ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

Cysticercus bovis: DEVELOPMENT AND EVALUATION OF SEROLOGICAL TESTS AND PREVALENCE AT ADDIS ABABA ABATTOIR.

By

NIGATU KEBEDE WUBIE

JUNE, 2004
DEBRE ZEIT, ETHIOPIA

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A Thesis Submitted to the Faculty of Veterinary Medicine, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Tropical Veterinary Medicine.

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ABBREVIATIONS

Ab	Antibody
Ag	Antigen
BSA	Bovine serum albumin
CBB	Carbonate bicarbonate buffer
CPB	Citrate phosphate buffer
ELISA	Enzyme linked immunosorrbent assay
F	Fat/fluid
FCS	Fetal calf serum
G	Group
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HRPO	Horseradish peroxidase
IHAT	Indirect hemagglutination test
IPB	Institute of Pathobiology
KCl	Potassium chloride
KH_2PO_4	Potassium phosphate
L	Lean
M	Meduim/membrane
MoA	Ministry of Agriculture
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Sodium phosphate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
OD	Optical density
OPD	Ortho-phenyl diamine
PBS	Phsphate buffer saline
PBS-T	Phsphate buffer saline Tween 20
PLC	Private limited company
PMI	Postmortem inspection
rpm	Revolution per minute
S	Scolex
SRBCs	Sheep red blood cells
T-20	Tween 20

ABSTRACT

This study was conducted to develop and evaluate serological tests for *C. bovis* for the diagnosis in live animals and determine the prevalence in Addis Ababa Abattoir. Postmortem inspection (PMI), indirect hemagglutinatio test (IHAT), indirect Enzyme-Linked Immunosorbent Assay (ELISA) and fecal examination techniques were conducted. A total of 743 serum samples, 522 from Addis Ababa Abattoir, 101 from Repi PLC Dairy Farm and 109 from Yeka-abado Farmers Association were collected and 11 negative controls from France. Postmortem inspection was conducted on cattle slaughtered from Addis Ababa abattoir, 39 (7.4%) were positive for *C. bovis*. The hearts of these animals were thoroughly inspected and live *C. bovis* cysts were collected for antigen preparation. The protein concentration of the cyst was measured using the Lowry method. Indirect ELISA was conducted on known positive and known negative samples and due to shortage and lack of fresh reagents ELISA was not conducted on the other samples. The cut-off was 0.84 with 100% and 81% sensitivity and specificity respectively. Parallely IHAT was conducted on positive and negative samples, and a titre of 1:64 and above was considered as positive. IHAT had 100% and above 91% sensitivity and specificity respectively and when compared with ELISA it showed better specificity. Based on this test 149 (28.5%) from the Abattoir, 33 (30.2%) from Yeka-abado Farmers Association and 8 (7.9%) from Repi PLC Dairy Farm samples were positive for *C. bovis*. The prevalence in the two management systems, namely Yeka-abado Farmers Association and Repi PLC Dairy Farm, was significantly different ($p<0.05$). The cyst fluid, protoscolex and cyst membrane were separately evaluated; for antigenic properties and the fluid was found to have a better discriminating titre. Postmortem inspection for *Cysticercus bovis* when compared with the serological tests was less sensitive. For fecal examination, the sedimentation and flotation techniques were conducted and cross reactivity with other helminths were either absent or very low. Further refinement and improvement of both serological test systems is necessary to increase the diagnostic potential.

KEYWORDS: Indirect ELISA, IHAT, Titre, OD, Postmortem inspection, Sensitivity, Specificity, *Cysticercus bovis, Taenia saginata.*

1. INTRODUCTION

Bovine cysticercosis is a muscular infection of cattle by the larvae of the human intestinal cestode, *Taenia saginata*. The parasite is cosmopolitan in its distribution (Minozzo, *et al.,* 2002) with varying of prevalence (Reinecke, 1983; Doyle, *et al.,* 1997). The adult Taenia infection in man is referred to as Taeniasis and that due to the larval stage cysticercosis (Hancock, *et al.*, 1989). The distribution of *Taenia saginata* is wider in developing countries where hygienic conditions are poor and where the inhabitants traditionally eat raw or insufficiently cooked or sun-cured meat (Florova, 1982; Symth, 1994; Minozzo, *et al.,* 2002). The infection is also a problem in developed countries where considerable "rare" (i.e. undercooked) beefsteak is consumed. It is important to note that eggs have been demonstrated to survive almost all stages of sewage treatment. It is significant; too, that even the high standard of meat inspection in abattoirs of highly developed countries that are expected to identify measly beef carcasses has not succeeded in eliminating this parasite (Florova, 1982; Symth, 1994

Cysticecosis was significantly more prevalent in feedlots and in traditional farming systems than in dairy farms. It is suggested that the continuous man to animal contact and the use of causal workers in feedlots may be factors that are conducive to *Taenia saginata* transmission (Dorny *et al*., 2002).

Taenia saginata/Cysticercus bovis is important from the standpoint of the health of cattle because of consequences for the meat supply and, more importantly, from the direct effects on the well-being of humans who, almost universally, consume beef as a source of protein and other minerals (Doyle, *et al*., 1997).

In Africa, inadequate health education and low availability of taenicides, are the major obstacles for the control of the disease (Pawlowski, 1996). The variations in the epidemiological patterns of Taeniasis/Cysticercosis throughout Africa are a reflection of the numbers and distribution of human and cattle populations (Harrison, *et. al.,* 1996). In East African countries prevalence rates of 30 to 80% have been noted (Tembo, 2001). In many developing countries, this disease constitutes a serious but sometimes less recognized public health problem (Minozzo, *et al.,* 2002).

In Ethiopia, the prevalence of *T. saginata/C.bovis* has been reported by a number of individuals. Florova in 1982 reported a prevalence of 100% which is the highest in Africa and also in the world. In some parts of Ethiopia, due to the habit of eating raw beef dishes such as *kourt* and *kitffo* that are served in raw or undercooked are the source of *T. saginata* infection in man (Teka, 1997). Tembo, (2001), reported prevalence of 89.41% in different agro-climatical zones of the country and she associated this high prevalence with the habit and/or culture of eating raw or undercooked beef. The prevalence of *C. bovis* in cattle reported by different individuals was 3.2% in different agro-climatic zones of the country (Tembo, 2001), 2.2-3.2% in Addis Ababa Abattoir (Tekka, 1997), 19.4% in Bahir Dar Alemu(1990), 21.17% in Nekemte Ahmed(1990), 13.85% in Debre Zeit Belayneh (1990) and 9.67% in Gondar Demissie (1989). Among 1,042,390 slaughtered cattle in different abattoirs of the country 1,308 whole carcass, 32, 630 portions, 30,656 heart, 21,917 heads, 7,462 tongues, 2,798 livers, 348 lungs, 26 spleens 21 kidnies have been condemned (MOA, 1973).

The nation's domestic meat consumption of about 45% comes from cattle, which generates export income mainly from the sale of live animals. In foreign trade, although the country is ideally placed to export live animals to the big markets of the Middle East and substantial markets of North and West Africa, export earning is relatively low. This is mainly due to the presence of a number of unimproved animal health problems, among which, *Taenia saginata/Cysticercus bovis* is one that remains a major public and animal health problem (EARO, 2000).

It is therefore important that sufficient emphasis be given to this problem so as to improve health, quality and quantity of beef that may satisfy the domestic requirements and increase the foreign export revenue. Thus the development of serological tests that capable of identifying infected animals before slaughter is helpful for diagnosis.

The serological tests used are indirect-ELISA and IHAT. ELISA is the primary binding test performed by allowing antigen and antibody to combine and then measuring the amount of immune complex formed. The basic principle of an ELISA is to use an enzyme to detect the binding of antigen-antibody. The indirect-ELISA is used to detect and quantitate antibody

(Crowther, 1995, Tizard, 1996). In IHAT antibodies can cross-link with particulate antigens, resulting in clumping or agglutination (Talwar, 1983). These tests help to estimate the prevalence of the disease and used to identify parasite free areas. The tests contribute to identify the source and control the prevalence of the disease.

Development of serological tests is necessary for diagnosis of the disease in live animals. The test can be used as an alternative tests for the diagnosis of *C. bovis* within the local setting and to qualify animals for international movement with an acceptable degree of confidence (Wrights, 1998).

Therefore the objectives include:

Primary objectives of this study are to develop and evaluate serological tests for the diagnosis of *C. bovis* in live animals and to estimate the prevalence of *C. bovis* at Addis Ababa Abattoir.

Specific objectives of this study are to:

1. Develop and evaluate IHAT and indirect ELISA for bovine cysticercosis
2. Conduct postmortem inspection and compare the results with the serological tests for bovine cysticercosis
3. Indicate the prevalence of *C. bovis* at Addis Ababa Abattoir
4. Conduct fecal examination to examine the presence of cross reactivity with other gastrointestinal helminths.

2. LITERATURE REVIEW

2.1. Description of the parasite

2.1.1.Taxonomy

Taenia saginata and its metacestode *Cysticercus bovis*, the unarmed beef tapeworm, belong to the class Cestoda order Cyclophyllidea Family Taeniida*e* and Genus Taenia (Soulsby, 1982; Symth, 1994; Urquhart *et al.*, 1996).

2.2.Morphology

2.2.1.Adult parasite

Taenia saginata, the beef tapeworm, is a large worm measuring 3-10 meters in length rarely the adult measures upto 15m (Soulsby, 1982; Reinecke, 1983; Urquahrt *et al*., 1996). It resides in the small intestine of humans where it attaches using its scolex and can survive for many years. The adult is ribbon-shaped, multi-segmented and hermaphroditic flatworm its body divided into three distinct parts consisting of scolex (head), neck and strobila (Gracey, 1981; Soulsby, 1982).

The scolex, measuring 1mm to 2mm in diameter, has four strong hemispherical suckers. There is no rostellum and hooks and the predilection site in the intestinal mucosa is in the proximal part of the jejunum (Gracey, 1981; Teka, 1997; O.I.E., 2000;). The neck is short unsegmented with a germinal structure immediately behined the scolex, which continiously produces proglottids (Urquahrt *et al*., 1996; Teka, 1997).

The strobila is a chain of segments made up of sexually immature, mature gravid segments in linear sequence. Each segment is called progllotid, strobilization occurs at the distal part of the neck (Soulsby, 1982).

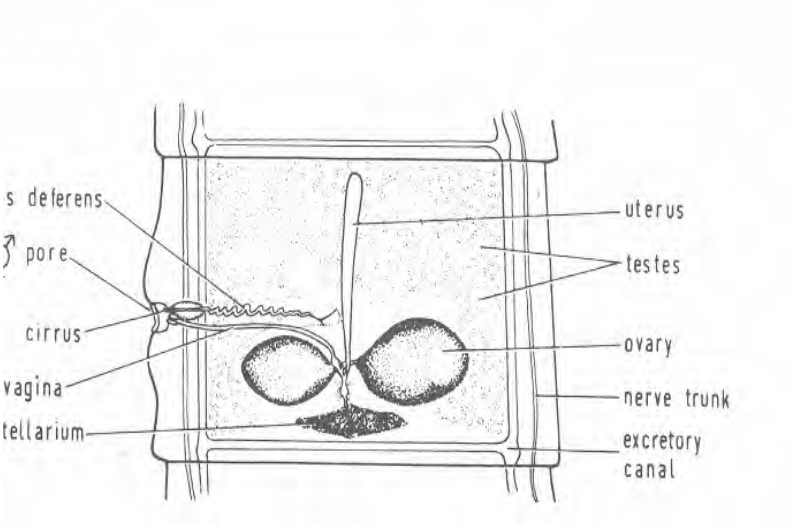
An adult *T. saginata* tapeworm has 600 to 2000 segments each of which is hermaphroditic with one set of reproductive organs and genital pores which open on the lateral margin(s) of the segment (Maeda *et al.,* 1996; Doyle *et al.,* 1997; Teka, 1997).

Self and cross fertilization between and among proglottids is possible. The gravid proglottids are 15 to 35mm long and 5 to 7mm wide and filled with eggs which detach from the strobila singly and leave the host via anus (Teka, 1997; Doyle *et al.,* 1997) (figure 1 and 3). This implies that coproscopic examination has a limited value in the diagnosis of *Taenia saginata* infection (Doyle *et al.,* 1997).

The graved segments, each containing branched uterus, are filled with thousands of eggs. The number of segments increase constantly as the tapeworm grows, forming long chains. The segments, which are formed first, are pushed towards the end leaving space for the new ones. The segments, which are found at the rear, are the oldest. These old segments periodically detached from the worms and discharged from the host's body with feces or independent of defecations (Teka, 1997; Maeda *et al.,* 1996).

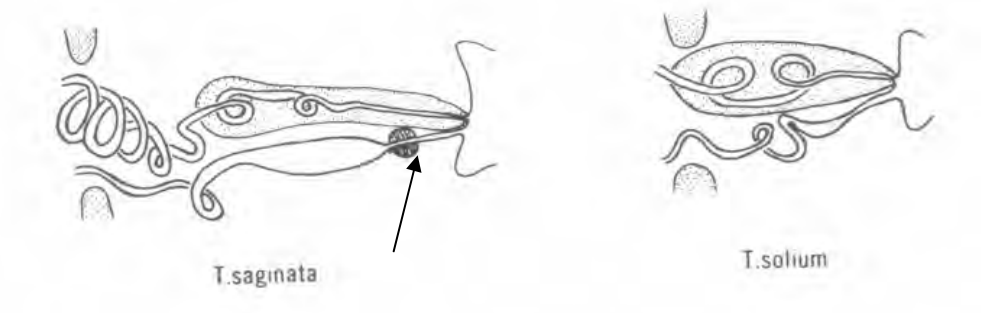
Each segment has a complete set of male and female reproductive organs in which eggs mature and develop (Symth, 1994). The mature proglottid/segement had vaginal sphincter muscle (Symth, 1994; O.I.E., 2000) (Figure, 1). It is estimated that each graved segment can contain as many as 80,000 to 100,000 eggs and an infected person may shed about 24 - 50 million eggs daily (Gracey, 1981; Teka, 1997). The ova from small number of carriers of the tapeworm can be widely distributed and infect large number of cattle (Harrison and Sewell, 1991). Once the mature eggs are excreted with the feces, they are capable of infecting the intermediate host, bovine (Teka, 1997; Minozzo *et al*., 2002).

Figure 1. Morphology of proglottids



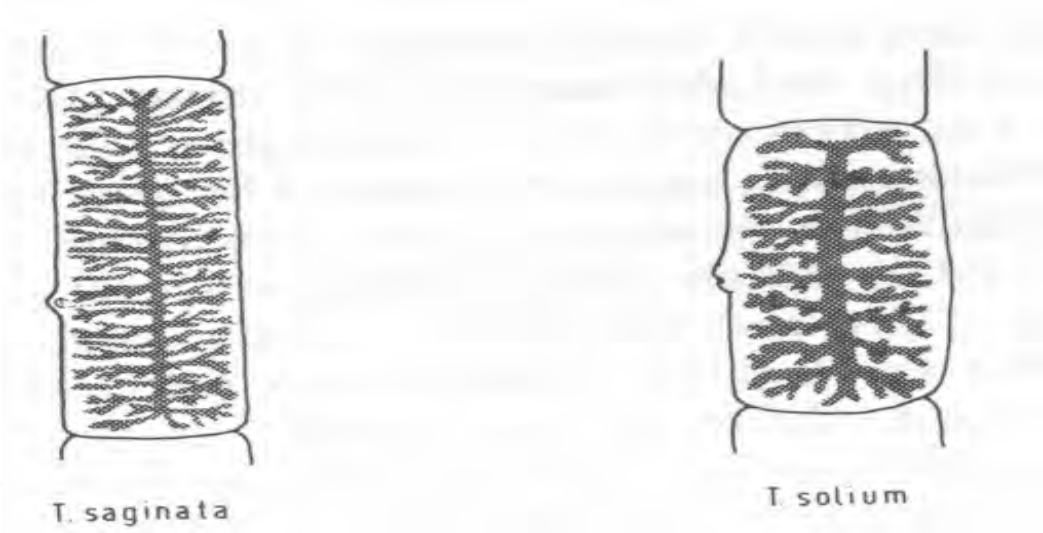
Source: Parija, 1996

Figure 2. Vaginal sphincters of *Taenia saginata* and *Taenia solium*



Source: Symth, 1994

Figure 3. Uterine branches of *Taenia saginata* and *Taenia solium*

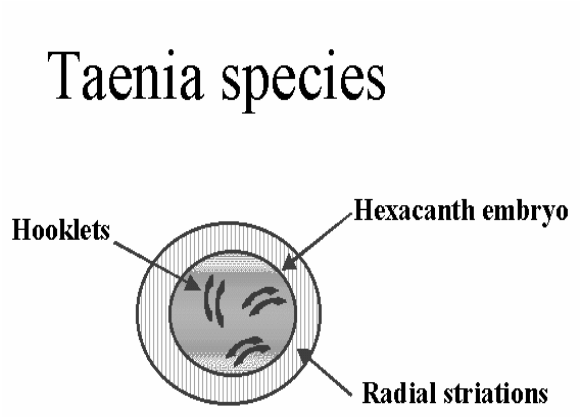


Source: Symth, 1994

3.2.2 Egg stage

Eggs passed in feces or discharged from ruptured gravid segments are subspherical to spherical in shape. The egg consists of the hexacanth (6-hooked) embryo (oncosphere), thick dark brown to yellow in color. There is an outer oval membranous coat, the true egg shell, which is lost in fecal eggs (Harrison and Sewell, 1991; Brown and Neva, 1983). It measures 30-41 micrometers in diameter and 46 to 50 micrometers in length (Soulsby, 1982; O.I.E., 2000). The eggs survive up to 200 days in moist manure, 33 days in river water, 154 days on pasture and are resistant to moderate desiccation, disinfectants and low temperature (4-5^{o}c) (Doyle *et al*., 1997).

Figure 5. Egg of Taenia species



2.2.3 Metacestodes or cysticerci

The larval stages, or metacestodes also referred to as "beef measles", are found in all striated muscles of the intermediate host. *C. bovis* is a small (pea-sized) oval in shape (O.I.E., 2000), semi-translucent cyst filled with dense white fluid containing an invaginated scolex. The metacestode is morphologically similar to the future adult tapeworm. It measures about 10 mm in diameter and 6 mm in length (Doyle *et al*., 1997). When incised, the cyst may be viable containing a thin fibrinous capsule or degenerate showing cream or green colored calcification (O.I.E., 2000). The cysticerci are formed over a period of 3-4 months after the egg is ingested. This form may remain viable in the intermediate host for up to 9 months or even up to the entire life of the host (Harrison and Sewell, 1991; Gracey, 1981). In the carcass *C. bovis* can survive for about 15 days at-5^{o}c, 9 days at –10^{o}c and 6 days at-15^{o}c to -30^{o}c (Harrison and Sewell, 1991;). If a carcass is found to contain cysts, it is required to be frozen at –10^{o}c for 10 days, or if the lesions are extensive, the entire carcass is condemned (Yoder *et al.,* 1994).

2.3 Epidemiology

*Taenia saginata,*Taeniasis occurs throughout the world with variable degree of prevalence (Harrison and Sewell, 1991). In the world there are 77 million bovine Taeniasis patients of which 32 million are in Africa, 11 million in Asia (excluding the former USSR) and about 3 million in the new world. Its prevalence could be classified into three groups (Doyle,*et al*., 1996; Frolova, 1982; Minozzo, *et al*., 2002).

a. High prevalence with Taeniasis exceeding 10%
b. Moderate infection rates (0.1-10%).
c. Low infection rate less than 0.1%.

Highly endemic areas include Central and East African countries (Ethiopia, Kenya, and Zaire), Argentina, Caucasian and South Central Asian republics of the former USSR and in the Mediterranean Region (Syria, Lebanon and Yugoslavia) (Florova, 1982). In some parts of Serbia and Montenegro, up to 65% of children have been reported to harbor *T. saginata* (Florova, 1982). Moderate prevalence is encountered in South East Asia (Thailand, India, Vietnam and Philippines), Japan as well as countries of Western Europe and South America while Canada, the USA, Australia and some countries of the Western Pacific have low prevalence (Harrison and Sewell, 1991).

In developing countries, cattle are reared on extensive scale, human sanitation is of comparatively lower standards and the inhabitants traditionally eat raw or inadequately cooked beef. The prevalence of Taeniasis is over 20% in certain areas of these countries. Based on routine carcass inspection the infection rate of bovine cysticercosis is often around 30-60% although, the real prevalence is considerably high (Tembo, 2001). *T. saginata* infections also occur in developed countries, where standards of sanitation are high and meat is carefully inspected and generally thoroughly cooked. Taeniasis/cysticercosis spreads in developed areas of the world through tourists enjoying the consumption of lightly grilled meat, mass migration of labor and the export

of meat unreliably passed by ‘’eye or knife’’ inspection or from live animals imported from endemic areas (Mann, 1984). Prevalence in these parts of the world is less than 1%. Occasionally, however, cysticercosis “storms” have been reported on particular farms. The cause of the storm has been attributed to the use of human sewage on pasture and the use of migrant labor (O.I.E., 2000). In developed countries, cattle of any age, are susceptible to infection since they generally posses no acquired immunity (Yoder *et al.,* 1994).

A high prevalence of *T. saginata/Cysticercus bovis* occurrs in Africa where cattle are kept in community grazing lands. The parasites appear to be specific to cattle, while wild animals play no part as intermediate hosts (Symth, 1994).

2.3.1. Mode of infection

Human feeding habits and modes of life are responsible for the spread of *T. saginata* infections. Man’s customs and traditions of consuming raw, sun-cured, inadequately cooked beef dishes like *steak tartar* in Europe, *shish kebab* and *tikka* in India, *shashlik* in the former USSR, *Ihab* in Thailand, *Yukhoe* in Korea and *kourt and kitffo* in Ethiopia containing viable bladder worms perpetuate human infection. Cattle are infected by ingestion of pasture and drinking water contaminated with *T. saginata* eggs, (Florova, 1982; Teka, 1997).

Dispersion of *T. saginata* eggs is favored by the following factors:

- Man’s indiscriminate defecation.
- The use of sewage effluents and sludge as fertilizer on pasture, the use of immigrant labor from countries with high prevalence of infection in feedlots.
- Scavenger birds (seagulls), earthworms, dung beetles, blowflies, oribatid mites, flooding water, etc.
- Age of the animal (Fertig *et al*., 1985; Symth, 1994).

2.3.2 Host range

Cattle are the preferred intermediate hosts and humans are the only final hosts of *T. saginata*. Cattle of all ages are susceptible; however young age groups are more susceptible. Parasitism is sometimes observed in other ruminants (sheep, goats, antelopes, gazelles, buffaloes) but cysticercus development is unlikely. Man cannot spread taeniasis to his own species. Management of animals in their natural environment predisposes them to infection. Cattle

grazing communally have a higher risk of picking up *T. saginata* eggs since they are frequently in contact with human feces compared to commercial herds, the risk of cattle coming into contact with *T. saginata* eggs is much higher when cattle are at pasture (Harrison and Sewell, 1991).

In developing countries cattle are reared on extensive scale, human sanitation is poorly developed which makes the incidence of *T. saginata* infection in humans very high. Calves are infected usually in early life, often with in the first few days after birth from infected stockmen whose hands are contaminated with Taenia eggs (Fertig *et al.,* 1985; Maedia *et al.,* 1996).

In Africa inadequate education of population and low accessibility to safe taenicides has favored the spread of *Taenia saginata* (Pawlowski, 1996). The prevalence of *C. bovis* in some selected African countries shows significant variation (table 1).

Table 1: Prevalence of *C. bovis* in some African countries

Country	**Prevalence in %**	**Source**
Zambia	6.1	Dorny, 2002
Namibia	6.2 communal, 2.3 commercial	Kumba, 2001
Egypt	0.23 in native cattle, 7.25 in imported cattle	Haridy, 1999
Kenya	33.02 14-18.2	Onyango-Abuje, 1996 Florova, 1982
Zaire	22.3	Florova, 1982
Chad	6.67	Florova, 1982
Nigeria	10.2	Florova, 1982
Ethiopia	2.2-3.2 3.2	Teka, 1997 Tembo, 2001

2.4 Life cycle

After the eggs are deposited in the soil or vegetation they are ingested by cattle or other herbivores. In ruminants, the thick embryophore of the ova remains un- affected in its passage through the first three compartments of the stomach. On reaching the abomasum it is exposed to the action of pepsin, which destroys the cementing substance (Symth, 1994).

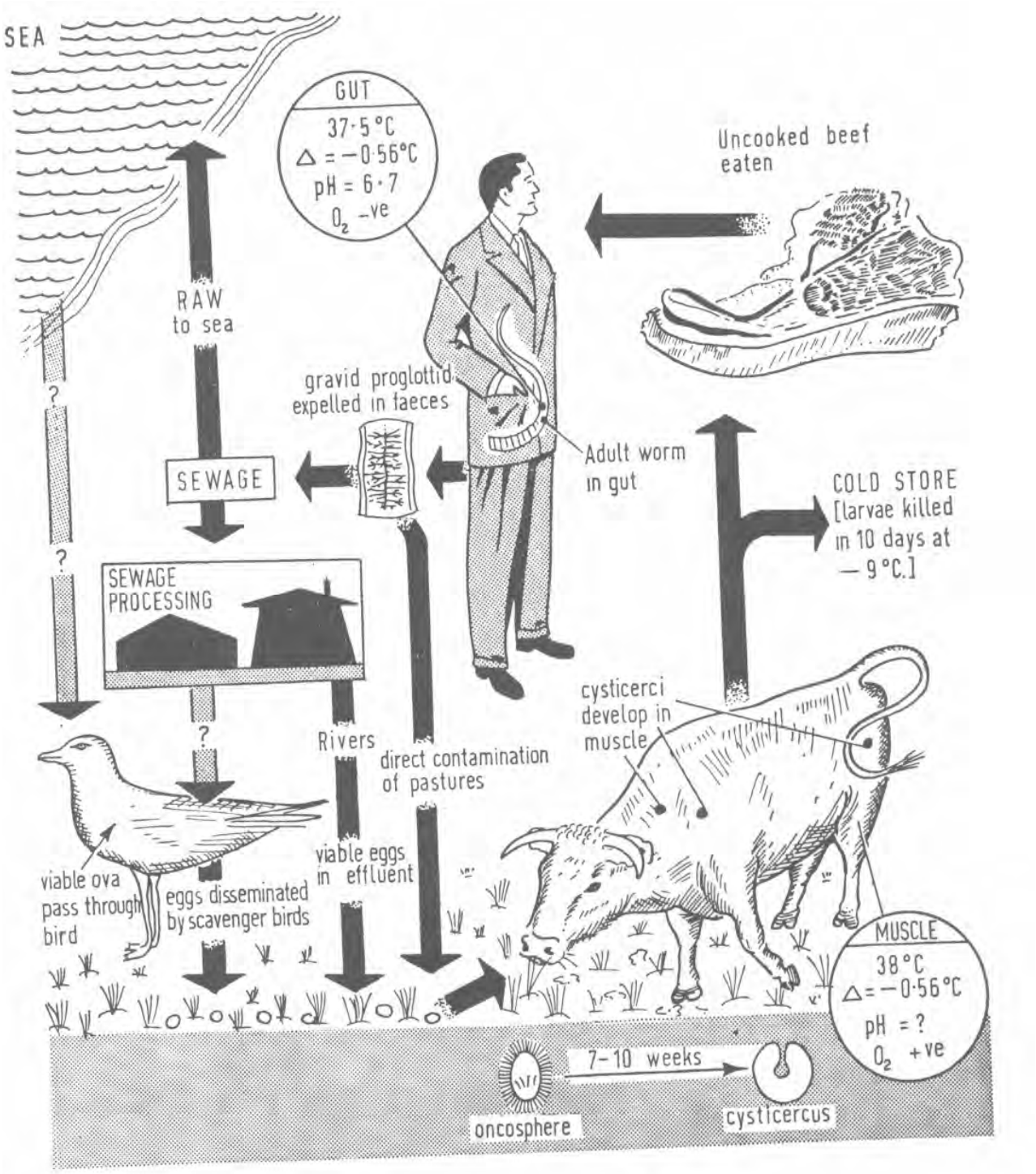
In the duodenum it is further affected by the pancreatic secretion and disintegrates releasing the onchosphere still contained within its ellipsoidal onchospheral membrane (Brown and Neva, 1983; Harrison and Sewell, 1991).

Histolytic secretion released by the onchosphere assists in invading the intestinal epithelium and is carried by vascular channels to the straited muscles in the hind limb, diaphragm and tongue. Here it is filtered out and transformed into an ovoid bladder warm or cysticercus over a period of 3-4 months. This form, which may remain viable for 9 months or more, measures about 5 mm by 10 mm and consists of a scolex held in a cyst like structure (Zivkovic *et al.* 1996; Teka, 1997; Harrison *et a*l, 1997; Loget *et al*, 1997; O.I.E, 2000; Santos *et al*., 2001).

Man ingests the cyst in raw or under cooked beef where the parasite develops to adult stage in the small intestine and completes the life cycle (Hancock *et al*., 1989). After ingestion of the cyst in raw or under cooked beef by humans about two months is required for the adult worm to develop in the intestine (Symth, 1994).

The adult parasite possesses unarmed scolex with four prominent suckers and between 600 - 2000 proglottids. When gravid proglottids come to rest on the ground, eggs extruded. The eggs are also present as a result of promiscuous defection (Soulsby, 1982; Brown and Neva, 1983; Teka, 1997). In recent review of *Taeniasis/Cysticercosis* control concluded that the difficulty in eradicating *T. saginata* may lie in their high biotic potential (Brown and Neva, 1983).

Figure 5: The life cycle of *Taenia saginata*



Source: Symth, 1994.

2.5.Clinicamanifestations

a. In man

The clinical manifestations in humans include abdominal pain, nausea, debility, weight loss, flatulence and diarrhea or constipation. A patient may have one or several of these symptoms and a high percentage of patients experience gastric hyposecretion. Individual reactions to the infection differ and may be influenced by psychogenic factors, since patients often notice symptoms only after they see proglottids (Symth, 1994). Signs like those of epigastric discomfort, hunger sensations and irritability were also observed in infested individuals (Harrison and Sewell, 1991).

b. In Animals

Light or moderate cysticercosis in cattle is not usually associated with any defined clinical picture. Heavy infections, those induced experimentally by 200,000 to 1,000,000 *T. saginata* eggs, may give rise to fever, weakness, profuse salivation, anorexia, increase heart and respiratory rate and a dose of one million or more eggs may cause death between 14 to 16 days due to a degenerative myocarditis (Oryan *et al.,* 1998).

2.6. Diagnosis

Definitive diagnosis is based on identifying the proglottid, since the eggs of *Taenia saginata* cannot be distinguished from those of other species of Taenia. The gravid proglottid of *T. saginata* has 15 to 35 lateral branches of the uterus on each side of the main uterine stem (Harrison and Sewell, 1991; Teka, 1997) a characteristic feature (Figure 3). If the gravid proglottid is treated with 10% formaldehyde and injected with india ink the uterine branches are very prominent. Uterine branches also can be seen by gentle pressing the proglottid between two microscope slides and holding them in front of a bright light (O.I.E., 2000). If the scolex is present, the four characteristic hookless suckers can be used as a distinguishing feature for identification (Symth, 1994).

The development of DNA probes has made it possible to distinguish *T. saginata* from *T. solium*. Sensitivity of serological tests varies depending on the particular method and the clinical form of infection (Doyle *et al.,* 1997). A “dip stick” technique based on an antigen capture ELISA, to detect coproantigens in feces has been developed for *Taenia* species in humans (Zarlenga *et al.,* 1999).

The metacestodes are readily visible in the organs or musculature at autopsy and therefore; diagnosis of bovine cysticercosis is usually made during postmortem examination in abattoirs and packing plants (Brown and Neva, 1983; Zivkovic *et al.,* 1996; Moreira *et al.,* 2001; Kumba *et al*., 2001; Reis *et al.,* 2000; Al-Sultan *et al*., 1998; Manhoso *et al.,* 1996; Gracey, 1981). Individual countries have different regulations regarding the inspection of carcasses, which usually attempts to reconcile the interests of owners and those of the consumers (Harrison and Sewell 1991; Gracey, 1981).

Meat inspection relies exclusively on visual examination of the intact and cut surfaces of the carcass (eye-and-knife method) in the slaughterhouse by meat inspectors who follow officially laid-down procedures (Yoder *et al.,* 1994). Individual countries have different regulations regarding the inspection of carcasses, but invariably the masseter muscle, tongue, and heart are

incised and examined. Several of these are also the sites at which the largest concentration of metacestodes is found in experimentally infected animals.

Diaphragm, muscles of the hind limb, liver, esophagus, lungs, kidneys, spleen and intercostal muscles are potential sites for cyst location (Dorny *et al*.,2000; Maeda *et al*., 1996). Classical meat inspection techniques cannot detect all of the carcasses infected with cysticerci (Dorny *et al.,* 2000; Harrison *et al*., 1997).

The effectiveness of meat inspection in the detection of *C. bovis* depends on the procedure used. The following are laid as normal routine inspection of carcasses by the Ministry of Agriculture in Ethiopian Meat Inspection Regulation Notice Number 428 of 1972 and the Meat Control Act of Kenya (MOA, 1972).

These are:

- Visual inspection and palpation of the surfaces and a longitudinal ventral incision of the tongue from the tip of the root.
- One deep incision into the triceps muscles of both sides of the shoulder
- Extensive deep incision into external and internal muscles of masseter parallel to the plane of the jaw.
- Visual inspection and longitudinal incision of the myocardium from base to apex. But more incision can be made when necessary.
- Visual inspection and 3 parallel incisions into long axes of the neck muscles on both sides
- Two parallel incisions on the thigh muscles of both hind legs
- Careful inspection, palpation and two parallel incisions into the diaphragmatic lobes of the lung through the lung substances.
- Visual examination of intercostal muscles and incisions when necessary
- One extensive incision into the fleshy part of diaphragm; visual examination, palpation and incision of kidneys, liver, oesophagus and associated lymph nodes.

However, minor infections are difficult to detect irrespective of laws and the skill of the inspector. If a *Cysticercus* is found in any of these sites and organs, thorough inspection of the whole carcass and offal should be done. The location, nature and number of cysts should be recorded (MOA, 1972).

2.6.1 Differential diagnosis

Differentiation of Taenia species is important in order to relate particular species and hence correctly determine the prevalence and incidence rates associated with each species. There are a number of methods to differentiate Taenia species. These are: morphological characteristics of the scolex in the adult tapeworm, the number of lateral branches of the uterus in the gravid proglottids, ovary and vagina; site of *Cysticerci* development and preferred intermediate hosts (Symth, 1976; Symth, 1994).

In cattle *C. bovis* should be differentiated from:
Cysticecus dromedarius (C. cameli) the larval form of *Taenia hyaenae.* The identification of *C. cameli* is by double row of hooks on the lateral invaginated scolex and its length being twice as large as *C. bovis* measuring 12-18mm in length and pearly white in color (Urquhart *et al*., 1996).

Sarcocystis bovifelis (Sarcocystis hirusta), which is a soft bradizoite cyst very large and visible to the naked eye whitish streaks running in the direction of the muscle fibers. The cyst ranges from 0.5mm to 5mm in length, localized in the esophagus, heart, in different muscular tissue (Minozzo, *et al*., 2002; Urquhart *et al*., 1996; Brown, 1995).

Onchocerca dukei measurs 3mm to 6mm in diameter, forms intra-muscular and subcutaneous nodules that are firm to touch and reveals worms surrounded by pus when sectioned (Tembo, 2001).
In humans *Taenia saginata* should be differentiated from the other two species of Taenia. These are:
Taenia solium, The adult *T. solium,* like that of *T. saginata*, is exclusively a parasite of human beings where the adult strobila develops in the intestine. The larval stage, *Cysticercus cellulosae* occurs in pigs and wild boars, but man can also be infected with serious and often fatal results (Giordano, 2003).

Asian/Taiwan Taenia (*Taenia saginata asiatica)* The larval form has a wider range of hosts than *C. bovis* (cattle, pigs, goats, wild boar, monkeys). Speciation of this parasite is in doubt as it shows some characteristics of both *T. saginata* and *T. solium* (Symth, 1994).

Table 2: Characteristics for differentiating *T. saginata, T.saginata asiatica* and *T.solium.*

Characteristic	***Taenia saginata***	***Taenia saginata asiatica***	***Taenia solium***
Intermediate host	Cattle, reindeer	Pig and wild boar, cattle, goat, monkey	Pig, wild boar
Development site	Muscle, viscera, brain	Mainly liver	Brain, skin, muscle
Scolex: Suckers Rostellum Hooks	 4 Absent Absent	 4 Present Present	 4 Present Present
Mature proglottids: Ovary Vaginal sphincter Egg size	 2 lobes Present 40x50 micrometer	 2 lobes Present 33x28 micrometer	 3 lobes Absent 40x50 micrometer
Cysticercus size	10 mm by 6mm	1,320 micrmeter x 3,219 micrometer	20 mm by 10 mm
Gravid proglottids: Uterine branches Passing of proglottids	 23(14-32) Spontaneously, singly	 17(12-26) Spontaneously, singly	 8(7-11) Passively in groups

Source: Symth, 1994

2.7 Prevention and Control

Lack of and improper use of latrine or open field defecation leads to contamination of grazing lands. The use of latrine reduces spread of *T. saginata* eggs. Controlled grazing, avoiding use of sewage effluent to fertilize pasture, prevents infection in cattle (Symth, 1994).

Adequate meat inspection, abstinence from eating raw or inadequately cooked beef (thorough cooking of meat at a temperature of 56 - 60^0c) and freezing the infected carcass at -10^0 c for 10 days prevent human infection. Chemotherapy in humans reduces the spread of eggs and infection in cattle (Solusby, 1982).

2.7.1. Treatment

There are a number of taenicidal drugs available in the market. However the drug of choice in treating Taeniasis is niclosamide (Niclocide, Yomesan). Adult dose rate of 2000 mg is effective in damaging the worm to such an extent that a purge following therapy often produces the scolex. Praziquantel (Bilitricide) at a dose rate of 5 to 10 mg per kg also has been reported highly effective (Doyle *et al.,* 1997) but the scolex is partially digested and often not recovered (Symth, 1994). Other drugs used in the treatment of *T. saginata* are mebendazole (Soulsby, 1982; Doyle *et al*., 1997) followed by purgative, for example magnesium sulphate (MSO_4) to expel the dead worms in toto (Soulsby, 1982).

In animals treatment with compounds such as albendazole (50mg per kg), praziquatel (50mg per kg), mebendazole (50mg per kg) can be given but they are considered not to be fully effective (Symth, 1994; Soulsby, 1982). Praziquantel is effective at 50mg/kg/day for four days but this treatment is impractical because of its high cost (Reinecke, 1983).

Recombinant vaccines have been developed using non-living antigens of the parasite, host protective responses can be induced readily in the intermediate hosts, may be used to control in cattle (Lightowlers *et al*., 1998).

The commonly used medical herbs in decreasing order of preference based on toxicity, higher potency and shorter worm expulsion time are: *Embelia schimperi, Cucurbito pepo, Thymus serrulatus, Hagenia abyssinica, Myrsine africana, Maesa lanceolata, Cynodon dactylon, Echinopis gigantean, Glinus lotoides, Silen macrosclen and Plantago lanceolata (*Kloos*, et al.*, 1978; Tembo, 200). The local and scientific names and the part of the plan used for treatment are listed (table 3).

Table 3: Traditional anticestodal drugs

Local name	**Scientific name**	**Part of plant**
Kosso	*Hagenia abyssinica*	Flower
Enkoko	*Embelia schimperi*	Fruit
Metre, Amkint	*Glinus lotoides*	Seed
Duba fre	*Cucurbita pepo; the pumpkin*	Seed
Gortteb	*Plantago lanceolata*	dn
Wogert	*Silen macrosclen*	Root
Dendero	*Echinopis gigantean*	dn
Serdo	*Cynodon dactylon*	dn
Kkelewa	*Maesa lanceolata*	dn
Tossigne	*Thymus serrulatus*	dn
Kkettechemo	*Myrsine africana*	dn

Source: Kloos*, et al.*, 1978 dn=Don't know

2.8 Zoonotic Importance

Man is the only final host where the adult *Taenia saginata* resides in the small intestine. The size reached by the adult worm is related to the number of worms present (Maeda *et al.,* 1996). In a single worm infection, a worm can develop longer and produce large number of proglottids (Smithy, 1994). Multiple infections upto 20 tapeworms in one host is often occurring in developing countries (Mann, 1984). The effect on human health is generally slight and symptoms may be vague or absent. Taenia has a debilitating effect on people who already have live of protein deficient diets suffer from iron deficiency and infected by hookworm etc. (Mann, 1984). The most noticeable symptom is the spontaneous discharge of one or several proglottids, which often show individual muscular activity. These may creep out of the anus onto the perianal skin and even migrate over clothes of the distraught host or on the ground, shedding eggs as they go (Reinecke, 1983).

Taeniasis causes various symptoms, which probably depend very much on the psychological and physical characteristics of the host. Some patients lose their appetite and thus lose weight while others tolerate the infection (Florova, 1982; Bessenovo, 1982). Sometimes the gravid proglottids of *Taenia saginata* migrate to different organs appendix, pancreatic duct, nasopharyngeal pathways and bile ducts producing obstruction and inflammation of the affected organs (Florova, 1982). Tapeworms can also cause intestinal obstruction (Doyle *et al*., 1997).

Taenia saginata in the small intestine of man absorbs digested food. From the day the cysticercus is ingested it may take 2-3 months for the parasite to produce ripe segments. As long as the scolices are attached to the intestinal mucosa of the victim new segments will continually grow to replace those, which are being detached from the worm (Teka, 1997).

2.9. Economic Importance

Attempts to reduce the prevalence of *T. solium* and T.*saginata* in humans and their cysticrci in animals (pigs, cattle) may have a considerable impact on the economics of meat production industries. Cysticercosis in domestic animals is a significant food safety problem and causes economic loss in food production. This will be particularly important where export industries are involved, since most importing countries have stringent regulation designed to prevent the importation of infected meat (Harrison and Sewell, 1991).

The cost implication can be broken down into those involved in treating human taeniasis and cattle carcasses (costs of freezing, boiling) or condemned, as well as the costs involved in the inspection procedures amount to millions of dollars (Mann, 1984). An annual losses due to treatment in USA was USD 100, 000 (Robert, 1995), in South Africa USD 428 million (Abdusslam, 1975). In Kenya and Botswana bovine cysticercosis resulted in annual losses of USD 4 million and USD 2 million respectively (Grindle; 1978). This mainly arose from the loss of value in abattoirs resulting from boiling the meat to kill the cyst, as the presence of cysticerci in the meat would be a serious obstacle to meet the import regulations of the recipient countries (Gracey, 1980). In feedlot cattle, the incidence may be as high as 40% or as low as3% (Reinecke, 1983).

Carcasses of cattle including the viscera infested with *Cysticercus bovis* shall be condemned if the infestation is excessive or if the meat is watery or discolored. Carcasses shall be considered excessively infested if incision in various parts of the musculature exposes on most of the cut surfaces (Hubert, 1974). Time regression analysis revealed a progressive increase in the incidence of taeniasis/ cysticercosis, which were related to a demographic increase (Reis *et al.,* 1996).

3. MATERIALS AND METHODS

3.1 Study area

Addis Ababa and its peri-urban areas have 62,166 bovine, 22,647 ovine, 7,531 equine, 5,597 caprine and 330,000 avian species. Addis Ababa Abattoir was established in 1956 as a private share company and was taken over by the government during the Derg regime. Now it is under Addis Ababa Municipality.

The main purposes of the Abattoir are processing of one or several classes of livestock into fresh meat for human consumption, hygienic processing and storage of meat and edible by-products, exercise close control over environmental conditions at all stages of processing and breakdown the transmission of zoonotic meat borne diseases through meat inspection.

At Addis Ababa Abattoir cattle, sheep, goats and swine are slaughtered and animals for slaughter come from different regions of the country. Daily 700 cattle, 250 sheep, and 75 goats are slaughtered. About 50 pigs are slaughtered per week and the source of pigs are Addis Ababa, Debre Zeit and Zeway farms. On average 153,000 cattle, 39,000 sheep, 3,200 goats and 750 pigs are slaughtered annually.

The Abattoir provides fresh meat for different institutions such as hotels, hospitals and butcheries. The Ministry of Agriculture (MOA) conducts the meat inspection procedure. The Abattoir, apart from fresh meat, produces meat and bone meal, tallow, glue, horn, hide and skin and tail. These products are distributed and/or sold to butcheries, enterprises, tanneries and soap factories.

Repi PLC Dairy Farm is located in southern Addis Ababa. The farm has 126 cattle, 125 cows and 1 bull. The animals are mostly house fed with occasional grazing in the restricted farm area. Hay is brought about 10km away from Addis Ababa and concentrate from Kaliti animal fed industry. The farm produces milk for sale and male calves are sold for veal. Replacement stock

is bred in the farm. Deworming is done every three months and additional veterinary service is delivered based on laboratory results. The farm has four permanent labor personnel and one assistant veterinarian. Hygiene facilities such as latrine, washing places, etc are available.

Yeka-abado Farmers Association is located about 10km away from Addis Ababa in the northern direction. Grazing and watering sites are communal. The inhabitants, living nearer to Addis Ababa, do have easy access to butcheries to eat meat raw or lightly cooked meat. There are no latrines in the area, bush defecation is practiced and animals often get in contact with human feces. Farmers visit the veterinary clinic once or twice in a year when they observe health problems in their animals.

3.2 Study animals

The study was conducted on 743 cattle from Addis Ababa Abattoir, Repi PLC Dairy Farm, Yeka-abado Farmers Association. The negative control sera (11) were obtained from Department of Parasitology, Toulouse, France. Serological tests were conducted on all animals. whereas coprological examination was conducted on 732 animals. Postmortem inspection was conducted on 522 cattle slaughtered at Addis Ababa Abattoir, which originate from neighboring localities and/or regions such as Orromia, Amhara, Souther Nation and Nationalities etc. 101 cattle from Repi PlC Dairy Farm and 109 cattle from the Yeka-abado Farmers Association were also used for the study. Particular attention was given to factors such as sex, age and body condition of the animals. Serum from naturally infected animals in the slaughterhouse with confirmed *Cysticercus bovis* cyst was used as positive control.

3.3 Study Design

Postmortem inspection, fecal examination and serological tests (Indirect ELISA and IHAT) were conducted. During the study period a total of 732 animals and 11 negative control sera were tested. From these 732 animals fecal samples for sedimentation and flotation techniques and blood for serological tests (Indirect ELISA and IHAT) were collected.

3.3.1. Postmortem inspection

Postmortem inspection was made on each of 522 animals slaughtered at Addis Ababa Abattoir where feaces and blood was collected. During postmortem inspection meat inspectors made the incision according to MOA (1972). The hearts of *C. bovis* infected animals were thoroughly inspected incised at 5 mm diameter.

3.3.1 Fecal examination

From those 732 animals fecal samples for sedimentation and flotation techniques were collected. The feaces was collected directly from the rectum and taken to the laboratory with tightly closed universal bottles and processed according to method described by Kofmanns (1996) and Sloss, *et al*.,(1994).

3.3.2. Serological tests

Blood samples were taken from the study animals and the serum separated using the method described by Dorny *et al* (2002) and kept at -20^0c until tested. For the serological tests two standard control sera were established; a strong positive and negative standard. These standards were used to determine the detection range and analytical sensitivity of the test method. Tests determined in this way are more likely to exhibit a diagnostic sensitivity and specificity. Comparisons were made between postmortem inspection and serological tests, IHAT and Indirect ELISA. Comparison was also made for the presence of cross reactivity with other gastrointestinal helminths and IHAT.

3.3.4. Preparation of Antigen

Cysticercus bovis infected tissues/organs were collected from Addis Ababa Abattoir and the cysts, dissected from the muscles were placed in petridish and washed intensively with saline. Some of the cysts were punctured using dissecting needles to obtain cyst fluid and protoscolices.

The intact cysts were ground using mortar and pestl (autoclaved at 121^0c for 15 minutes and cooled to -20^0c). A small amount of distilled water was added and the material was further

homogenized using tissue grinder and sonicator. The homogenate was centrifuged with suprafuge 22 at a speed of 10000rpm, temperature 4^{o}c and time 35minutes. The supernatant and sediment were separated into different Nunc® Cryo tubes and stored in liquid nitrogen. The fluid (F), membrane (M) and the scolices (S) were also similarly harvested, placed in separate vials and stored in liquid nitrogen.

Protein concentration was determined using the Lowry method (Annex1). The saline extract was used as antigen to coat the ELISA plates for indirect ELISA and sheep red blood cells for indirect hemagglutination tests.

3.3.5. Equipment and reagents for serological tests

Equipment and reagents used are listed in Annex 3 and 4. Different solutions prepared and used in the tests (Annex 5).

3.3.6. Procedures for serological tests

3.3.6.1. Indirect ELISA

The optimal dilution of the serum, antigen, conjugate and H_2O_2 were determined by checkerboard titration. The cut-off OD value was calculated according to IAEA (1992).

The assay involved coating of polystyrene ELISA plates (costar®) with 50µl of antigen at a protein concentration of 5µlgml^{-1} in carbonate-bicarbonate buffer at PH of 9.6 per well; incubation was overnight at 4^{0}c. After washing four times with 0.1% Tween-20 in PBS (PBS-T) blocking was done with 75 µl per well 1%BSA in PBS (1h, 37^{0}c) and washed four times. 50 µl of test sera diluted 1:100 were added and incubated (1h, 370c). Each sample was tested in duplicate. On each plate thirty positive serum samples from cattle with *T. saginata* cysticercosis infection and ten serum samples from *T. saginata* cysticercosis free cattle (negative control) was analyzed. Washed four times and 50 µl goat anti-bovine IgG conjugated to horseradish peroxidase enzyme diluted 1:3000 was added per well and incubated (1h, 370). After washing four times substrate solution (OPD, CPB, H_2O_2) 50 µl was added per well and incubated for 30

minutes in darkness. To stop the reaction 2.5N sulphuric acid 25 µl per well was added. Color development was measured using titertekmultiscan spectrophotometer at 492 nm wavelengths.

3.3.6.2. IHAT

Formalinization of SRBCs

Sheep blood was aseptically collected by venipancture into equal volumes of Alsever's solution (Alsever's solution pH 6.1, adjusted with 10% citric acid). The fresh red cells in Alsever's solution was washed five times with phosphate buffer saline (PBS) pH 7.2 and packed. 25ml of packed cells suspended with 200ml PBS (pH 7.2) and placed in 500ml conical flask. Then 50 ml of commercial formalin (40% formaldehyde) was introduced in to a length of dialysis tubing, which is then tied off so that air is excluded, the tube was only two-third full. The filled dialysis tube was submerged into the red cell suspension. The flask was placed on a shaker and gently agitated at 1000rpm at room temperature. After three hours the swollen dialysis sac was punctured, to allow the formalin to escape and the tube is removed removed. Gentle mixing was then continued for overnight. The dark brown suspension produced was filtered through muslin to remove seam and debris. It was washed with PBS (pH 7.2) five times to remove the formalin and stored at 4^0c until use.

Sensitization and coating of sheep red blood cells

One hundred µl of 25% glutaraldehyde was mixed with 0.5 ml packed SRBCs in 10ml PBS (pH 7.2) and this mixture incubated at 37^0c water bath for 20 minutes. Centrifuged at 2000rpm for 5 minutes at 4^0c (GPR Beckman GPR Centrifuge) removed the supernates and resuspend with 10ml PBS (pH 7.2) and wash two times with 10ml PBS (pH 7.2) and packed.

Coating involves gentle mixing of 250µl antigen at a concentration of 5mg/ml to sensitized 0.5ml SRBCs in 4ml PBS (pH 6.4). The mixture was incubated at 37^0c water bath for 30 minutes, by shaking twice. This mixture was centrifuged and washed three times with PBS (pH 7.2) containing 1% fetal calf serum (FCS) and finally suspended in the same serum saline to 10ml. This gives 5% suspension ready to use for the test. Centrifugation was done using

BECKMAN GPR Centrifuge made in UK at speed of 2000 rpm for 5 minutes at a temperature of 4^0c.

Equal volume of test sera and uncoated sensitized SRBCs were incubated in water bath at 37^0C for 20 minutes and centrifuged at 2000 rpm for 5 minutes using Biofuge A centrifuge. This is to avoid non-specific (group agglutinins) reaction.

FCS (fetal calf serum), stabilizer, heated to 56^0c for 20 minutes to destroy complement.

3.3.6.3. Test Procedure

To the V-shape griener microplate® 50 µl PBS (pH 7.2) was distributed to each well except the 2^{nd} column. 100 µl of test sera diluted 1/8 in PBS (pH 7.2) were added to the 2^{nd} column. Serial double fold dilution of sera was made by transferring 50 µl serum to the other wells (1:8, 1:16, 1:32,…,1:4096). Finally 50 µl of 5% sensitized and coated SRBCs was added to the respective wells and kept on the bench undisturbed from 3hrs to overnight at room temperature. The hemagglutination results were read by settling patterns. The end point titre was determined one well before the first well of agglutination ceased to exist identical to the control well of the first column.

3.4. Body condition scoring

Body condition scoring of the study animals was conducted according to Nicholson and Butterworth, (1986) (Annex 2).

3.5. Data analysis

The data entry and cleaning was done using MS Excel and statistical analysis was done using Epi Info version 6 and SPSS version 11.5.

The data obtained from postmortem inspection, fecal examination, body condition, indirect-ELISA and IHAT were entered into MS Excel. Results obtained from postmortem inspection and were compared with that of IHAT using cross tabulation.

The test agreement between PMI and serological tests was compared using the Kappa statistic where Kappa value between 0.8 and 1.0 denotes very good agreement between the assays, a value between 0.6 and 0.8 substantial agreements, and a value between 0.4 and 0.6 moderate agreements.

The non-parametric Kruskal-Wallis test was used for analysis, the data was not normally distributed. Therefore the cut-off for serological tests was calculated taking the median (for indirect ELISA) and 75^{th} interquartile range (for IHAT) as an average. The cut-off for indirect ELISA was calculated as the median absorbance OD value of the negative controls plus two times standard deviation like the method described by IAEA (1992). The 75^{th} interquartile range of the negative control IHAT titre plus three times standard deviation was the IHAT cut-off titre. Descriptive statistics and graphs were also used to compare the cut-off titre and OD in *C. bovis* confirmed and normal control cattle. Histogram was used to describe and compare the titre in tested cattle.

Chi-square tests were applied to see if statistically significant association exists between IHAT titre and other gastrointestinal helminths, management systems (Repi PLC Dairy Farm and Yeka-abado Farmers Association), sex, age and body condition. According to the work of Atlas link inc. coefficient of variation was used to check repeatability and reproducibility of the IHAT.

4.RESULTS

4.1.Postmortem Inspection

Of the total of 522 animals inspected, 39 animals were positive for *C. bovis* at postmortem inspection (PMI). Based on this the prevalence was 7.5% in the abattoir. Thirty-seven of the hearts had viable *C. bovis* cysts whereas the remaining two had calcified cysts. The number of *C. bovis* cyst obtained per heart varies from two to fourteen.

4.2. Fecal Examination

The flotation and sedimentation techniques were conducted on all samples. Prevalence of gastrointestinal parasites from samples in the abattoir, Repi PLC Dairy Farm and Yeka-abado Farmers Association are shown on table 4. The prevalence in the Yeka-abado Farmers Association (32.11%) was significantly different from Repi PLC Dairy Farm (4.95%), $p < 0.05$. No significant difference was observed, $p > 0.05$, between fecal examination results of Yeka-abado Farmers Association and the Abattoir (Table 4).

Table 4: Prevalence of gastrointestinal helminths at Addis Ababa Abattoir, Repi PLC Dairy Farm and Yeka-abado Farmers Association.

Source of cattle	No. Positive	% Positive	Trematodes	Nematodes	Mixed
A.A. Abattoir (n = 522)	210	40.22%	13.02%	27.2%	16.47%
Dairy (n = 101)	5	4.95%	4.95%	0%	0%
Yeka-abado(n = 109)	35	32.11%	13.76%	18.34%	11%
Total, n= 732	250	34.15%	12.02%	22.13%	13.38%

The trematode species identified during fecal examination were Fasciola species and paramphistomum species. The nematodes species identified were Trichostrongylus Oesophagostomum and Trichuris. Mixed nematode and trematode infestation were observed in Addis Ababa Abattoir and Yeka-abado Farmers Association.No nematodes were identified at Repi PLC Dairy Farm.

Out of those 39 *C. bovis* positive animals four, 10.25% infested with Fasciola species and sixteen, 41.02% infested with nematodes (Table 5).

Table 5: Results of fecal examination on *C. bovis* positive animals during postmortem inspection in the Abattoir.

No.*C.bovis* positive animals in PMI	**Sedimentation**		**Flotation**		**Total**	
	Positive	Negative	Positive	Negative	Positive	Negative
39	4	35	16	23	20	19

4.3. Serological tests

Comparisons made between postmortem inspection and serological tests, IHAT and Indirect ELISA indicated that the serological tests were more sensitive than postmortem inspection. Tests made for the presence of cross reactivity with other gastrointestinal helminths and IHAT, demonstrated that there was no or minimal cross reactivity. Development and comparison of serological tests conducted using crude saline extract antigen of *C. bovis* cyst showed that the cyst fluid had better antigenic properties.

4.3.1. Indirect ELISA

The results of indirect ELISA were described (Table 6) and the OD distribution of group I and group III described (Figure 6).

Table 6: Comparison of Indirect-ELISA between *C. bovis* positive Abattoir samples and negative control cattle.

Indirect-ELISA	**G I**	**G III**	**Total**
Positive	39	2	41
Negative	0	9	9
Total	39	11	50

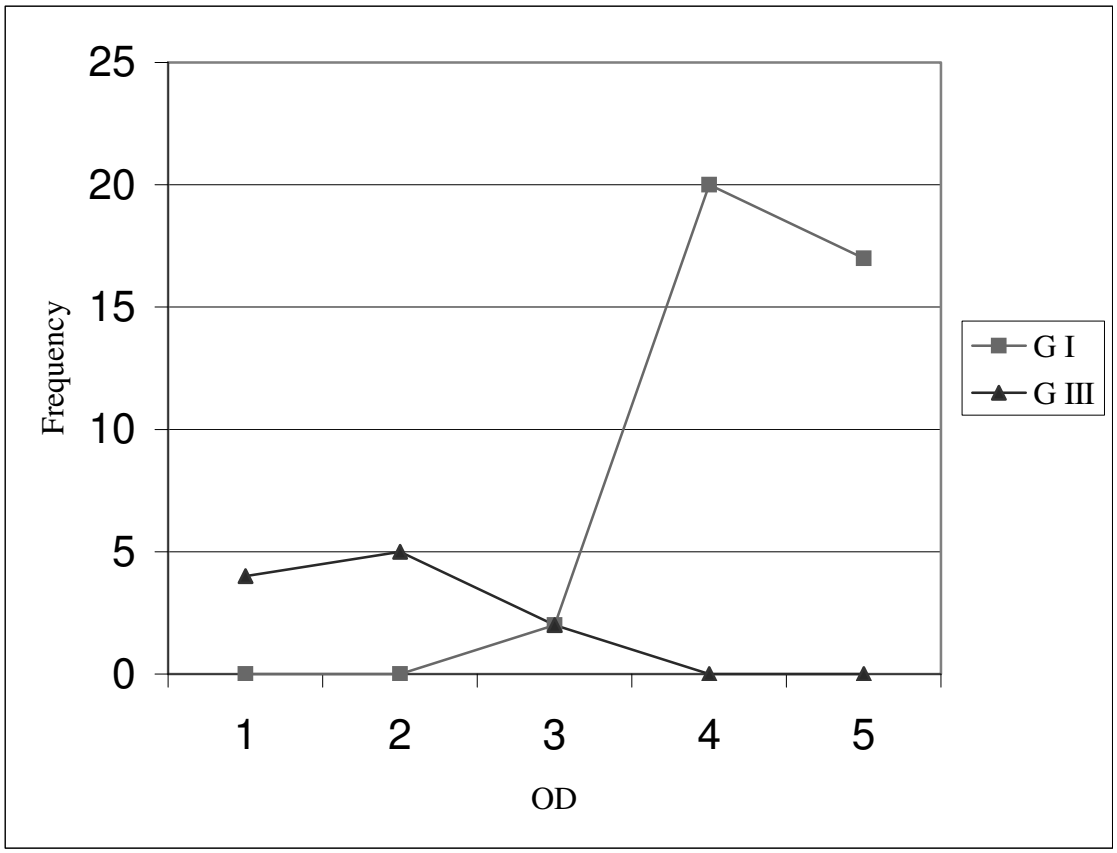
G I = *C. bovis* confirmed cattle

G III = negative control cattle

Sensitivity = 100%

Specificity = 81%

Figure 6: Frequency of distribution of OD of *C. bovis* confirmed and negative control cattle.



1 = 0.4, 2 = 0.6, 3 = 0.8, 4 = 1, 5 = 1.2

G I = *C. bovis* confirmed cattle

G III = negative control cattle

The OD value was measured at 492nm wavelength. The cut-off OD, the median OD of the negative controls plus two times standard deviation, was 0.84.

4.3.2.IHAT

The result of IHAT with confirmed *C. bovis* and known negative sera were described (Table7).

Table 7: Comparison of IHAT between C. *bovis* confirmed and normal control cattle.

IHA	G I	G III	Total
Positive	39	0	39
Negative	0	11	11
Total	39	11	50

G I = confirmed C. *bovis* positive serum

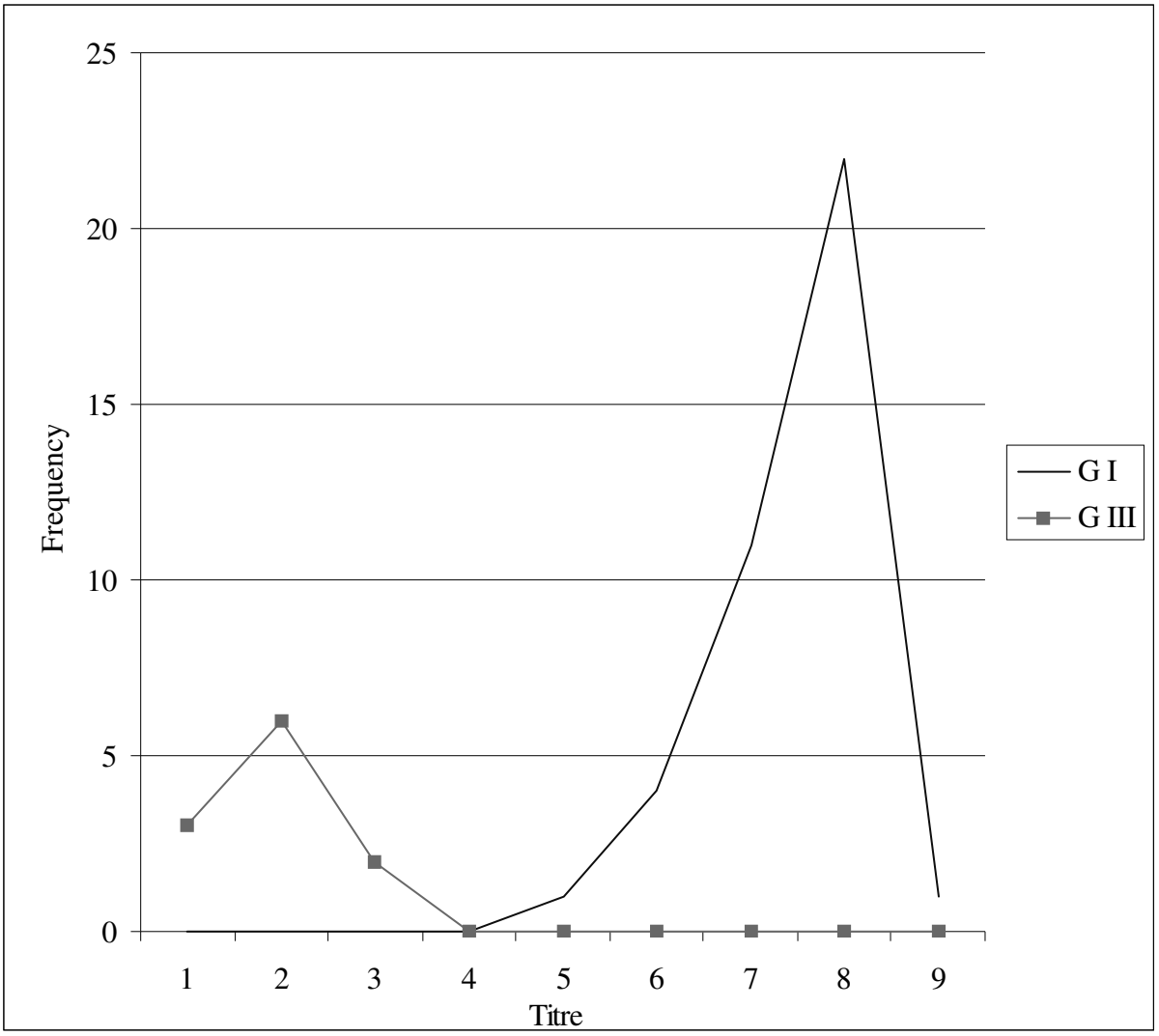
G III=known *C. bovis* negative serum

In this study, for ease of analysis the titres were transformed into small integers and an IHAT titre of 6, as calculated according to Thrusfield (1995), and above were regarded positive (Table8). The IHAT titre distribution in group I and group III poles apart (Figure 7).

Table 8: IHAT titre and transformed integers.

Titre	Integer
1:8	3
1:16	4
1:32	5
1:64	6
1:128	7
1:256	8
1:512	9
1:1024	10
1:2048	11

Figure 7: Frequency of distribution of IHAT of *C. bovis* confirmed and negative control cattle.



In the graph 1 = titre 3, 2 = titre 4, etc. 9 = titre11 of table 9.

G I = *C. bovis* confirmed sera

G III = *C. bovis* negative control sera

Cut-off titre = 75^{th} percentile of the negative controls plus three times standard deviation

4 + (3 x 0.7) = 4 + 2.1 = 6.1, (75^{th} percentile = 4 and SD = 0.7) = 6.

Comparison between postmortem inspection and IHAT result were made for samples collected from Addis Ababa Abattoir (Table 9).

Table 9: Comparison between postmortem inspection and IHAT on samples from Addis Ababa Abattoir

IHA	PMI		
	G I	G II	Total
Positive	39	110	149
Negative	0	373	373
Total	39	483	522

G I = confirmed C. *bovis* positive during PMI

G II = *C. bovis* unknown, postmortem inspection negative.

Kappa = 0.336

Out of 39 animals, infected with *C. bovis* during postmortem inspection and positive for IHA test, 19 were negative by fecal examination for helminth parasites and among those samples negative on postmortem inspection but positive for IHAT 74 were negative by fecal examination (Table 10).

Table 10: Comparison between IHA test and fecal examination negative Abattoir samples.

Fecal negatives	No. Positive	% Positive
Group I, n= 19	19	100%
Group II, n =293	74	25.25%
Total, n = 312	93	29.8%

n = number of animals negative for fecal examination

G I = *C. bovis* confirmed samples

G II = *C. bovis* negative samples during PMI

Comparison between fecal examination positive results and IHAT was conducted for group I group II animals slaughtered at Addis Ababa abattoir. No significant difference was observed, $p > 0.05$(Table 12).

Out of 39 animals, infected with *C. bovis* during postmortem inspection and positive for IHA test, 20 were positive by fecal examination for helminth ova and among those samples negative on postmortem inspection but positive for IHAT 36 were positive by fecal examination (Table 11).

Comparison between fecal examination results and IHAT was conducted for group I group II animals slaughtered at Addis Ababa abattoir. No significant difference was observed, $p > 0.05$.

Table 11: Comparison between IHAT titre and fecal examination positive Abattoir samples.

Fecal positives	**IHAT**	
	No. Positive	% Positive
Group I, n = 20	20	51.28
Group II, n = 190	36	18.94
Total, n = 210	56	26.6

P = 0.13

n = number of animals negative for fecal examination

G I = *C. bovis* confirmed samples

G II = *C. bovis* negative samples during PMI

Comparison between fecal examination results and IHAT was conducted for all samples. No significant difference has been observed in animals infected with gastrointestinal parasites and non-infected cattle on IHA test result, $p > 0.05$ (Table 12).

Table 12: Comparison between IHAT and gastrointestinal parasite infection.

Results of fecal examination	**IHAT**	
	No. Positive	%Positive
Positive, n=250	65	26
Negative, n=482	125	25.9
Total, n= 732	190	51.8

P value =0.12.

The prevalence of *C. bovis* was 7.5%using postmortem inspection and 28.5% using IHA test result in animals slaughtered at Addis Ababa abattoir. The prevalence using IHA test is 3-4 times greater than the conventional method of inspection (Table 9).

The prevalence of *C. bovis* at Repi PLC Dairy Farm and Yeka-abado Farmers Association were compared using IHA test, in Repi PLC Dairy Farm, it was7.9% and at Yeka-abado Farmers Association, it was 30.2%. The prevalence in these two farms is significantly different ($p<0.05$) (Table 13). No significance difference was observed between Yeka-abado Farmers Association

and Addis Ababa Abattoir, where the prevalence was 30.2% and 28.5% respectively using IHAT, $P > 0.05$ (Table 13).

Table13: Results of IHA test using crude *Cysticercus bovis* extract on sera collected from different groups of cattle.

Group	No. Positive	% Positive
I, n = 39	39	100%
II, n = 483	110	22.77%
III, n = 11	0	0%
IV, n = 101	8	7.9%
V, n = 109	33	30.27%
Total, n = 743	190	25.57%

Group I = known *C. bovis* positive

Group II = *C. bovis* negatives abattoir samples during PMI

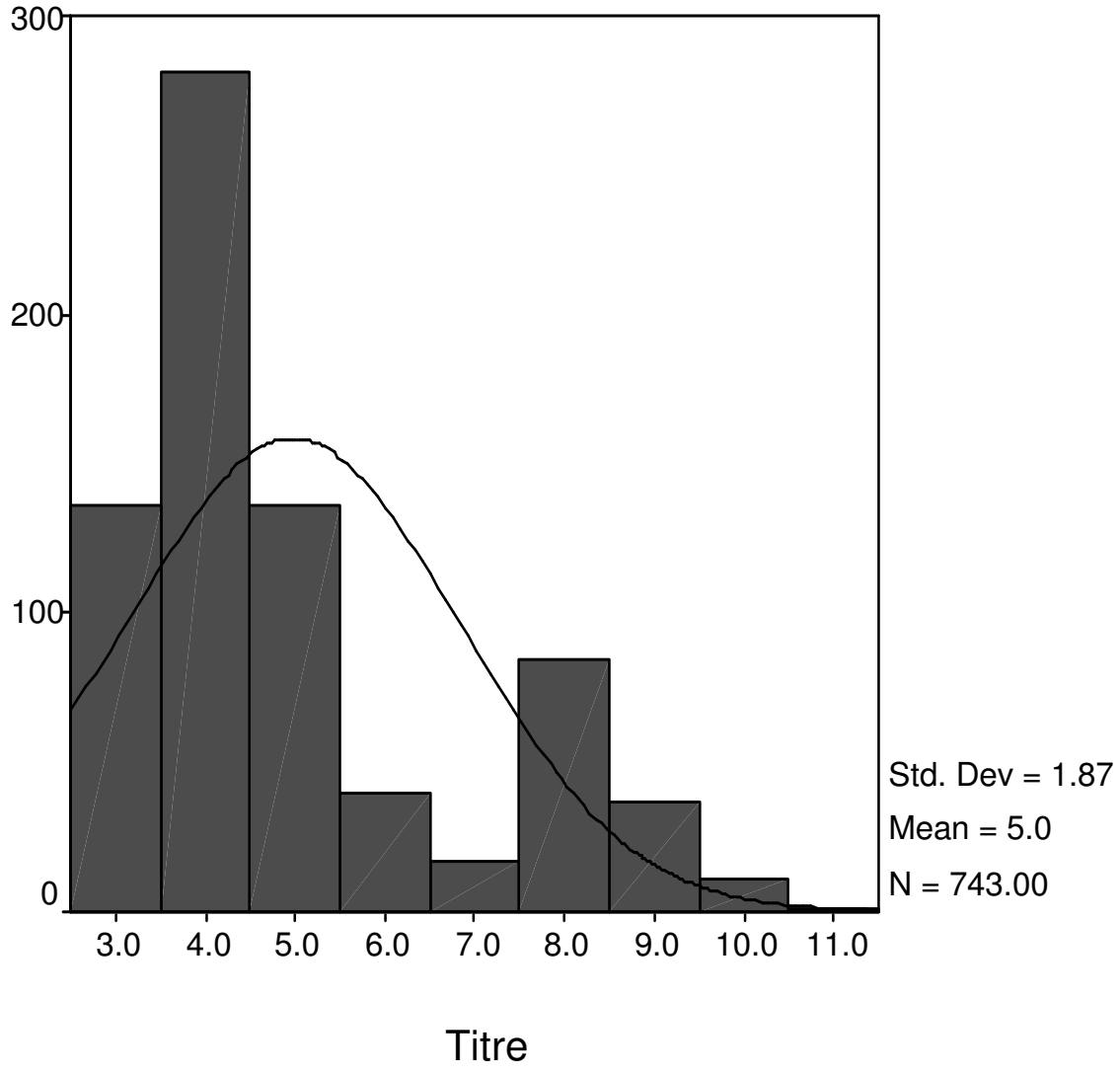
Group III = known *C. bovis* negatives

Group IV = Repi PLC Dairy Farm

Group V= Yeka-abado Farmers Association

The titer in IHA test was evaluated in each group of samples. The highest were in-group I, 1:2048 (2^{11}) seen in one sample. The lowest was 1:8, foud in all groups except in-group I where all samples were with confirmed cases of *C. bovis* during postmortem examination. There is significant variation between groups I, II or V, III and IV ($p< 0.05$). The variation between group II and group V was not significant (Figure 8).

Figure 8: Frequency of distribution of IHAT titres in sera from *C. bovis* infected, unknown samples and negative control cattle.



4.4 Body condition and titre

Body condition score of animals examined was correlated with fecal examination results. Most of the animals positive for flotation and sedimentation techniques had condition scoring less than six, $p < 0.05$. However, no significant difference observed between titre and body condition, $p > 0.05$.

4.5 Antigenicity

Antigenicity of the cyst fluid, cyst scolex and cyst membrane was determined. The test was conducted on known positive and negative samples, as the procedure used for the crude antigen. The cyst membrane did not react to any of the five positive samples, whereas the cyst fluid and cyst scolex discriminated 80% and 60% respectively (Table 14).

Table 14: IHAT titre on the cyst fluid, cyst scolex and cyst membrane of *C. bovis.*

Sample	**Number of IHAT positive (%positive)**		
	Fluid	Scolex	Membrane
PMI Positive (n = 5)	4(80%)	3(60%)	0(0%)
Negative control (n = 3)	0(0%)	0(0%)	0(0%)

4.6. Repeatability and reproducibility

Repeatability of IHAT was determined by running the same positive and negative samples for three consecutive days. The first and second tests were conducted on the same batch of SRBCs whereas the third test was conducted on another batch of SRBCs. The test was conducted on 104 samples in which 101 (97%) of the samples showed the same titre and therefore the test was 97% repeatable.

Reproducibility of the test was determined by running the test on 56 positive and negative samples. Three different individuals conducted the test for three consecutive days. The first and

the second individuals conducted the test using a batch of SRBCs and the third one used another batch of SRBCs. Of the samples tested 54 (96.4%) showed the same titre and therefore the test was 96.4% repeatable.

4.7. Diagnostic evaluation

Predictive values of positive and negative results, diagnostic accuracy, specificity and sensitivity of the IHAT was calculated as the method described byThrusfield, (1995).

$$\text{Positive predictive value of the test} = \frac{\text{true positives}}{\text{Total positives}} \times 100 = \frac{149}{149} \times 100 = 100\%.$$

$$\text{Negative predictive of the test} = \frac{\text{true negatives}}{\text{Total negatives}} \times 100 = \frac{373}{373} \times 100 = 100\%$$

$$\text{Diagnostic accuracy (efficacy)} = \frac{\text{true negative + true positives}}{\text{Grand total}} \times 100 = \frac{373 + 149}{522} \times 100 = 100\%$$

Sensitivity and specificity of the test was 100%.

5. DISCUSSION

The study compared the detection of *Cysticercus bovis* cyst in the muscle and antibodies in sera of 522 animals slaughtered at Addis Ababa abattoir using serological tests (indirect ELISA and IHAT) and the conventional meat inspection.

Based on postmortem inspection bovine cysticercosis was detected on 7.5 % of cattle presented for slaughter at Addis Ababa abattoir. This finding is greater than the findings of Tembo (2001) and Tekka (1997), in which the prevalence was 3.2% and 2.2 - 3.2% respectively. The reason for the increase prevalence may be attributed to difference in improved quality of inspection and increased consumption of raw meat. The conventional method of meat inspection is less sensitive to pick all animals that are infected with *Taenia saginata* metacestode, which allows transmission between man and cattle (Dorny, *et al*., 2002; Minozzo, *et al.,* 2002). In this study only 39 animals were identified as infected with *C. bovis* out of 522 animals inspected at Addis Ababa Abattoir, cysticerci are easily missed during inspection because the cyst may not be present on the routine cuts. According to Dorny *et al.* (2002) and Minozzo, *et al.,* (2002) 5-50 times higher prevalence rates could be found by complete slicing of the predilection site than routine inspection. According to experimental study conducted by Minozzo, *et al.,* (2002) 85.9% of *C. bovis* were found in the skeleton muscles that are not inspected during the routine inspection. The other reason is that postmortem inspection is less sensitive when the infection is at initial stage.

Using the serological tests, which include indirect ELISA and IHAT, known positive and negative sera were tested. All cattle that were positive for cysticercosis during postmortem inspection (PMI) were positive for both serological tests. Indirect ELISA test was conducted only on those known positive and negative samples due to shortage and lack of fresh reagents. The indirect ELISA had 100% sensitivity and 81% specificity and this low specificity is may also be due to lack of fresh reagents. On the other hand the IHAT had 100% sensitivity and 91- 100 % specificity. When the total postmortem and IHAT titre results were compared, there was no agreement, k=0.336, between IHAT and PMI. The observed discrepancy between meat

inspection and serological tests can be attributed to poor quality of the conventional method of inspection since cysticerci are easily missed as they may not be present on the routine cuts and most cases of cysticercosis may be light infections.

The results of this study confirm that, in spite of the time and effort taken by meat inspectors for looking for cysticerci at predilection sites, this method is less sensitive (Dorny, *et al*.,2001; Minozzo, *et al.,*2002). This is further complicated by the fact that live cysts are translucent and often pinkish in color and may therefore be less conspicuous in the meat than dead cysts that usually form white and fibrotic lesions (Onyango Abuje *etal* 1996). The inefficiency of detecting infestations by classical meat inspection is one of the reasons for the persistence of the infection in the human population of Ethiopia. In Ethiopia bush defecation, habit of eating row beef (*kifto, Kourte, Gored-Gored*) and village slaughter might have contributed much for the increase in prevalence of cysticercosis in cattle.

When the two serological tests are compared indirect ELISA has lower specificity than IHAT where as IHAT is more specific. This agrees with the findings of OnyangoAbuje *et.al*., (1996) where he found the specificity of indirect ELISA to be low. Both IHAT and indirect ELISA are sensitive for older infections where the cysticerci are calcified. This indicates that the antibody titre is detected but drops slowly though the cysticerci are not viable.

Studies conducted to develop such serological tests in Ethiopia are rare and this is the first of its kind for bovine cysticercosis. In order to reduce the infection risk of humans there is an urgent need to assessing the risk factors for infection in cattle. The IHAT can be a useful tool for sero-epidemiological surveys and can be conducted on live herds. It may be considered as part of an integrated quality control programm for determining the quality of beef products and developing

certain standards of hygiene. It is relatively cheaper and can be done in most laboratories, as it does not require sophisticated equipment.

There was no significant association between IHAT and fecal examination results. This indicates the cross reactivity between cysticercosis of cattle and other gastrointestinal helminth infestations may be absent or very limited. When the IHAT is compared with total number of animals acquiring gastrointestinal helminth parasite in each management system cross reactivity for Cysticercosis was minimal.

Significant difference in prevalence of bovine cysticercosis was found between Repi PLC Dairy Farm and Yeka-abado farmers Association, ($p<0.05$). An overall sero-prevalence of 7.9% and 30.2% was measured respectively. The habit of eating raw meat and poor sanitation at the Yeka-abado Farmers Association might have resulted in spreading of eggs from tapeworm carriers in the environment is probably the most important reason for the higher prevalence of *C. bovis* in the area. Another important reason is the long survival time of eggs of *Taenia saginata* in the external environment, studies have indicated that on the soil may remain infective for 5 ½ to 9 ½ months (_Dorny *etal* 2000). Also, it has been shown that eggs may stay alive in the effluent from swage treatment plants and that cattle may be at risk when having access to streams carrying this effluent (Dorny *et al.,* 2002). The Repi PLC Dairy Farm, on the other hand, is relatively well managed and employs permanent personnel that are living on the farm premises with relatively good hygienic conditions. Replacement stock is usually bred on the farm. This system apparently reduces the risk of infection. One would thus expect low prevalence of bovine cysticrrcosis.

No significance difference ($p > 0.05$) in prevalence was observed between samples from Addis Ababa Abattoir and Yeka-abado farmers Association. The over all sero-prevalence were 28.5%

and 30.2% respectively. Animals slaughtered at Addis Ababa Abattoir could have been brought from such farms or from feedlots where the continuous man to animal contact is maintained. The use of causal casual workers in feedlots may be factors that are conducive to *Taenia saginata* transmission (Dorny, *et al*.2000).

Significant association was not observed between IHAT and body condition of the animals and this might be attributed to less pathogenic effect of the metacestode on the general health of the intermediate host. On experimental studies conducted in Brazile by Minnozo,*etal*.,(2002) the effect of cysticercosis on the condition score of infected experimental animals was not significant except in few individuals dosed with more than 2 $x10^4$ of *Taenia saginata* eggs. Clinical illness in such cyst carrier animals was observed (increased heart and respiratory rates and even death from myocardial degeneration). The other reason was that the animals were adults that have develop immunity. It is an established fact that animals infected acquire immunity for about 9 months (Parija, 1996).

Animals slaughtered at Addis Ababa Abattoir were 98.6% males whereas in Repi PLC Dairy Farm the animals sampled were 99.09% females. In both cases all were adults. It was not possible to compare between IHAT and age. At Yeka-abado Farmers Association the number of both sexes were almost equal, 56% female and 44% male, no significant difference was observed. As described above and based on the results of this study the difference in prevalence between Yeka-abado Farmers Association and Repi PLC Dairy Farm was not attributed to age or sex but differences in management. Due to uncontrolled grazing at the Yeka-abado Farmers Association animals have frequent chance of contact with human feaces and ingest *Taenia saginata* eggs. In Repi PLC Dairy Farm, the animals graze occasionally in the restricted farm area and the chance of getting contact with human faeces and ingesting the *Taenia saginata* eggs is lower.

6. CONCLUSIONS AND RECOMMENDATIONS

The wide distribution of *Taenia saginata/ Cysticercus bovis* is associated with several factors including: raw and under cooked beef consumption, bush defecation and poor waste disposal, poor sludge and swage treatment system, low level of public awareness and presence of backyard (village) slaughtering practices.

Conventional meat inspection technique is less sensitive (pick only 7.5% of infected cases) and time consuming, Lightly infected carcasses can be easily missed and passed for human consumption thus the infection transmission is maintained between humans and cattle. Thus taeniasis /cysticercosis, remains a widespread zoonosis that affects human health and economy through condemnation, quality degradation of frozen beef, cost of refrigeration, cost of human therapy, lowering productivity of infected workers who may be absent from work or reduce their working efficiency by creating uneasiness.

The IHAT with 100% sensitivity and 91-100% specificity can be used as a diagnostic test for epidemiological surveys, to map infected and disease free areas and to estimate the national prevalence of the disease. The test is relatively cheaper and can be conducted in most laboratories. The cyst fluid produced better discriminating titre than the scolex and membrane, demonstrating better antigenic properties.

The prevalence of *Cysticercus bovis* was higher in animals slaughtered at Addis Ababa Abattoir and Yeka-abado Farmers Association compared to Repi PLC Dairy Farm using IHA. Based on this study, cross reactivity with other gastrointestinal helminths was either absent or minimum.

The IHA test, with further improvement and evaluation, can be used to qualify animals for international movement, provide a degree of confidence that those animals that give negative test results are free of *Cysticercus bovis*. Thus the test can serve for the diagnosis of *Cysticercus bovis* within the local setting for import/export of animals.

Therefore to reduce the transmission of taeniasis/cysticercosis health education, consumption of cooked meat, improved standards of human hygiene and to increase the diagnostic potential of the tests (IHAT, ELISA) further refinement and improvement of both test systems are recommended.

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ANNEX

Annex 1: Reagents and procedures for measurement of protein concentration

The Folin Ciocalteu (Lowry) method was used to determine protein concentration. The principle is alkaline copper reaction on tryptophan and tyrosine, where phosphomolybdotungstate is reduced to heteropolymolybdenum blue by copper catalyzed reaction of aromatic amino acids. The reaction results in strong blue colour, which depends partly on the tyrosine and tryptophan content (Walker, 1997)(Annex 1).

Reagents: solution A= 2%NaCO3 in 0.1N NaOH

Solution B= 1% CuSO4 .5H_2O in distilled water (pentahydros copper sulphate)

Solution C= 2% Sodium potassium tartarate in distilled water

Solution D=Mix 50ml A + 0.5ml B + o.5ml C

Solution E= Folin Ciocalteu

Standard= dissolve 10mg BSA in 10mg PBS (Final conc. 1mg/ml)

Procedure: Test tube method

1.add to glass tubes, in duplicate

0.2ml PBS (blank)

0.2ml standard, several concentrations, from 0.4-0.025mg/ml) in PBS

0.2ml unknown, four serial dilutions in PBS

2. add 1ml reagent D. Mix well. Let to stand for 10' at room temperature
3. add 0.1ml reagent E Mix well. Let to stand for 30' at room temperature
4. read the optical density (OD) at 500-700nm (650nm).

5.determine the protein concentration in the unknown sample by comparison with the calibration curve of OD's of standards (Walker, 1996; Lowry*, et al.,* 1951).

Annex 2: Description of body condition scores

Score	Condition	Features
1	L-	Marked emaciation (animal would be condemned at ante mortem examination)
2	L (Lean)	transverse processes project prominently, neural spines appear sharply.
3	L+	Individual dorsal spines are pointed to the touch; hips, pins tail-head and ribs are prominent. Transverse processes visible, usually individually.
4	M-	Ribs, hips and pins clearly visible. Muscle mass between hooks and pins slightly concave. Slightly more flesh above the transverse processes than in L+.
5	M (Medium)	Ribs usually visible, little fat cover, dorsal spines barely visible.

6	M+	Animal smooth and well covered; dorsal spines cannot be seen, but are easily felt.
7	F-	Animal smooth and well covered, but fat deposits are not marked. Dorsal spines can be felt with firm Pressure but feel rounded rather than sharp.
8	F(Fat)	Fat cover in critical areas can be easily seen and felt; transverse processes cannot be seen or felt.
9	F+	Heavy deposits of fat clearly visible on tail-head, brisket and cod,; dorsal spines, ribs, hooks and pins fully covered and cannot be felt even with firm Pressure (Nicholson and Butterworth, 1986).

Annex 3: Equipment used for serological tests

Equipment and supplies

a. ELISA

-Micro plates

-Micro plate reader/spectrophotometer capable of reading at wavelength of 492nm

-Micro pipettes 5 to 200micoliter

-multichannel pipettes for dispensing antigen, conjugate, substrate and stop solution.

-Plate washing system

-Incubator

-Pipette tips

-Disposable basin

b. Hemagglutination

-Micro plates

-Pipettes

-Tips

-Cover folium

-Centrifuge

-Conical tubes (50ml)

-Bottles

-Incubator9water bath)

-Dialysis tube

Annex 4: Reagents used for serological tests

a. ELISA

Distilled water

Antigen

Coating buffer PH 9.6(carbonate bicarbonate buffer)

Blocking reagent, 1% BSA (bovine serum albumin)

Sera

Conjugate, goat anti-bovine IgG conjugated to HRPO (horseradish peroxidase)

Substrate-OPD (o-phenyldiamine)

-H_2O_2 (hydrogen peroxide)

-CPB (citrate phosphate buffer) PH 5.2

PBS PH 7.2

Tween-20 (T-20)

Stop solution H_2SO_4(sulphuric acid)

b. Hemagglutination

Antigen

25% glutaraldehyde

40% formaldehyde

Alsever's solution

Sheep red blood cells

PBS

FCS

Annex 5: Preparation of diferrent solutions

1. PBS: NaCl 8.0g
   KCl 0.2g
   Na_2HPO_4 1.44g
   KH_2PO_4 0.24g
   Distilled water 1000ml,
   Dissolve in 800ml and adjust PH to 7.2; then fill to one litre.

2. Coating buffer: Na_2CO_3 0.398g
   $NaHCO_3$ 0.733g
   Distilled water 250ml,
   Dissolve in 225ml and adjust PH to 9.6; then fill 250ml.
   Store at 4^0c.

3. Citrate-Phosphate buffer (CPB): Citric acid 1.16g
   Na_2HPO_4 1.8g
   Distilled water 250ml.
   Store at 4^0c.

4. 2.5NH_2SO_4 Concentrated H_2SO_4 70ml
   Distilled water 930ml.

5. Substrate solution: OPD 5mg
   CPB 10ml
   30%H_2O_2 20µl

6. Preparation of Alsever's solution

D-(+)-Dextrose, anhydrous 2.05g
Sodium citrate 0.8g
Sodium chloride 0.42g
Distilled water 100ml, the PH is adjusted to 6.1 using 10% citric acid

CURRICULUM VITAE

1. **Personal Details**

Name: Nigatu Kebede Wubie

Birth date: 1970

Birthplace: Kuandisha

Sex: male

Marital status: single

Nationality: Ethiopian

2. **Educations and Training**

School/university	Duration	Award	Instruction Media	Remark
Chara Elementary School	5yrs	6th complete	Amharic	
B.M.J Junior Secondary School	2yrs	8th complete	English	
Dangila S.S. High School	4yrs	12th complete	English	
Addis Ababa University, Faculty of Veterinary Medicine	6yrs	DVM (Doctor of Veterinary Medicine)	English	
	2yrs	M.Sc. in VPH (Veterinary Public Health)	English	
Kaliti A.I. Center	40days	Certificate	English	

3. **Language Skills**

Language	Speaking	Read and write	Remark
Amharic	Excellent	Excellent	Mother tongue
English	Very good	Excellent	Educational Language

4. **Work experiences:**

Field Veterinarian in Amhara Nationa Regional State from : 1993-1994 Alefa Tacussa Woreda, 1995-2000 Guangua Woreda and 2001 Team leader In Awi zone Deparment of Agriculture. Trainer (gust teacher) for Animal Health Technicians from March- July, 1998, March- July, 1999 and March 2000, at Kombolcha Agricultural Training Center.

Research: Prelimenary Survey of Mange Mites of Sheep and Cattle in and around Addis Ababa. DVM Thesis. Addis Ababa University, Ethiopia.

Cysticercus bovis: Development and Evaluation of Serologica Tests and Prevalence at Addis Ababa Abattoir.

5. **References:**

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