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

BY

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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
lth and production of livestock, worldwide. The adult worm causes taeniasis in humans
le the larvae causes cysticercosis in cattle. The economic losses accruing from these
ctions are substantial.

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

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

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

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

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

The level of agreement between the two methods was, on average, lower in urally infected animals than in experimental calves.This was because in natural ctions, there were more light infections than in experimental infections and these

ld not be detected by meat inspection method but could be detected by either Ag-
SA or total dissection.

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

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION:

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

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

This parasite is cosmopolitan in distribution, particularly in beef-eating countries. It ndemic in several developing countries, especially those of Asia, Africa and Latin America re the level of poverty is too high and that of hygiene too low to prevent transmission cycle he parasite from defintive host to intermediate host and vice versa. Many authors have orted varying prevalence rates between and within continents and countries. For instance,in 7 Stoll estimated that 39 million people had taeniasis and in 1965 Froyd estimated the re at 40 million people. These figures drastically rose to 61 million persons almost two ades later (Peters and Gilles, 1982). In 1972, Pawlowski and Schultz, estimated a ticercosis prevalence rate of 10% in many parts of the world including Kenya, Uganda, eria, South Africa, and Chad. In 1975, Muller recorded a human prevalence rate ranging

0% in Africa (Kenya) to 0.03% in Europe (Poznan) for taeniasis and 2% for bovine rcosis. Cheruiyot,(1981),while working in Kenya, recorded a cysticercosis prevalence ging from 0.74% in the Coast Province to 18% in Kisii District, Nyanza Province. In nd 1991, the bovine cysticercosis prevalence rates were 8.8% and 1.1%, respectively t provinces of Kenya, (Kang'ethe,1995).

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

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

Infection in man is usually acquired by ingestion of raw or poorly cooked bee contains viable cysticerci. The cysticerci evaginate in the human small intestine (u jejunum mainly), attaches to the mucosa by its scolex, and grows to become an adult pa in about 3 months, occupying and following the small intestine loops for much of its l (Neva and Brown, 1994). The parasite may survive for as long as 20 years (Botero, 198 upto 25 years (Neva and Brown, 1994) in man. After 3 months of post-ingestion of cysti the mature worm begins shedding gravid or ripe proglottids (segments with only u containing eggs) which may crawl independently out of the anus or may be passed out feacal bolus (Neva and Brown. 1994). Each gravid proglottid is known to contain 200,000 eggs (Anon., 1997). Almost immediately after the patient passes these progl they expel a milky fluid full of eggs from their anterior border by muscle contraction parasite,thus contaminating the environment with the eggs. In varying environments, eggs have been known to remain viable as follows:- in liquid manure for 71 days, in unt sewage for 16 days, on grass for 159 days or 180 days (Chandler and Read, 1961; Schmi Roberts, 1985) and in river water for 33 days (Soulsby, 1982). The deposited eggs m disseminated in the environment by wind, flies, earthworms, birds, rains and even by b that roll away the faeces (Chandler and Read, 1961; Silverman and Griffiths, 1955). Th also may be disseminated by man when untreated sewage is used as fertilizer in cattle g fields.

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

d workers feeding neonatal calves with contaminated hands, present major routes of gestion by cattle (Chandler and Read, 1961; Dewhirst et al., 1967; Slonka et al., 1975; lt and Roberts, 1985; Neva and Brown, 1994). The contamination of food for example, d water given to neonatal calves also increase chances of cattle being infected (Slonka 975). In Kenya, calves have been reported to be infected in the first week of their life h contaminated food and hands of the workers (Urquhart, 1958 and 1961; Troyd, 1964 oyd and Round, 1960). Ginsberg (1958) and Mc Manus (1960 and 1963), reported that tal infections can also occur although not common. It is possible that such prenatal ions are confused with early neonatal infections (Soulby, 1982).

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

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

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

and Collins, 1992). Using six experimentally confined Thomson's gazelles fed with ggs of T. saginata, Fay, (1972) failed to produce an infection in any of them. The role 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 fact that the parasite, (both at larval and adult stages) present health hazard to man. the adult tapeworm does not cause serious diseases in man, its presence may result in ic pain, vague abdominal discomfort, nervousness, vertigo, nausea, vomiting, ea, either increased or loss of appetite, intestinal obstruction, mucosal lesions, icitis, pancreatic necrosis, moderate eosinophilia and increased levels of serum IgE, ving a debilitating effect on man (Botero, 1989; Neva and Brown 1994). The resulting es due to medical treatment are therefore great. Economic losses to agricultural and livestock industries are incurred through:- (1) condemnation of heavily infected es (2) downgrading of lightly infected carcasses (3) losses due to costs by refrigeration sts of boiling (5) costs of storage after refrigeration and boiling and (6) costs of ort of carcasses for example, to places where carcasses are processed. In Kenya, ana and Great Britain, annual losses have been estimated at £1.0 million, £0.5 million le, 1978) and 1.2 million (Gracey and Collins, 1992), respectively. In the African ent, Mann (1983), reported an annual loss of US$ 1.8 billion where an overall infection as found to be 7%. In South America, where an overall infection rate was estimated at bovine together with porcine cysticercosis caused an annual loss of US$ 428 million 1997). More important also is the loss of potential export markets of beef from endemic

f the World (Harrison et al., 1989).

he control of this parasite is therefore necessary. However, the control measures which een and are still in use such as the ones listed below, have never been able to eliminate asite and even control it in some places like East Africa where infection pressure is still Harrison et al., 1984). These control measures include:- meat inspection and subsequent sing of infected carcasses, irradiation of carcasses and improved sanitation (the use of ries and observation of hygiene of feedlots and zero grazing stalls). Other control ures are:- the use of treated human faeces to fertilize pastureland, keeping animals in d pastures and education of the public on the parasite life cycle , its transmissional anisms and its importance. Human diagnosis followed by treatment of infected persons, ugh cooking of meat at 57°C (especially if suspected to be infected) until it has lost its eddish tinge, pickling infected meat in 25% salt solution for 5 days and avoiding to buy from informal/unregistered butchers are some of the effective control measures if strictly wed. Even in countries with very strict meat inspection regulations like Kenya (Hughes, 6) and favourable socioeconomic conditions (Walther and Koske, 1980), the parasite is a problem. Vaccination and chemotherapy following ante-mortem diagnosis has proved ible but is still at the experimental stage and so remains the ultimate hope for the future odor et al., 1971; Rickard and Adolph, 1976; Pawlowski et al., 1978; Clegg and Smith, 8; Gallie and Sewell, 1978,1983; Walther and Koske, 1979; Harrison et al.,1984; Babiker-iba 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

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

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

cisions can be made as whether to treat the infected animals or improve farm/ranch ent practices, with a view to curbing T. saginata transmission (Harrison and Sewell, y such control strategy, the farmer does not incur losses due to downgrading or ation of infected carcasses. To date, there is no test which reliably diagnoses bovine cosis disease in cattle.

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

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

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

LITERATURE REVIEW:

Immunodiagnosis of bovine cysticercosis.

For the last three and a half decades, serology of parasitic diseases has been expanding any publications have appeared in the scientific literature regarding its application in the l of parasitic infections. Although highly valuable immunoassay systems have emerged, annot readily be applicable to the diagnosis of many tropical parasitic infections due to short comings. Some methods for example, immunofluorescent assays, complement on tests and radioimmunoassays, have been reported to have high clinical value in the osis of some infections such as cysticercosis, fascioliasis, filariasis, trichinosis, hydatid, tosomiasis, malaria, amoebiasis, pneumocystis, leishmaniasis, trypanosomosis and plasmosis (Voller and De Savigy, 1981; Fleck and Moody, 1993). Others such as antibody-antigen-ELISA have been shown to be useful in seroepidemiological surveys of bovine cercosis (Craig and Rickard, 1981b; Geerts et al., 1981; Harrison et al., 1989; Hughes et 993; 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; vhirst et al., 1967; Walther and Koske, 1980), much effort has been put into finding a able immunodiagnostic method for cysticercosis and other helminth infections. Since in ne parasitic infections, an immune response (whether humoral or cell-mediated) may be only evidence of indicating an infection in a live organism, the need of developing these munodiagnostic techniques is therefore very important. This has resulted into the velopment and application of quite a number of immunodiagnostic techniques as an

alternative to meat inspection techniques. These immunodiagnostic techniques have bee reviewed by Geerts et al., (1977), Gathuma (1977), Onyango-Abuje (1984) and Kamanga-soll (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 Gathum 1977). Since then, IDT has been one of the immunodiagnostic tests that has been widely us in the diagnosis of many parasitic diseases (Fife, 1971). It is a sensitive test and results a obtained within a few hours. Limitations such as non-specificity (Fife, 1971; and Froyd, 196 frequent false positive (Bugyaki, 1961) and negative (Kagan, 1968) reactions, were report However, Dewhirst (1960, 1967) and his co-workers found the test to be very fast a sensitive. Later, Mechnicka-Roguska and Swierz (1970), while working on hum cysticercosis found that the problems of IDT mentioned above could be attributed to the of impure and heterologous antigens for which Enyenihi (1970) suggested that the us 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 develo for diagnosing parasitic infections. Soulsby (1963) described the sensitivity of CFT t relatively high in diagnosing calcified and encapsulated T. saginata cysticerci. He also f that the test could diagnose prenatal infections more efficiently than neonatal infection

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, 1960; Froyd, 1964a).

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

Precipitin test is also amongst the antibody test that have been used in the serologic 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

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

:4. Latex agglutination test (LAT).

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

1:5. Indirect haemagglutination test (IHAT).

Indirect haemagglutination test has been extensively used for immunodiagnosis of

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

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

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

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

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

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

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

8. Radioimmunoassay (RIA).

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

. **Enzyme - linked immunosorbent assay (ELISA).**

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

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

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

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

ucing surface turn-over, excretory and secretory products as antigens in infected cattle
ua 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 mum detection level to be approximately 200 live cysticerci. They also found that the gens appeared in circulation, 4-5 weeks post-infection. Brandt et al., (1992) also working surface/excreted/secreted glycoproteins but using a different monoclonal antibody, came vith a minimum number of living cysticerci that could be detected by thei. Ag - ELISA em to be 88. In a different experiment, Bogh et al., (1996) using a different monoclonal body, detected between 2 and 22 live cysts but the same test was unable to detect several r animals which haboured between 2 and 41 viable cysts at slaughter. In naturally infected le, Onyango-Abuje et al., (1996) found the sensitivity of the McAb HP10 Ag - ELISA test e 83% of those with 30 or more live cysts and 22% of those with 1-29 live cysts but failed letect cattle harbouring less than 4 cysticerci. Therefore, as reported by Onyango-Abuje l., (in press, c) the assay presently may be used in the field to identify herds with high or infection with bovine cysticercosis but with low infections there are either false positive false negative results. At its present state therefore, antigen-ELISA is not specific and sitive enough to diagnose cysticercosis in individual animals. From the above literature,the act number of live cysticerci that can be reliably detected by Ag-ELISA using McAb HP10 therefore not yet known. Therefore, further work is necessary to evaluate on the exact mber of live cysticerci that the assay can reliably detect in the infected animal and rrelate 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 ements in hygiene, adequate sanitary installations, proper animal management and dry practices, the development of specific and sensitive diagnostic procedures such as igen-ELISA would facilitate the execution of reliable epidemiological surveys which he basis for pinpointing target control areas and evaluating control measures. Such s, are also essential for the design of environmentally appropriate control strategies, ing the introduction of recombinant vaccines.

CHAPTER TWO

MATERIALS AND METHODS

THE PARASITE EGGS:

Collection of the eggs:

Taenia saginata proglottids were collected from human excrement in Mathare Valley of Nairobi area and brought to NVRC laboratories. The proglottids were collected in ological saline (0.15M NaCl) containing 200 units/ml of Crystapen benzylpencillin o Laboratories, U.K.); 0.2mg/ml Streptomycin Sulphate (Glaxo Lab., U.K.) and 5ug/ml zone (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 250nm, 150nm and 30nm apertures, respectively. The 30nm aperture sieve retained the which were then transferred into a universal bottle containing physiological saline and iotics stored at 4°C untill required.

2. Testing the viability of the eggs:

This was done in accordance with Stevenson's method (1983) . This process was first ed at removing the embryophore using Sodium hypochlorite. Equal volumes of egg ension and 10% Sodium hypochlorite solution were mixed together in a 15ml graduated ic centrifuge tube and shaken vigorously for 2 minutes. Immediately, the tube was filled e 15ml mark with physiological (normal) saline and centrifuged for 5 minutes at 4xg on MSE Minor Centrifuge. The supernatant was drawn off leaving approximately 0.25ml to

hich normal saline was added to the original mark of 15ml and centrifuged again. This was peated before a freshly made hatching solution (1.17g Sodium hydrogen carbonate + 0.05g ypsin in 100ml of deionised water) was added upto the 2ml mark. One ml of bovine bile was ded and the tube was transferred to a 37°C water bath and incubated for 45 minutes. Every 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 amined under the microscope using both low (x10) and high (x40) power objective lenses. e number of motile oncospheres was counted in a group of a 100 oncospheres to determine ir percentage. The counting was done 2 or 3 times and an average obtained. No longer than minutes was spent examining one slide, otherwise death of the oncospheres would occur e to drying of the slide.

:3. Egg counts.

A bottle containing eggs was gently shaken and 0.5ml was removed (using a 1ml inge) and transferred to a universal bottle marked at 15ml. Normal saline was added upto ml mark. After shaking the bottle well, a pasteur pipette was used to fill both chambers of McMaster slide which was allowed to stand for 2-3 minutes. Eggs within the grid in both mbers of the McMaster slide were counted using a microscope with x2.5 objective. The nber of eggs in the volume of egg suspension was calculated by adding the number of eggs both chambers (a volume of 0.3ml) and multiplying by the dilution factor of 50. Three nts were made and cross-checked by at least 2 other people using a tally counter. An

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

:4. Evaluation of infectivity of the eggs.

This was done according to the methods of Silverman (1956). The measure of the ctivity of the eggs was evaluated by comparing the number of larval cysticerci which eloped, with the number of potentially infective eggs fed. The number of potentially ctive embryos in a suspension of tapeworm eggs was caculated from the percentage of acanth embryos in a sample, which appeared motile after treatment with hatching solution verman,1954a and Stevenson, 1983). The following infectivity formula was used:-

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

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

. THE ANIMALS:

:1. Naturally infected cattle:

Naturally infected Zebu herds were identified through history and reports from the trict Veterinary Officer in Samburu District. Ninety six animals were bled for serum. od samples were transported to the laboratory in a cool box, kept overnight at 4°C and

entrifuged at 2500g for 30 minutes in a refrigerated centrifuge.The resultant sera were spirated and stored in eppendorf tubes at -20°C till required.The serum samples were tested or circulating antigen by Ag- ELISA (High Ag-ELISA reading usually meant a high likelihood f positivity of bovine cysticercosis). Based on the Ag-ELISA optical density (OD) values, venty five steers (about 1-1½ years old) were selected, bought and brought to the National eterinary 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 onth 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 w OD readings (negative cattle), were utilized in the experiment. They were feacal sampled or nematode and fluke infections using modified McMaster egg counting and Boray edimentation techniques, respectively. This was done in accordance with the standard perating methods in helminthology laboratory at NVRC, Muguga. Those animals which were ound infected were treated with a wormicide.

The animals were slaughtered in the 3rd month and examined for cysticerci first by outine meat inspection procedures as stipulated by Kenya Meat Control Act - 1977 and then llowed by total dissection of a half of the carcass. The Total dissection was done by thinly icing the entire masculature of the carcasses in order to recover the cysticerci. The number f cysticerci obtained in one half following symmetrical dissection of the entire carcass, was oted and doubled to get the total number of cysticerci in the whole animal.

Experimentally infected calves:

Thirty two neonatal calves, 3 to 34 days old, were bought from Konza Ranch at Kapiti s Estate, Machakos District and brought to NVRC in groups of 16 in two separate nces. Immediately, they were bled and the serum tested for circulating T. saginata ens as explained in **2:3:1.** below. The calves were kept worm/cysticercosis free in pens ed on milk initially and later on calf weaner pellets and hay. Unfortunately, 2 calves died e infection with T. saginata eggs. The infection of the calves was staggered. The first 15 s were given eggs earlier than the second lot but the eggs were always administered when alves were 2 - 2½ months old.

Serial dilutions were made from the egg suspension to get the number of eggs required nfection per animal in each group. The first 15 calves were divided into 4 groups of 3, 4, 4 calves which were given varying doses of T.saginata eggs as follows:- group 1 received ggs (control), group 2 received 2500 eggs each, group 3 received 5000 eggs each and p 4 received 10,000 eggs each. The calves were bled just before administered with T. ata eggs and thereafter they were bled every two weeks till slaughtered in the 15th week. second group of 15 calves was treated similarly.

3. Post - mortem examination:

3:1. Meat inspection:

Meat inspection was done in accordance with the Kenya Meat Control Act-1977 ch stipulates that the cheek muscles (masseter-external muscles and Pterygoid-internal

les), tongue, heart and Masculus triceps brachii (shoulder muscles) must be incised and nined for the presence of C. bovis. For cheek muscles, two deep linear incisions were parallel to the mandible from its upper muscular insertion. The tongue (also examined lpation) was incised lengthwise on the lower surface from base to root while the heart split from base to the apex and further incisions made into the muscles. Three deep and lel transverse incisions were made above the point of the elbow in the shoulder muscles.

3:2. Total dissection:

Half of each carcass of naturally infected cattle was cut and divided into the wing regions:- head, tongue, neck and hump, fore legs, pelvis, hind legs, lumbar, rumen, , heart, liver, kidneys and diaphragm. The muscles of these parts were cut into very thin parent slices of about 1mm, for recovery of cysticerci. The cysticerci encountered g slicing were counted, doubled (for the whole carcass) and recorded. It was assumed that ysticerci were evenly distributed in the carcasses. However, visceral organs were not ed. In experimental calves, the whole carcass was examined for cysticerci because the als were small in size.

SEROLOGICAL TESTS:

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

The ELISA method utilized in the study was based on screening serum samples for ulating T.saginata antigens using a mouse monoclonal antibody (McAb), HP10, as

ribed by Harrison et al., (1989). The assay involved sensitization of Linbro polyvinyl ride microtiter plates (Dynatech Laboratories, Inc., Virginia) with a monoclonal antibody, 0) at the protein concentration of 10ug/ml in borate buffered saline (BBS), pH 8.4 , 100 r well. The plates were incubated at 4°C overnight or for 4 hours at room temperature. plates were washed three times with normal saline/Tween 20 with 3 minutes interval een washes, blocked using 200 ul of phosphate buffered saline, (PBS) pH 7.4 with bovine m albumin (BSA)/Tween 20, and incubated for 1 hour at room temperature. The plates e again washed three times, allowing to stand for 3 minutes between washes. The luted test serum samples were added, 100 ul per well. The plates were then incubated at for 30 minutes and thereafter, washed three times as above. Biotin - conjugated McAb 0, diluted at 1:500 to 1:1000 (depending on the batch of the reagent) in PBS/BSA/Tween was added 100 ul per well and incubated at 37°C for 30 minutes. The plates were washed e times as above and Streptavidin biotinylated horseradish peroxidase conjugate at 1:1000 BS/BSA/tween 20 was added 100 ul per well and the plates incubated at 37°C for 30 utes. The plates were then washed as above and 100 ul of the substrate, 3'3'5'5'-methylbenzidin (TMB),was added to each well and the incubation allowed to go on for 0 minutes. The reaction was stopped with 100 ul of 0.2M H_2SO_4 (ARISTAR)per well and optical density was read at 450nm on an ELISA reader (Titertek Multiscan Plus MK11).

STATISTICAL ANALYSIS:

1. Negative cut - off point.

An optical density cut-off value to distinguish between positive(+ve) and negative(-
results was taken as the mean of the negative controls plus three standard deviations
) (Onyango - Abuje et al., 1996).

2. Correlation Analysis:

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

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

vhere 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.

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

3. Kappa statistic

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

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 ven test, a method or diagnostic technique which is biologically independent of the nods used to define the true health status of the animals, should be used as a gold standard rtens et al., 1987). In this study, the true status of C. bovis infection was established by t-mortem examination (Total dissection) done by thinly slicing the masculature of the casses to recover the cysticerci.

A four-fold table (Martens et al., 1987) shown below was used to evaluate sensitivity, cificity, accuracy, prevalence and predictive value of Ag-ELISA and meat inspection

od.

	Gold standard		
t under study	Positive	Negative	Total
itive	a	b	a + b
gative	c	d	c + d
tal	a + c	b + d	a+b+c+d

e a - True Positive
c - False Negative
b - False Positive
d - True Negative.

the table,

ivity = $\frac{a}{a+c}$ Specificity = $\frac{d}{b+d}$

ctive value = $\frac{a}{a+b}$

racy = $\frac{a+d}{a+b+c+d}$ Apparent prevalence = $\frac{a+b}{a+b+c+d}$

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

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

ode of Cattle	Total number of cysticerci recovered by total dissection.		
	Live	Dead	Total
	2	6	8
	37	17	54
	5	6	11
	31	22	53
	25	19	44
	2	6	8
	8	13	21
	7	8	15
	7	12	19
	24	19	43
	22	9	31
	55	22	77
	0	1	1
	0	2	2
	0	8	8
	0	15	15
	5	8	13
	2	4	6
	1	2	3
	55	39	94
	2	10	12
	5	11	16
	42	46	88
	24	5	29
	0	0	0
d total	361	310	671

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

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

. Antigen-ELISA findings in naturally infected animals.

The results of the Antigen-Enzyme-linked Immunosorbent Assay (Ag-ELISA) mmarized in Table 2. Out of 25 animals selected from the field, 16 animals were ted seropositive for bovine cysticercosis while nine animals were seronegative. eropositivity and negativity of the animals were based on the negative cut off s as shown in Table 2. Based on negative cut off point at slaughter, the Ag-ELISA ted 18 animals as seropositive for bovine cysticercosis. There were 2 animals were seronegative at selection but later converted to be seropositive. There t any animal which was seropositive at selection and later found to be egative at slaughter.

Out of 9 seronegative animals at selection from the field, only four (animals he following code numbers:- 723, 720, 976 and 714) were without any cysticerci ne (976) out of these four was found to be seropositive at slaughter (Table 2). emaining five animals (animals with the following code numbers:- 715, 956, 972, nd 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 egative (live cysticerci were recovered by total dissection but tested seronegative Ag-ELISA) and 2 other cases of false seropositive (no live cysticerci were ered by total dissection but tested seropositive with Ag-ELISA). The Ag-ELISA ble to detect seropositive cases for bovine cysticercosis in animals harbouring 2 living cysticerci upto 55 cysticerci. However, there were other 3 cases (animals he following code numbers:- 956, 972 and 971) with 2 live cysticerci each that not detected as seropositive (Table 2).

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

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

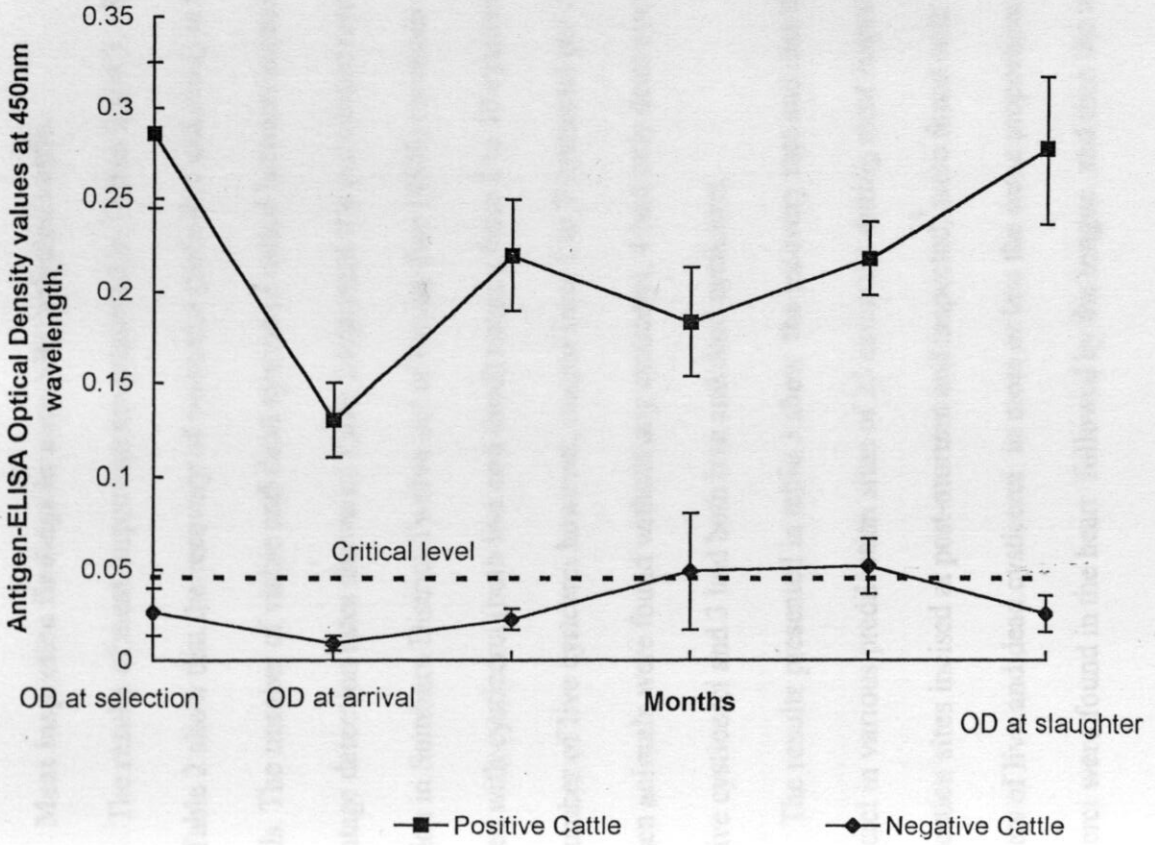
2. Post-mortem findings of 25 animals selected from the field together with Antigen-ELISA readings (Optical Density values) at 450nm wavelength.

No.	Total Dissection			Meat Inspection	AG-ELISA READINGS (OD VALUES)	
	L	D	T	T D L	AT SELECTION	AT SLAUGHTER
	2	6	8	0 0 0	0.110 (0.150)	0.060
	37	17	54	1 0 1	0.334 (0.064)	0.116
	5	6	11	0 0 0	0.390 (0.064)	0.283
	31	22	53	2 0 2	0.268 (0.118)	0.094
	25	19	44	1 1 0	0.353 (0.150)	0.172
	2	6	8	0 0 0	0.061 (0.064)	0.013
	8	13	21	2 1 1	0.391 (0.064)	0.429
	7	8	15	4 0 4	0.186 (0.064)	0.108
	7	12	19	0 0 0	0.244 (0.064)	0.171
	24	19	43	0 0 0	0.685 (0.150)	0.565
	22	9	31	2 2 2	0.299 (0.150)	0.166
	55	22	77	8 1 7	0.282 (0.150)	0.285
	0	1	1	0 0 0	0.046 (0.150)	0.014
	0	2	2	0 0 0	0.096 (0.150)	0.005
	0	8	8	0 0 0	0.003 (0.064)	0.090
	0	15	15	10 10 0	0.008 (0.150)	0.001
	5	8	13	0 0 0	0.076 (0.064)	0.145
	2	4	6	0 0 0	0.037 (0.064)	0.018
	1	2	3	0 0 0	0.020 (0.150)	0.010
	55	39	94	3 1 2	0.266 (0.118)	0.311
	2	10	12	1 1 0	0.091 (0.064)	0.026
	5	11	16	0 0 0	0.266 (0.118)	0.663
	42	46	88	2 0 2	0.105 (0.064)	0.220
	24	5	29	3 3 0	0.315 (0.064)	0.484
	0	0	0	0 0 0	0.098 (0.064)	0.237(0.043)
	361	310	671	39 20 19	16 +ve CATTLE	18 +ve CATTLE
tion	80	96	96	48 32 28	64	72

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

res in parentheses represent negative cut-off points.

e percentages were based on 25 animals selected from the field.


Antigen-ELISA Optical Density values at 450nm wavelength.
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Critical level
OD at selection
OD at arrival
Months
OD at slaughter
Positive Cattle
Negative Cattle

3. Meat inspection findings in naturally imfected cattle.

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

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

3: Frequency of cysticerci in predilection sites of naturally infected cattle

g meat inspection.

lection sites for ction according enya Meat rol 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
t	20(5)	2.98(0.75)*	9(4)*	36(16)
lder muscles	7(2)	1.04(0.30)	4(2)	16(8)
ue	12(12)	1.79(1.79)	3(3)	12(12)
eter and goid muscles	0(0)	0(0)	0(0)	0(0)
d total	39(19)	5.81(2.83)	12(7)	48(28)

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

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

. 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 us doses of eggs of T. saginata was very variable. The results indicate that the tion rate of the eggs in different calves was very poor. On average, group 2 and 3, s dosed with 2500 and 5000eggs, respectively, showed the highest infection rate an infectivity index of 0.02 whereas calves in group 4 dosed with 10,000 eggs, ed the lowest infectivity rate with an index of 0.01.

4. The mean infectivity of eggs of Taenia saginata in 30 rimental calves.

No.of calves per group	Mean egg dose	Mean No. of cysticerci Recovered			Mean infectivity Indices*
		Live	Dead	Total	
6	0000	0	0	0	0.00
8	2500	33	13	46	0.02
8	5000	44	39	83	0.02
8	10,000	35	48	83	0.01

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

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

2. Recovery of Taenia saginata cysticerci in experimentally infected es.

The recovery rate and distribution of cysticerci in various sites of the 24 infected es at slaughter, is shown in table 5 (a). All the sites sliced including visceral organs ng total dissection of carcasses of the infected calves were found with varying bers of live and dead cysticerci, except in the kidneys. Total dissection, like in rally infected animals, was used as the gold standard and revealed cysticerci in all 24 calves administered with various doses of T. saginata eggs. Total dissection aled live cysticerci in 16 out of 24 (66.67%) calves. Both visceral organs and etal muscles harboured considerable number of live and dead cysticerci. There e more dead cysticerci in the visceral organs (liver, heart, rumen, diaphragm and gs) than there were in the skeletal muscles (hind legs, fore legs, trunk and ribs). nerally, more live cysticerci were recovered than the dead ones.

The number of cysticerci recovered from the carcasses of the individual calves hown in table 5 (b). The number of viable and dead cysticerci also varied between casses of individual calves. Twenty four of the calves administered with various es of T. saginata eggs, were found with cysticerci (either live, dead or both) and y one calf did not habour any cysticerci. One of the control calves was found ected with one live cysticercus at autopsy (Table5 (b)).

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

E	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]
	40(11)[3]	2.40 (0.66)[3]	9 (5)[3]	37.5(20.83)
ue	42(27)	2.52(1.62)	10 (7)	41.67(29.17)
legs	222(163)	13.35(9.80)	15 (10)	62.50(41.67)
& Hump	100(59)	6.01(3.55)	14 (10)	58.33(41.67)
s	73(55)	4.39(3.31)	13 (9)	54.17(37.50)
legs	250(168)	15.02(10.10)	14 (10)	58.33(41.67)
	108 (75)	6.49(4.51)	13 (10)	54.17(41.67)
oar	68 (37)	4.09(2.22)	11 (8)	45.83(33.33)
en	9 (3)	0.54 (0.18)	6 (2)	25.00(8.33)
s	65 (37)	3.91 (2.22)	12 (8)	50.00(33.33)
t	285(116)	17.13(6.97)	12 (8)	50.00(33.33)
	366(93)	22.00(5.59)	21(10)	87.50(41.67)
eys	0(0)	0.00(0.00)	0(0)	0.00(0.00)
hragm	35 (20)	2.10 (1.20)	8 (5)	33.33(20.83)
d total	1663(864)	100.00(51.95)	24(16)	100.00(66.67)

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

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

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

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

percentages were based on the grand total of cysticerci obtained in all the calves

percentages were based on the 24 experimentally infected calves.

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

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

ode No.	Egg dose	Number of cysticerci recovered at autopsy		
		Live	Dead	Total
	0000	0	0	0
	0000	0	0	0
	0000	1	0	1
	0000	0	0	0
	0000	0	0	0
	0000	0	0	0
	2500	152	1	153
	2500	17	18	35
	2500	76	12	89
	2500	3	4	7
	2500	0	28	28
	2500	0	10	10
	2500	14	31	45
	2500	0	0	0
	5000	0	8	8
	5000	124	38	162
	5000	0	22	22
	5000	2	36	38
	5000	0	4	4
	5000	1	14	15
	5000	193	2	195
	5000	0	187	187
	10,000	62	29	91
	10,000	59	26	85
	10,000	0	249	249
	10,000	12	61	73
	10,000	0	8	8
	10,000	55	4	59
	10,000	1	5	6
	10,000	93	1	94
d total	140,000	864	800	1664

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

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

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

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

Antigen-ELISA findings in experimentally infected calves.

Table 6 shows post-mortem results of each of the 30 experimental calves used with the Antigen-ELISA optical density readings before and at slaughter. All experimental calves were seronegative for bovine cysticercosis before tration with various doses of Taenia saginata eggs. The seropositivity and ity values were based on the seronegative cut off points as shown in table 6. At er, with an OD negative cut-off point of 0.059, the Ag-ELISA detected 9 s (37.50%) as seropositive for bovine cysticercosis.The controls never showed tectable levels of antigenemia.

Basing on the recovery of live cysticerci by total dissection at slaughter, Ag- gave 7 cases of false negative (live cysticerci recovered by total dissection but gative with Ag-ELISA). Unlike naturally infected animals, there was no case of positive (no live cysticerci recovered by total dissection but seropositive with Ag-A). The lowest number of cysticerci detected by Ag-ELISA in these calves was 14 ysticerci. Antigenemia could not be demonstrated in animals habouring only cysticerci or those harbouring less than 14 live cysticerci. However, there was one (with calf code number 4153) with 17 live cysticerci was not detected by the Ag-A.

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

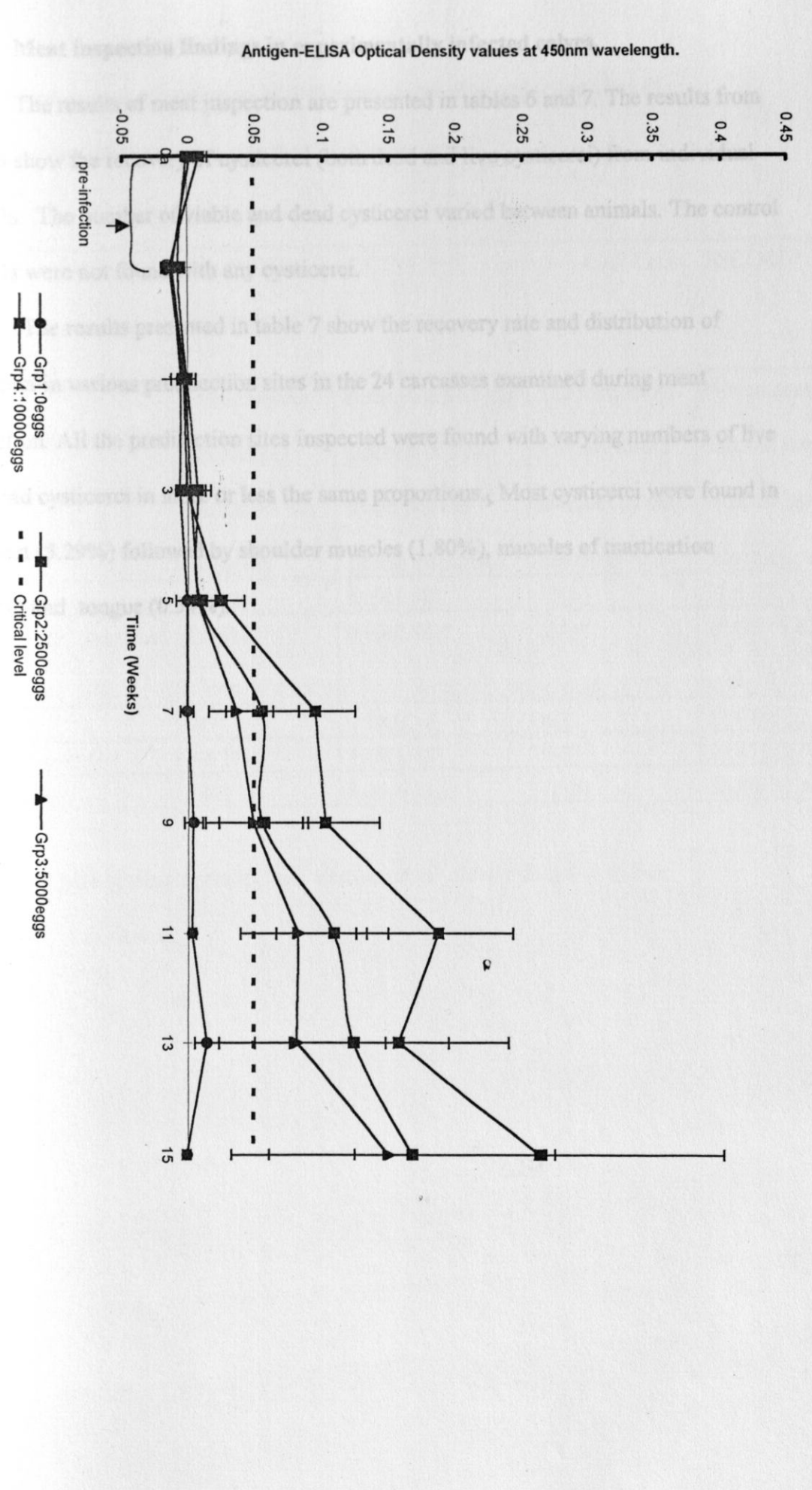
True seropositive and seronegative cases correlated well with live cysticerci ens, both at selection and at slaughter. By using Spearman rank - order correlation

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

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

	Egg No	Post-mortem finding		Ag- ELISA readings
Number		Total Dissection L D T	Meat Inspection L D T	Before Infection At slaughter
	00000	0.....0.....0	0.....0.....0	-0.002 .....-0.003
	00000	0.....0.....0	0.....0.....0	0.001.....0.001
	00000	1.....0.....1	0.....0.....0	-0.004.....0.006
	00000	0.....0.....0	0.....0.....0	-0.024 .....-0.004
	00000	0.....0.....0	0.....0.....0	-0.011... .....-0.010
	00000	0.....0.....0	0.....0.....0	-0.030.....0.003
	2500	152.....1.....153	0.....0.....0	-0.004.....0.783
	2500	17.....18.....35	0.....0.....0	0.007.....0.003
	2500	76.....12.....89	4.....0.....4	0.005.....0.387
	2500	3.....4.....7	0.....0.....0	0.003.....0.009
	2500	0.....28.....28	0.....0.....0	0.002.....-0.004
	2500	0.....10.....10	0.....2.....2	-0.027.....0.025
	2500	14.....31.....45	4.....0.....4	-0.022.....0.050
	2500	0.....0.....0	0.....0.....0	-0.023.....-0.012
	5000	0.....8.....8	0.....0.....0	0.006.....0.000
	5000	124....38.....162	2.....0.....2	0.000.....0.317
	5000	0.....22.....22	0.....0.....0	0.000.....-0.006
	5000	2.....36.....38	0.....0.....0	0.003.....-0.00
	5000	0.....4.....4	0.....0.....0	-0.018.....-0.004
	5000	1.....14.....15	0.....4.....4	-0.043.....0.024
	5000	193.....2.....195	10.....0.....10	-0.028.....0.872
	5000	0.....187.....187.	0.....49.....49	-0.018.....0.002
	10000	62.....29.....91	0.....0.....0	-0.003.....0.060
	10000	59.....26.....85	3.....0.....3	0.000.....0.697
	10000	0.....249....249.	0.....10.....10	-0.002.....-0.006
	10000	12.....61.....73	1.....3.....4	0.005.....0.002
	10000	0.....8.....8	0.....0.....0	-0.039.....-0.007
	10000	55.....4.....59	17.....0.....17	-0.045.....0.879
	10000	1.....5.....6	0.....0.....0	-0.015.....-0.010
	10000	93.....1.....94	9.....0.....9	-0.012.....0.486
l		864....800...1664	72..... 68.....140	(0.032).....(0.059)
etection*		66.67 95.83 95.83	33.33 20.83 50	0.00 .....37.5

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


Antigen-ELISA Optical Density values at 450nm wavelength.
-0.05
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
pre-infection
3
5
7
9
11
13
15
Time (Weeks)
Grp1:0eggs
Grp2:2500eggs
Grp3:5000eggs
Grp4:10000eggs
Critical level

Meat inspection findings in experimentally infected calves.

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

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

7. Frequency of cysticerci in different parts of predilection sites of mentally infected cattle during meat inspection.

ection sites for ction according to a Meat Control 977	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
	88(32)	5.29(1.92)	10(6)	41.67(25.00)
eter and pterygoid les	11(8)	0.66(0.48)*	6(4)*	25.00(16.67)
ue	9(3)	0.54(0.18)	4(2)	16.67(8.33)
lder muscles	30(27)	1.80(1.62)	5(3)	20.83(12.50)
d total	138(70)	8.30(4.21)	12(8)	50.00(33.33)

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

EFFICACY OF ANTIGEN-ELISA:

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

1. Diagnostic test evaluation tables for naturally infected animals.

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

Evaluation of meat inspection in naturally infected animals.

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

m the table, False +ve = 0

False -ve = 12

True +ve = 7

True -ve = 6 and therefore,

Sensitivity of the test = 7/19 x 100=36.84%

Specificity of the test = 6/6 x 100=100.00%

Predictive value of the test = 7/7 x 100=100.00%

Accuracy of the test = 13/25 x 100=52.00%

Apparent prevalence of the infection

= 7/25 x 100 = 28.00%

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

valuation of an Antigen-ELISA in naturally infected animals.

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

n the table, False +ve = 2

False -ve = 3

True +ve = 16

True -ve = 4 and therefore,

ensitivity of the test = 16/19 x 100 = 84.21%

pecificity of the test = 4/6 x 100 = 66.67%

redictive value of the test = 16/18 x 100 = 88.89%

ccuracy of the test = 20/25 x 100 = 80.00%

pparent prevalence of the infection

= 18/25 x 100 = 72%

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

comparison between an Antigen-ELISA and meat inspection in naturally fected animals.

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

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

2. Diagnostic test evaluation tables for experimentally infected calves.

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

Evaluation of meat inspection in experimentally infected calves.

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

the table, False +ve = 0

False -ve = 7

True +ve = 8

True +ve = 9 and therefore,

ensitivity of the test = 8/15 x 100 = 53.33%

pecificity of the test = 9/9 x 100 = 100.00%

redictive value of the test = 8/8 x 100 =100.00%

Accuracy of the test = 17/24 x 100 = 70.83%

Apparent preva;ence of the infection

= 8/24 x 100 = 33.33%

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

Evaluation of an Antigen-ELISA in experimentally infected calves.

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

n the table, False +ve = 1

False -ve = 7

True +ve = 8

True -ve = 8

Sensitivity of the test = 8/15 x 100 = 53.33%

Specificity of the test = 8/9 x 100 = 88.89%

Predictive value of the test = 8/9 x 100 = 88.89%

Accuracy of the test = 16/24 x 100 = 66.67%

Apparent prevalence of the infection

= 9/24 x 100 = 37.5%

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

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

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

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

3. A summary of comparison of the diagnostic methods, for both naturally perimentally infected animals based on live cysticerci only.

eters		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 (%)
ivity of the test		36.84	84.21	-	53.33	53.33	-
ficity of the test		100.00	66.67	-	100.00	88.89	-
ctive value of st		100.00	88.89	-	100.00	88.89	-
racy of the test		52.00	80.00	-	70.83	66.67	-
rent prevalance the test		28.00	72.00	-	33.33	37.50	-
a Statistic(k)		0.218	0.482	0.262	0.461	0.374	0.727
als ted th	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

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

CHAPTER FOUR

DISCUSSION, CONCLUSIONS, OBSERVATIONS AND RECOMMENDATIONS

DISCUSSION

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

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

. and Table 2.

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

The results obtained by thinly slicing the carcasses, were in accordance with the ious studies conducted by Mitchell (1978), Walther and Koske (1980), Gallie and ell (1983), Harrison et al., (1984), Kyvsgaard et al.,(1990) and Gracey and Collins 2) in terms of distribution of the cysticerci in various parts of animals. However, there variations with regard to the existence and ranking of sites occupied by cysticerci in e. These variations depend on a number of factors. For instance, Gracey and Collins 2) stated that the distribution of cysticerci is purely mechanical by the volume and nsity of the arterial blood owing to day-to-day activities of the animals in consideration. he other hand, the results obtained by this investigation were in contrast to the views Gracey and Collins (1992) with regard to predilection sites for cysticerci. This is ause, Gracey and Collins (1992) stated that there are no predilection sites for cysticerci he animals, while the results shown in Tables 1(a) and 5(a) clearly indicated that erent organs and muscle groups of a given carcass did not have the same number of ticerci and therefore, they could be ranked according to the number of cysticerci overed from each one of them. For instance, there was a decreasing number of both live dead cysticerci recovered from the fore legs, hind legs, liver, ribs, heart, lumbar, vis, tongue, lungs, neck and hump, head and diaphragm in that order which was in eement to that of Mango and Mango (1972). In naturally infected animals, no

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

Since the distribution of the cysticerci is purely mechanical by the volume and ılation of the arterial blood (Gracey and Collins, 1992), any factor affecting them mines the distribution and location of the cysticerci in the animal. For instance, blood ing through a capillary network is partly controlled by the action of sphincter muscles ıe arterioles and its tension is regulated by nerves and hormones. By dilation ansion) of the arterioles in one part of the organism's body and their constriction in her, the flow of blood in which cysticerci are carried is adjusted and distributed ording to the needs of various regions or parts of the organism (Pures and Orians, 7). Due to this internal physiological mechanisms in the cattle, more cysticerci antigens be available in the serum if during sampling more blood was from muscles heavily sted with live cysticerci which were actively secreting and excreting their metabolic ducts into blood circulation. These physiological mechanisms coupled with blood tics and external environmental conditions of the cattle, may help explain why some cted animals tested negative with the Antigen-ELISA while their counterparts (with ılar number of live cysticerci) tested positive.

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

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

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

In the experimentally infected calves, one control calf was found with one live
cercus in the hind leg at autopsy (Table 6). This could have resulted from accidental
mination during oral administration of eggs to the calves.

On average, poor recovery rates of cysticerci were realized at autopsy in

ental infections. For instance, mean recovery rates from 2500, 5000 and
ggs of T. saginata, were 46, 83 and 83 cysticerci, respectively, which were
. These low recovery rates were manifested in poor infectivity indices as
n Table 4 and which resulted in very low recovery rates or complete lack of
ci in some carcasses (Table 6). There are several possible reasons to explain
r infectivity indices. The ability of tapeworm eggs to produce an experimental
n in the appropriate intermediate host is dependent on:- the state of maturity of
, and the resistance of the host by innate and specific acquired immunity as
l by Silverman (1956). The availability of hatching (gastric juice) and
on (bile salts) stimuli at optimal conditions in the alimentary canal of cattle
nes the hatchability of oncospheres to cause the infection (Silverman, 1954).
man error which might have occurred during percentage motility determination,
g of the number of eggs for infection, affected the recovery rate of cysticerci at
, and greatly contributed to the poor infectivity indices obtained (Table 4).
contrast to post-mortem techniques, the monoclonal antibody (McAb coded as
based antigen detection ELISA system was designed to detect cattle harbouring
cysticerci, thereby indicating potential source of human infection (Harrison et al.,
The assay was highly specific for Taenia saginata and Taenia solium infections.
sults obtained in this investigation, using McAb, HP10, correspond very closely
e observation of Harrison et al., (1989) and Onyango-Abuje et al., (1996), using
ne monoclonal antibody (McAb) HP10. McAb, HP10 reacts with a repetitive
ydrate epitope on the glycoproteins found on the surface and in the secretions
cretions of the cysticerci in cattle. Using Antigen-ELISA, 16 out of 25 animals
ly infected were diagnosed as seropositive, at the field thereby giving a

alence of 64%. The 16 animals which had been diagnosed as seropositive at tion, remained seropositive throughout the monitoring period of three months slaughter (Figure 1). This showed a very good precision of the Antigen-ELISA in group of animals and 15 of these were true positives at autopsy, that is, they were d with live cysticerci and only one did not habour any live cysticerci (Table 2). At ghter, 18 of these 25 animals were diagnosed as seropositive thereby giving a alence of 72%. The two additional animals (715 and 976) which tested positive at slaughter increasing the number of seropositive animals from 16 to 18 , from a group which had been diagnosed as seronegative at selection (Table 2). seroconversion of these two animals from the seronegative group was not rising because there was a corresponding rise in the level of antigenemia in these negative animals above the critical level two months before slaughter (Figure 1). re are possible reasons to explain this discrepancy. The animals might have become cted between initial serum sampling and when they were purchased. Since it is well wn that the larvae of taeniid tapeworms generate a strong host immunity with ost complete resistance to reinfection (Soulsby, 1972), the antibodies resulting from mounted immune responses formed complexes with the antigens thereby removing antigens from the circulation and therefore, making the animal test seronegative le actually they had live cysticerci. Freeing parasite antigens from antigen-antibody nplexes might be the ultimate solution to, not only avoiding the occurrence of the ve mentioned discrepancy, but also increasing the sensitivity of this assay. Brandt 92) and his co-workers attempted to free parasite antigens from the antigen-body complexes according to the method of Weil and Liftis (1987), but they did not ceed. Their method involved mixing one part of serum and three parts of 0.1M-Na_2

A (pH4) and after boiling for 5 minutes and then centrifuging twice at 9000g for 3 es, the supernatant was recovered for use in ELISA. The cysticerci wall, ating the parasite and the host tissue, might prevent the exit of the parasite cts (antigens) into circulation thereby resulting into false negative cases but later, ntigens leak through the wall and get into circulation thereby resulting int. true ive cases. Surprisingly, the encysted cysticerci already present are often unaffected e host's immune responses, in spite of evidence that globulins can diffuse through vall around the cysticerci, indicating that they are able to resist or avoid the unological attack (Muller, 1975).

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

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

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

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

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

In experimentally infected calves, the antigenemia levels were first detected at 7- veeks post-infection (Figure 2). From week 9, the antigen level remained above the off point and kept on increasing for the entire period of 15 weeks in all the cted groups of calves. The control group never showed any detectable level of icercal antigens throughout the period and the antigen level remained far below the off point (Figure 2). Likewise, in naturally infected animals, the animals diagnosed eropositive at selection, had antigenemia level well above the cut off point ughout the monitoring period of three months thus indicating that the cysticerci e still alive (Figure 1). Between the second and third month of monitoring the

rally infected animals, the antigenemia level rose slightly above the cut off point in group of animals which had been serodiagnosed as negative at selection. This rise e antigenemia level was an indication that the cysticerci antigens might have been ent in the 9 animals serodiagnosed as negative at selection (Appendix 5). The ibution of the antigen level in both seropositive and seronegative groups of animals some times overlap as stated by Martens et al (1987). This supports the results vn in Fgure 1. This therefore showed that it is difficult to rule out absolutely the cted and the noninfected individual animals when using the Antigen-ELISA. ough with flactuations, both Figures 1and 2 showed that the cysticerci antigens ain present in the circulation once produced until removed from circulation at a which is not yet known. This is because, from Figures 1 and 2, there are no nals which were serodiagnosed as positive and after sometimes, they tested negative. This could have been manifested in their graphs going below the cut off nt. The flactuations of the antigen level in the animals and the removal of these gens from circulation, undoubtedly corresponds with the constantly occurring ctions between antibody and antigens. As the parasite produces metabolic products tigens), the host responds by producing antibodies which react with the antigens ming antigen-antibody complexes. These are then removed from circulation thereby ucing the antigenemia level or completely removing the antigens from circulation. these reactions, initially seropositive animal may eventually test seronegative and e versa.

When using Antigen-ELISA , the cut off point is very important in making isions about the infected and noninfected animals. In both Figures 1 and 2, if the cut level was increased by moving it upwards, the chances of false-positiveness

ing increased, hence, decreasing specificity and increasing sensitivity of the test. ity, false-positive cases which do not have cysticerci and get excluded from a t, are preferred to false-negative cases which have live cysticerci and when led in a feedlot, enhance the transmission cycle between humans and bovines in vironment. It follows therefore that for the test to have favourable perfomance in eld and to serve its purpose as a test geared towards helping in the control of both n taeniasis and bovine cysticercosis, the cut off point should be relatively very A high cut off point would help eliminate most if not all cases of false-negatives tens et al., 1987). Therefore, the Figures 1 and 2 are very important in making ions about the cut off point when validating the Antigen-ELISA.

There was considerable variation in the diagnosis of bovine cysticercosis when g three methods, namely, Antigen-ELISA, total dissection and routine meat ection. In 25 naturally infected cattle, 80, 72 and 28 per cent of the animals were cted positive of bovine cysticercosis by total dissection, Antigen-ELISA and meat ection, respectively. In experimentally infected calves, the order of these valence rates were, 66.67, 37.50 and 33.33 per cent for total dissection, Antigen-SA and meat inspection, respectively. Except for meat inspection, the corresponding es of prevalence rates were higher in naturally infected animals than in erimentally infected calves. These results indicated that although total dissection used as a gold standard, in practice, it may not have been 100% efficient in the ection of bovine cysticercosis. Total dissection cannot be used for detecting ticerci in slaughterhouses during inspection of meat because it is a tedious and time suming method. Furthermore, it would greatly lower the quality of meat should the ults obtained indicate that the carcass under inspection was fit for human

umption. The antigen detection assay was more sensitive than meat inspection od for it diagnosed more animals as positive for bovine cysticercosis than the in both naturally and experimentally infected animals. Although the prevalence of Antigen-ELLISA was just slightly above that of meat inspection by 4.17% in rimental calves, it was almost three times as high as that of meat inspection in al infections (Table 8). Meat inspection method can only be used to detect tion after the death of the animal when it is too late to make any decisions over ment or improving the public hygiene of the environment. Meat inspection method the least sensitive of the three methods. As stated before, meat inspection method ysically designed to observe, identify cysticerci at specified predilection sites, ght to have high density of the cysticerci than elsewhere in the carcass (Kyvsgaard,). This therefore means that an animal could be diagnosed as negative even if cerci were located elsewhere in the carcass being examined. During the inspection arious carcasses, it was realised that except for the dead, degenerate or calcified icerci which often formed spots of white and fibrotic lesions, a careless meat ector is most likely to miss out quite a number of viable cysticerci which blend with ranslucent and pinkish-red colour of the background and pass on for human sumption. This significantly lowers the sensitivity of the meat inspection method hence its unreliability and low detection rate previously observed especially in tly infected animals (Dewhirst et al.,1967; Walther and Koske, 1980). The Antigen-SA which was designed to detect products of viable cysticerci in live animals, is efore superior to meat inspection method and, becomes such an important gnostic test for bovine cysticercosis because, it is more effective than meat ection method in helping to break the life cycle thereby helping in the control

gies of the parasite.

rom the results of diagnostic test evaluation Tables summarised in table 8, the miological usage of the parameters (sensitivity, specificity, predictive value, acy, and prevalence) was considered when comparing the diagnostic methods. The ing of these parameters have been defined in Appendix 1. In naturally infected als, the antigen assay displayed a sensitivity of 84.21% (in animals harbouring 2- e cysticerci) while meat inspection method had 36.84% (in animals harbouring 1- cysticerci) when total dissection was used as a gold standard. The results ated that the assay was at least more than twice (that is, 2.3 times) as sensitive as inspection method in natural infections. These results compared favourably with obtained previously by Onyango-Abuje et al (1996) using the same assay. In imentally infected calves, the sensitivity of the assay and meat inspection method he same (53.33%) (in animals harbouring 14-193 and 1-17 live cysticerci, ctively) (Tables 6 and 8) when total dissection was used as a gold standard. discrepancy might have been due to poor infectivity of the eggs in the calves and was the possible reason also for the low prevalence rates obtained in these calves the Antigen-ELISA. Although the Antigen-ELISA had the same sensitivity as meat ction method in experimentally infected calves, it is advantageous to use the gen detection assay to avoid incurring economic losses. Otherwise, in both natural experimental infections, the prevalence rates for meat inspection method were r than those of Antigen-ELISA because the meat inspection method identified r animals as positive for bovine cysticercosis at slaughter than the Antigen-ELISA. In both experimentally and naturally infected animals, specificity and predictive es were 100% for routine meat inspection method while for Antigen-ELISA, the

es ranged from 66.67% (for the assay specificity in natural infections) to 88.89% redictive value(in natural and experimental infections) and specificity (in rimental infections) (Table 8). This is because the meat inspection method does ive false-positive cases like the Antigen-ELISA. Accuracy which measures the all preformance of a test in the laboratory or in the field, was higher in natural tions than in experimental infections for Antigen-ELISA and vice versa for meat ection (Table 8). This indicated that the Antigen-ELISA gave a true measure of the tion in the animals more than the meat inspection method because meat inspection hod leaves out many lightly infected carcasses which are detected by Antigen-SA. However, this was the case in natural infections but not in experimental tions (Table 8). Therefore, the Antigen-ELISA may be the most appropriate hod of diagnosing bovine cysticercosis infection but on a herd basis because of its - positive and negative reactions in light infections. However, in heavily infected s, the assay can be used for individual diagnosis.

The Kappa statistic (k) was used to measure the level of agreement between any diagnostic methods for bovine cysticercosis infection in cattle. Of the 25 animals cted from the field, 28% cases were detected by both meat inspection and total ection ($k = 0.218$; $p > 0.05$), 64% cases by both Antigen-ELISA and total ection ($k = 0.482$; $p > 0.05$) and 28% cases by both Antigen-ELISA and meat ection ($k = 0.262$; $p > 0.05$) (Table 8). In all these three comparisons, the Kappa sure of agreement was poor except between Antigen-ELISA and total dissection re the agreement was moderate. This suggests that the methods were not detecting same animals as either positive or negative for bovine cysticercosis infection. Of the experimentally infected calves, 33.33% cases were detected by both meat

tion and total dissection ($k = 0.461$; $p > 0.05$), 33.33% cases by both Antigen-
A and total dissection ($k = 0.374$; $p > 0.05$) and 29.17% cases by both Antigen-
A and meat inspection ($k = 0.727$; $p > 0.05$) (Table 8). In all these three
arisons, the Kappa measure of agreement was good except between Antigen-
A and total dissection where the agreement was moderate. This therefore,
sts that the methods were in most cases, detecting the same animals as either
ve or negative for bovine cysticercosis. The Kappa statistic values were on
ge lower in natural infections than in experimental ones. This was because in
al infections, there were more light infections which could not be detected by meat
ction method but could be detected by either Antigen-ELISA or total dissection,
in experimental infections. Generally, in both naturally and experimentally
ted animals, there was little overlap between animals diagnosed positive for
ne cysticercosis by the three methods except between Antigen-ELISA and total
ction in naturally infected animals. This discrepancy was due to a variety of
ons, some of which, in the case of total dissection and meat inspection, have
dy been explained and discussed before in the above paragraphs. As for the
gen-ELISA, it still gives false-positive cases owing partly to the fact that it is not
eally known when the cysticerci antigens disappear from circulation following the
h of the cysticerci. Also contributing to the above discrepancy, were the antigen-
body reactions that remove antigens from circulation by forming undissociated
plexes thereby resulting into false-negative cases. These are possible reasons to
explain the above discrepancy.

CONCLUSIONS.

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

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.

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.

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.

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

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.

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GLOSSARY OF HELMINTHOLOGICAL AND IMMUNOLOGICAL TERMS

dity) : Is the proportion of all the tests, both negative and positive that are correct and ess the overall performance of a diagnostic test.

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

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

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

: A foreign molecule that induces an adaptive immune response.

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

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

: Existence of parasitic organisms on the outside of the body of the host or in the ues.

bility) : Is the ability of the test to give a consistent measure upon repeated testing of the

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

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

Negative predictive value: Is the probability that the animal does not have the en test gative (normal).

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

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

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

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

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

n : The number of worms present in the host (this is often estimated from the egg

: Infection or disease naturally transmitted between man and other vertebrates

EAGENTS USED FOR ELISA.

d saline (BBS) pH 8.2

6.18 g

OH_20 9.54 g

4.38 g

I_20 to 1 litre, adjust with HCI if necessary.

ffered saline (PBS) pH 7.3

10.11 g

0.362 g

0.362 g

1.449 g

I_20 to 1 litre.

saline (TBS) pH 7.5

se 2.42 g

29.22 g

H_20 to 1 litre, adjust with HCI.

Tween 20

9.0 g

) (Sigma) 0.5 g

H_20 to 1 litre.

BSA

erum albumin (BSA) 10.0 g (Sigma A4503)

) 0.5 g

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

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

n acetate/Citric buffer pH 6.0.

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

strate.

5mg of 3,3, 5, 5. Tetramethylbenzidine (TMB) in 0.5 dimethl sulfoxide

Make up to 50 ml with 0.1M sodium acetate/citric acid buffer pH 6.0,

l 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:																									Total no. of cysts
	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	
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

X 5.

TEM FINDINGS IN NATURALLY INFECTED CATTLE TOGETHER WITH THEIR MEAN ANTIGEN-DINGS AT SELECTION AND SLAUGHTER.

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

- Optical density
- Total
- Dead
- Live

+ ve - positive
- ve - negative
H - High
M - Moderate

ures) - represent negative cut - off points.

IX 6.

CTIVITYOF THE TAENIA SAGINATA EGGS IN INDIVIDUAL CALVES

EGG DOSE	NUMBER OF CYSTICERCI RECOVERED			INFECTIVITY INDICES*
	LIVE	DEAD	TOTAL	
0000	0	0	0	0.00
0000	0	0	0	0.00
0000	1	0	1	0.00
0000	0	0	0	0.00
0000	0	0	0	0.00
0000	0	0	0	0.00
2500	17	1	35	0.02
2500	151	2	153	0.07
2500	76	23	99	0.05
2500	3	4	7	0.00
2500	0	10	10	0.01
2500	14	31	45	0.02
2500	0	0	0	0.00
2500	0	28	28	0.01
5000	0	8	8	0.00
5000	124	38	162	0.04
5000	0	22	22	0.01
5000	2	36	38	0.01
5000	0	4	4	0.00
5000	1	13	14	0.00
5000	193	2	195	0.05
5000	0	187	187	0.05
10000	62	29	91	0.01
10000	59	25	84	0.01
10000	0	249	249	0.03
10000	12	61	73	0.01
10000	0	8	8	0.00
10000	55	4	59	0.01
10000	1	5	6	0.00
10000	93	1	94	0.01

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

rmular was:-

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

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)**

***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 standard deviation (S.D.) values is = 0.045

X 12.

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

ttle 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
						$\sum d^2 = 960$

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

ank-order correlation coefficient (r)

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

960/25(625-1=624)

0/15600

59230769

76923

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

= 25, the number of pairs of values.

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

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

re, H_o : is rejected and H_I : accepted , thereby concluding that there is a statistically ant correlation

erpretation of these values of r:-

lues range from -1 to +1.

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

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

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.

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.

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
58	0 -0.003	23.5	21	-2.5	6.25
57	0 0.001	23.5	18	-5.5	30.25
51	1 0.006	15	13	-2	4
70	0 -0.004	23.5	23	-0.5	0.25
47	0 -0.01	23.5	28.5	5	25
44	0 0.003	23.5	14.5	-9	81
53	17 0.003	9	14.5	5.5	30.25
60	151 0.783	2	2	0	0
54	76 0.387	5	5	0	0
52	3 0.009	12	12	0	0
173	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
172	0 -0.004	23.5	23	-0.5	0.25
162	0 0	23.5	19	-4.5	20.25
161	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
					$\Sigma = 910$

$\Sigma d^2) / n(n^2 - 1)$

910) / 30(900 - 1)

60 / 26970

202447163

52836

alue at significance level of $\alpha = 0.05$ is = 0.306, when n = 30, the

airs of values.

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

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

H_0 : is rejected and H_1 : accepted, thereby concluding that there is a

significant correlation