	0.145
Average O.D		0.286	0.13	0.22	0.184	0.219	0.278
Group 2 cattle Selected as Negative	720	0.096	0.001	0.03	0.027	0.095	0.005
	956	0.061	0.006	0.012	0.009	0.006	0.013
	723	0.046	0.031	0.024	0.282	0.109	0.014
	972	0.037	0.022	0.005	0.01	0.011	0.018
	717	0.02	0.001	0.01	0	0.097	0.01
	971	0.001	-0.005	0.01	0.006	0.01	0.026
	976	-0.003	0.003	0.038	0.11	0.067	0.09
	714	-0.008	-0.011	0.015	0.006	0.012	0.001
	715	-0.011	0.009	0.06	0.09	0.064	0.06
Average O.D		0.027	0.009	0.023	0.049	0.052	0.026

The overall mean negative cut-off point of the above Antigen-ELISA Optical Density (O.D.) values is = 0.045.

APPENDIX 12.

Correlation analysis between the number of living cysticerci recovered during total dissection and the antigen- ELISA reading optical densities (O.D) values at slaughter of naturally infected cattle (Steers).

Cattle No.	No. of Live Cysticerci recovered at slaughter (X)	Antigen-ELISA readings at Slaughter at 450nm wavelength(Y)	X- rank	Y -rank	d (y-x) ranks	d^2
715	2	0.06	16.5	18	1.5	2.25
705	37	0.116	4	14	10	100
708	5	0.283	13.5	7	-6.5	42.25
988	31	0.094	5	16	11	121
713	25	0.172	6	10	4	16
956	2	0.013	16.5	22	5.5	30.25
969	8	0.429	10	4	-6	36
958	7	0.108	11.5	15	3.5	12.25
724	7	0.171	11.5	11	-0.5	0.25
716	24	0.565	7	2	-5	25
721	22	0.166	8.5	12	3.5	12.25
718	57	0.285	1	6	5	25
723	0	0.014	22.5	21	-1.5	2.25
720	0	0.005	22.5	24	1.5	2.25
976	0	0.09	22.5	17	-5.5	30.25
714	0	0.001	22.5	25	2.5	6.25
968	1	0.145	19	13	-6	36
972	2	0.018	16.5	20	3.5	12.25
717	0	0.01	22.5	23	0.5	0.25
701	55	0.311	2	5	3	9
971	2	0.026	16.5	19	2.5	6.25
707	5	0.663	13.5	1	-12.5	156.25
989	42	0.22	3	9	6	36
965	22	0.484	8.5	3	-5.5	30.25
970	0	0.237	22.5	8	-14.5	210.25
						$\sum d^2 = 960$

The negative cut-off point for the above O.D. values is = 0.043

Spearman rank-order correlation coefficient (r)

$r = 1 - 6(\sum d^2)/n(n^2 - 1)$

$r = 1 - 6x960/25(625-1=624)$

$r = 1-5760/15600$

$r = 1-0.369230769$

$r = 0.63076923$

Tabulated value at significance level of $\propto = 0.05$ is = 0.329 (Olds, 1938).

Where n = 25, the number of pairs of values.

Hypothesis: H_o = x and y values are independent of each other.

H_1 = x and y values are not independent of each other.

Therefore, H_o : is rejected and H_I : accepted , thereby concluding that there is a statistically significant correlation

The interpretation of these values of r:-

The values range from -1 to +1.

+1----→ corresponds to a perfect positive linear association between the two variable.

-1----→ corresponds to a perfect negative linear association between the two variables.

0----→ no linear relationship between the two variables.

near ±1----→ a strong linear association between two variables.

near 0----→ little or no linear association between two variables.

APPENDIX 13.

Correlation analysis between the number of living cysticerci recovered during total dissection and the antigen- ELISA reading optical densities (O.D.) values at slaughter of experimentally infected calves.

Cattle No.	No. of Live Cysticerci recovered at slaughter (X)	Antigen-ELISA readings at slaughter at 450nm wavelength (Y)	X- rank	Y -rank	d(y-x)ranks	d^2
4158	0	-0.003	23.5	21	-2.5	6.25
4157	0	0.001	23.5	18	-5.5	30.25
4151	1	0.006	15	13	-2	4
4170	0	-0.004	23.5	23	-0.5	0.25
947	0	-0.01	23.5	28.5	5	25
944	0	0.003	23.5	14.5	-9	81
4153	17	0.003	9	14.5	5.5	30.25
4160	151	0.783	2	2	0	0
4154	76	0.387	5	5	0	0
4152	3	0.009	12	12	0	0
4173	0	0.05	23.5	10	-13.5	182.25
951	14	0.125	10	7	-3	9
945	0	-0.12	23.5	30	6.5	42.25
4172	0	-0.004	23.5	23	-0.5	0.25
4162	0	0	23.5	19	-4.5	20.25
4161	124	0.317	3	6	3	9
4166	0	-0.006	23.5	25.5	2	4
4155	2	-0.002	13	20	7	49
4171	0	-0.004	23.5	23	-0.5	0.25
4167	1	0.024	15	11	-4	16
950	221	0.087	1	8	7	49
948	0	0.002	23.5	16.5	-7	49
4164	62	0.06	7	9	2	4
4165	59	0.697	6	3	-3	9
4163	0	-0.006	23.5	25.5	2	4
4159	12	0.002	11	16.5	5.5	30.25
4175	0	-0.007	23.5	27	3.5	12.25
4169	55	0.879	8	1	-7	49
949	1	-0.01	15	28.5	13.5	182.25
946	93	0.486	4	4	0	0
						$\sum = 910$

$r = 1 - 6(\sum d^2) / n(n^2 - 1)$

$r = 1 - 6(910) / 30(900 - 1)$

$r = 1 - 5460 / 26970$

$r = 1 - 0.202447163$

$r = 0.797552836$

Tabulated value at significance level of $\alpha = 0.05$ is = 0.306, when n = 30, the number of pairs of values.

Hypothesis H_0 : x and y values are independent of each other.

H_1 : x and y values are not independent of each other.

Therefore, H_0 : is rejected and H_1 : accepted, thereby concluding that there is a statistically significant correlation