



DEVELOPMENT AND DISTRIBUTION OF *TAENIA SAGINATA* METACESTODE/ CYSTICERCUS BOVIS IN HOST AND NON-HOST ANIMALS

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ABSTRACT

Taeniasis due to *Taenia saginata* is an intestinal infection of human beings acquired by consumption of infected beef harbouring its larvae (cysticercus bovis). The objective of this study was to demonstrate the development of metacestode (cysticercus bovis) of *T. saginata* in host (calves) and non-host animals infected with eggs of *T. saginata*. Each animal was given an aliquot of $2 \times 10^5 T. saginata$ eggs in order to experimentally demonstrate the development of the cysticercus larvae. The total number of recovered cysticerci were 243 (mean = 81 cysts/animal) from all the calves. The anatomical distribution of cysticerci in the infected calves were in the following proportion: (heart 82 (33.74%), tongue 10(4.11%), diaphragm (2.47%), lungs 2(0.82%), Liver 7(2.88%), brain 2(0.82%), oesophagus 5(2.05%): 144(4.98%); skeletal muscles (masseters 22(9.05%), front limbs 28 (11.52%), hind limbs 22(9.05%), paraspinal muscles 20(8.23%), trunk 37(15.22%): 129(53.08%). The present study indicates that young calves are an ideal host model for experimental studies of maturation and development of metacestode stage of *T. saginata*.

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INTRODUCTION

Taeniasis saginata is an intestinal infection of man, acquired by consumption of infected beef harbouring its larvae (cysticercus bovis). Taeniasis is an economically significant disease in human beings responsible for high morbidity than mortality especially in developing regions of the world. Low hygiene in cattle rearing, straying of cattle, use of human feces as manure in agriculture farming, open defecation and improper disposal of human feces make cattle easily accessible to infection with the eggs of *T. saginata* and are the main cause behind its exclusively infection in human population of developing nations especially muslim regions. Taeniasis due to *Taenia saginata* occurs in two different hosts, the ultimate one are the humans (adult parasite is present) and the intermediary are cattle (cysticercus bovis is present) including cow (*Bos taurus*); buffalo (*Bos buffelus*); Zebu (*Bos indicus*), Yak (*Bos grunniens*). However, Machul Skii (1941) also diagnosed cysticerci bovis found in the cardiac and spinal musculature of the gazelle (*Gazella gutturosa*). Shpilko (1956) evaluated reindeer as a possible intermediate host of *T. saginata*. Therefore, in order to evaluate the relation as an intermediate

with normal (usual) host and non-host animals, in this regard an experimental trial was carried out with the objective to demonstrate the development of metacestode of *T. saginata* so as to get a better understanding of its life cycle and the possible influence on the epidemiology and treatment options for taeniasis.

MATERIALS AND METHODS

Preparation of inoculum of *Taenia saginata* eggs

Gravid proglottids of *Taenia saginata* were obtained from non-treated local taeniasis patients, who were enrolled for epidemiological study, at temporary constituted laboratory, at Safapora Manasbal of District Ganderbal, Kashmir, India. Around 55 segments from different patients were identified as *T. saginata* by compression between two glass plates and the microscopic analysis of uterine ramification (15 to 32 ramifications) in *T. saginata* (Joao Carles et al., 2002; Fan et al., 1992; Hayunga et al., 1991; WHO, 1983). These identified segments were opened with the small sterilized needles of different sizes (micro dissection) and were put into 0.85 % NaCl solution. The egg number was estimated with a

Neubauer chamber. An aliquot of 2×10^5 eggs were put in test tubes containing 50 ml of saline solution (Joao Carlos *et al.*, 2002; Smith *et al.*, 1991; Kyvsagaard *et al.*, 1991; Fan *et al.*, 1989, 1992; Hayunga *et al.*, 1991).

Experimental animals, their management and administration of infection

Ten healthy parasite free animals including 3 sheep, 3 goats and 4 calves ranging all in the age group of 2-6 months were used for this experimental study. All animals belonged to local breeds. The minimum age of animals were 2 months, calf (n = 1), goat (n = 1) and rest animals (n = 8) including 3 calves, 2 goats and 3 sheep were above 4 months but less than 6 months.

All the ten animals were raised in the temporary set laboratory attached with an animal house at Safapora, Manasbal of District Ganderbal Kashmir. Initially they were supplemented with milk (n = 2); 1 goat and 1 calf and rest (n = 8) animals; 2 goats, 3 calves and 3 sheep were fed with leaves of locally available forage plants and pellets ration was later introduced as feeding supplements for the animals.

Animals (n=9) including calves (n=3), sheep (n=3) and goats (n=3) were infected with *T. saginata* eggs and one non-infected calf was used as control. Each animal was given an oral dose of $2 \times 10^5 T. saginata$ eggs kept ready in separate test tubes containing 50 ml each of saline solution (0.85%). Then following the inoculation, they were kept for 5 days in individual stalls. During this time, their faeces/pellets were collected and kept until the fermentation and decomposition of the organic matter. The biophysico-parameters (body weight, rectal temperature, pulse) were recorded before and after inoculation.

Slaughtering of animals and muscular/organ necropsy

All the ten experimental animals were slaughtered commonly as the animals in Kashmir are being slaughtered in slaughter houses. First day one sheep, one goat and one calf were slaughtered, then after one week animals (n = 3), including one sheep, one goat and one calf were slaughtered. Then finally after one more week remaining animals (n = 4), including one infected calf, one sheep, one goat and one non-infected controlled calf were slaughtered. The whole slaughtering took place after 9th, 10th and 11th week of infection. The carcasses and organs, except the intestinal viscera, were removed and brought to locally set laboratory where a careful inspection took place using the total slicing technique.

All organs and animal muscles were carefully sliced each 0.5 cm as earlier adopted by Joao Carles *et al.* (2002); Fan *et al.* (1989, 1992), Hayunga *et al.* (1991), Kyvsagaard *et al.* (1989, 1991); Smith *et al.* (1991). First a careful observation of whole carcass was made using hand lenses after deskinning the animal to detect any surface nodule, cyst, or lesion. Then the carcasses were cut in individual organs.

The predilection sites were examined more carefully as being the most favourite regions for development and concentration of cysts. Head, particularly tongue and masticating region, heart and skeletal muscles were screened then the rest of organs were examined. For slicing very thin, sharp razor edged knives, cutters were used for smooth and thin slice cuts. The collected cysts were counted for each organ and site.

Classification and identification of recovered cysticerci

All recovered cysticerci were classified as alive or degenerated during the necropsy. Fully transparent cysts were considered as viable and other as degenerated, degenerated cysts were of cheesy type (its contents were yellowish and smooth) (Joao Carlos *et al.*, 2002; Fan *et al.*, 1992; Walter and Koske, 1980; Kyvsagaard *et al.* (1989). All the collected cysticerci were preserved in 4 % formalin for future scientific use.

RESULTS

The experimental infection in animals with *Taenia saginata* eggs revealed that in all the infected calves (n=3), body temperature rose to 39.8–41.9°C, with accelerated pulse and respiration, on the 2nd – 4th day of infection, and remained high until 5th – 7th. However no such change was observed in other infected animals including sheep (n=3), goats (n=3) and non-infected controlled calf. The general condition of infected calves was impaired; appetite was poor, emaciation, and rumination absent for 4 days from infection. Then after a week all these symptoms subsided spontaneously. In the 8th week of infection all the three infected calves manifested general body weakness with weight loss. Rest of the experimental animals were found healthy at this stage of post infection.

The total number of recovered cysticerci were 243 from all the experimental animals used for this trial, from the non-host animals N=6 including sheep (n=3) and goats (n=3) none of the cysticerci were recovered after adapting the same slicing technique as in rest of the infected host calves (n=3). So all the recovered cysticerci n=243 (mean=81cysts/animal), were exclusively recovered from these infected calves, however, from controlled calf (n=1) none of the cysticerci could be detected (Image 1, Table 1, Fig. 1).

From the total of 243 recovered cysts, 175 (72.01%) were considered live and 68 (27.98 %) were found degenerated. The cysticerci found in the infected calves were anatomically distributed as organs (heart 82 (33.74%), tongue 10 (4.11%), diaphragm (2.47%), lungs 2 (0.82%), Liver 7(2.88%), brain 2 (0.82%), oesophagus 5 (2.05%): 144 (46.91%); skeletal muscles (masseters 22 (9.05%), front limbs 28(11.52%), hind limbs 22 (9.05%), Para-spinal muscles 20 (8.23%), trunk 37 (15.22%): 129 (53.08%). However, there remains an apprehension to miss some of the cysticerci due to their misidentifications and degeneration. The diagnosis of bovine cysticercosis infection with *cysticercus bovis* was routinely determined by visual inspection through specific slices in the

Table 1 Distribution and concentration of recovered cysticerci from the carcasses of infected animals

Calf No.	Masseters	Tongue	Heart	Liver	Esophagus	Front limbs	Hind limbs	Lung	Diaphragm	Brain	Para spinal region	Trunk skelton	Total	%age
1	4	3	22	1	2	8	3	X	X	1	6	9	59	24.2
2	7	2	31	2	1	9	6	1	1	1	5	11	77	31.6
3	11	5	29	4	2	11	13	1	5	X	9	17	107	44.0
Total (%)	22 (9.0)	10 (4.1)	82 (33.7)	7 (2.8)	5 (2.0)	28 (11.5)	22 (9.0)	2 (0.8)	6 (2.4)	2 (0.8)	20 (8.2)	37 (15.2)	243 (100)	99.8



Image 1 Representation of various Images 1-8 showing the necropsy of various organs and muscle for the recovery of developing metacestodes.

carcasses in the slaughter houses. From the total of 243 recovered cysts, 175 (72.01%) were considered live and 68 (27.98 %) were found degenerated.

Two of the viable cysts of *C. bovis* obtained from the experimental calves were fed to two human volunteers and the manifestation of symptoms i.e., passage of gravid proglottids were obtained between 85th and 93rd of infection, other symptoms included increased appetite, nausea and abdominal discomforts

DISCUSSION

The changes in the bio-physico parameters in infected calves were found in accordance with previous workers. Ershov (1933) in the same way observed manifestations of acute cysticercosis in experimentally infected calves and adult bulls as we noted in this experimental trial. In all the experimental infected calves and bulls he found body temperature rose to 39.8 - 41.8°C on the 2nd - 4th day of infection, and remained high until 6th - 7th day. The general condition of these infected animals was impaired; the animal lay moaning; appetite was

poor, rumination absent the proventriculus atonic, constipation present. According to Neumann, (1892), Zurn noted in an experimentally calf, the temperature rose 4°C, with accelerated pulse, abdominal distention, emaciation, and difficulty in standing up.

transparent. Usually the cysticerci in the heart are subject to earliest degeneration.

As in the current experimental study the maximum cyst density was found in heart, thus may be attributed to the

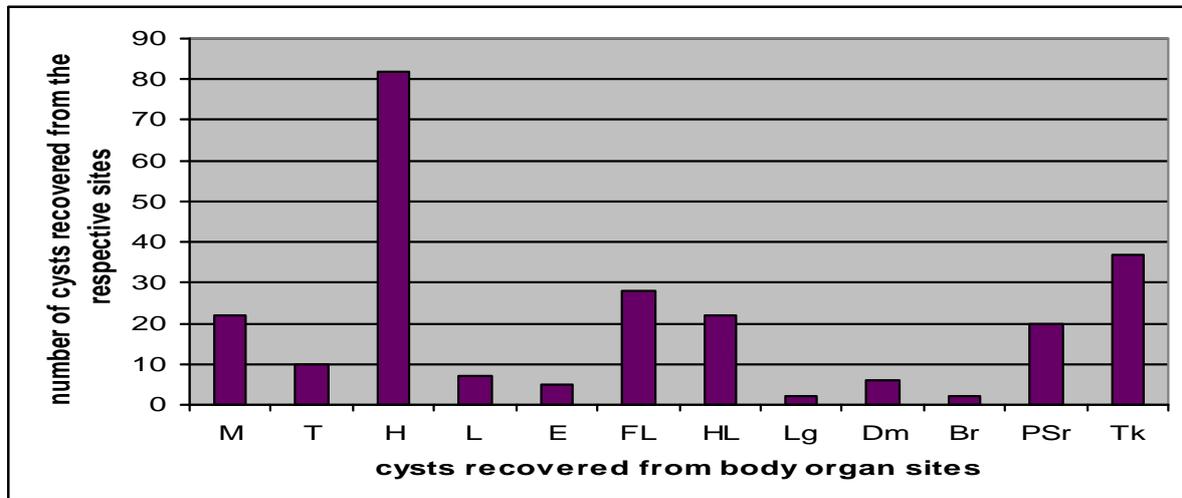


Fig 1 Graphical representation of recovered number of cysticercusbovis from their respective organ sites

(M)Masseters, (T)Tongue, (H)Heart, (L)Liver, (E)Esophagus, (L)Limbs, (FL)Fore limbs, (HL)Hind limbs, (Lg)lung,(Dm)Diaphragm, (Br)Brain, (PSr) Para spinal region, (Tk) trunk

In this experimental study the maximum cyst concentration was found in heart 82 (33.74%) followed by skeletal muscles of trunk regions 37 (15.22 %), Front limbs and Hind limbs with 28 (11.52%) and (9.05%) respectively. These results resembles with the observations of (Fan *et al.*, 1989, 1992; Smith *et al.*, 1991; Kyvsagaard *et al.*, 1991; Hayunga *et al.*, 1991; Joao Carlos *et al.*, 2002;). An experiment conducted by Fan *et al.*(1988) with infection in four month old calf that showed cysticerci were in the heart (16.67%) and the skeletal muscle (83.33%). Wanzala *et al.* (2003) found same results for distribution of cysts and found most of cysticerci in the heart. Walter and Koske (1980) found that only 60 of cysticerci infected bovines were detected through a regular inspection and the remaining 37 (61.7%) were identified only by the slicing technique. Data on the localization of cysticerci in cattle are varied; Borodin (1940) observed infection of the tongue in 48% of the cases. Of the neck muscles in 46%, of the heart in 42% and of the jaw muscles in 18%. The results of total dissection were in agreement with previous studies (Gallie *et al.*, 1983; 1987; Kyvsgaard *et al.*, 1990; Gracey *et al.*, 1994). It presents low sensitivity when the infection level is initial or low (Murrel *et al.*, 1986; Kyvsgaard *et al.*, 1989; Wanzala *et al.*, 2003). So, there remains every possibility to miss the detection of these little number of cysts thus humans remain susceptible; and unfortunately in Kashmir Valley where there is no concept of bovine cysticercosis in municipal and health departments and beef is being sold without prior inspection in the slaughter houses thus human infections due to *Taenia saginata* are endemic and in moderate levels (2.23 to 3.98%), thus eradication is almost impossible. From the total of 243 recovered cysts, 175 (72.01%) were considered live and 68 (27.98 %) were found degenerated, the cysticerci may die in the cattle. Upon death, the cysticercus walls and fluid become opaque. The neck and scolex become yellow. The connective tissue capsule usually remains unaffected. Sometimes, on the other hand, caseous masses are observed in the capsule when the cysticercus is alive and seems

maximum number of deaths in cattle due to cysticercosis. As also reported by Gracey (1992) attributed heavy infestation by the larval of *Taenia saginata* in cattle to cause myocarditis or heart failure, responsible for heavy economic losses. The metacestodes were found to cause extensive damage resulting in infiltrative, degenerative changes, haemorrhages, necrosis and exudation mainly in the vicinity of cysts.

According to Moscow meat control stations (Katkova, 1957), cysticerci were found in the heart in 19.4% of the cases. In the jaw muscles in 15%, in the omanconeus muscles in 29.4%, in the lumber muscles in 8.7% and in the neck muscles in 0.9%. According to routine inspection few selected tissues or regions (predilection sites) are only partially investigated, therefore fewer slices are made. A more careful examination conducted in these tissues could result in the great economic loss. In routine inspections only a partial slicing up to 50% of the inspected tissues would make possible to find only 7.05% of the present cysticerci (Walther and Koske, 1980; Joao Carlos *et al.*, 2002; Wanzala *et al.*, 2003). The limitations of the bovine cysticercosis are evident mainly if the infection is in a low level. Santos (1993) observed that 96.7% (4,222) of (4,366) infected bovines presented only one cysticercus; however other cysticerci must be present in skeletal muscles. While as, according to our results, 31% of the cysticerci were found in the routinely infected tissues, probably due to this limitation in the inspection the most efficient sanitary system could not interrupt the parasite disease cycles, particularly in Kashmir were beef inspection is almost absent. Thus in order to prevent the continuous parasite cycle, it would be necessary to encourage and improve the beef inspection. This could be achieved by extending the examinations to some other tissues or sites, therefore, it would be necessary to include some less important muscles simultaneously with an increase in the number and the depth of incisions, moreover the inspector's awareness of the correct identification and treatment of infected carcasses to prevent the development of new *Taenia saginata* infections.

Thornton (1951) reported that in Kenya after examining 5000 heads of infected cattle and never found young and old cysticerci simultaneously. However, young and old cysticerci were often found together. The author suggests that apparently there are two types of immunity in animals: temporary, caused by a short-term infection, which lasts for the lifetime of the parasite, and fairly permanent immunity. In present study where the single dose of *Taenia* eggs were given to experimental animals degenerated and live cysticerci were found together and immunity was felt playing an important role as found by Thornton (1951).

Young calves are considered to act as an ideal host model for experimental studies of metacystode stage of *T. saginata* as also used in the current experimental study. Calves have also been found to be very susceptible to *Cysticercus bovis* infection in their young ages and adult cattle are to some extent immune to this infection, this is worth to mention here that people of Kashmir region having common belief that Taeniasis is mostly due to the consumption of beef from older animals were as small calves if eaten even in crude form can not infect the masses. These results are also in accordance to the observations of Peel (1961) who established after many experiments that in West Africa calves are infected in the first weeks of their life, and subsequently become immune to the reinfection for several years. After 80 days the calves were immune to experimental infection. The author claims this is due to the acquisition of hereditary and individual immunity. Yet in Australia adult cattle were quite susceptible to infection. Peel suggests that the biological difference exists between the strains of *C. bovis* in Australia and in West Africa. The cysticerci may die in the cattle. Upon death, the *Cysticercus* walls and fluid become opaque. The neck and scolex become yellow. The connective tissue capsule usually remains unaffected. Sometimes, on the other hand, caseous masses are observed in the capsule when the cysticercus is alive. Usually the cysticerci in the heart are subject to earliest degeneration as also found in this experimental study.

During the routine beef inspection during current study more than 5% of the slaughtered cattle were found infected with *C. bovis* with distribution of cysts in the same manner as in experimentally infected calves with slight variations it was found that cattle of every age group were found infected and usually get infection in summer and autumn. The reason attributed might be the conducive weather for the survival of eggs and easy access of animals to acquire infection while grazing as in Kashmir cattle move at their will and are always vulnerable to infection of *Taenia* eggs. These observations are favoured by the findings of Gracey (1981) who recorded the highest incidence in autumn and summer in some European countries. The reason he attributed was the conducive weather for the survival of eggs and easy access of animals to acquire infection with grass. Moreover other good reasons in the Epidemiology of *Taenia saginata* in cattle of Kashmir were the suitable temperature and high humidity during spring and autumn which prolong the age of the eggs, however age had no significant effect on prevalence in this study which suggested that once infected, the animals acquired life-long immunity to super-infection. Oryon *et al.* (1994) who carried out a study in a 3-year period. Of 9501 cattle examined, 736 (7.7%) were infected with cysticerci of *T. saginata*. The endemic foci were identified and prevalence was significantly higher ($P < 0.005$), Kenarch (10.0%) and Shiraz area (8.5%) than elsewhere. The prevalence was significantly higher ($p < 0.005$) during spring

and autumn seasons. There was no variation in the infection rate in animals of different age groups, suggesting that immunity was acquired to super-infection. The most common sites were muscle of the shoulder (26.3%). Pharynx oesophagus and diaphragm showed 0.9, 0.5 and 0.4% infection, respectively. The metacystodes were found to cause extensive damage resulting in infiltrative, degenerative changes, haemorrhages, necrosis and exudation mainly in the vicinity of cysts. They also found that infection was the cause of condemnation of 34.6% of infected Carcasses. The rejected Carcasses and infected organs were valued at 100.1 million Rials over the 3-year period. Our results are also in accordance to Rickerd and Adolph (1977) who studied the prevalence of cysticerci of *Taenia saginata* in cattle reared on sewage-irrigated pasture and found the heart, masseter muscle, tongues and laryngeal muscles from 200 cattle aged 10 to 11 months, and 100 cattle aged 20-21 months which had been reared on sewage-irrigated pastures at Melbourne and Metropolitan Board of Works Farm, Werribee, Victoria, were examined for infection with cysticerci of *Taenia saginata* by slicing in the laboratory and the result obtained were compared with those recorded during normal meat inspection procedures at the abattoir, of the 10-11 month old cattle 51-5% were found to be infected and 8% of the total animals harboured viable cysticerci. Of the 20-21 month old animals 33% were infected, and even at this age, 8% of the animals still carried viable cysticerci. On time meat inspection at the abattoir detected significantly fewer infections than did laboratory slicing. Slonke (1978) reported 4.75% of the animals sent to slaughter from a Southern California-feed lot during a 9 month period were found to be infected with the *Cysticercus* of *T. saginata*. Dewhirst *et al.* (1978) reported that meat inspection is a useful for detecting heavily infected Carcasses. However, lightly infected Carcasses can easily be missed and passed on for human consumption. The same results were observed by Walter *et al.* (1980).

During current study it was found that cysts of *C. bovis* were found in deep muscle layers suggesting that it is very difficult to detect these deeply embedded cysts during routine meat inspection thus increasing the chances of human infection if cooked in bigger cuts without proper heat treatment these observations can be justified also by Eystein Skjerve (1999) who worked on Monte Carlo risk assessment model to estimate the public health risk of importing prime cuts of beef infected with *T. saginata* to Norway from an endemic area in Southern Africa. The model predicted that 21 (lower 5%=1), (upper 95%=56) viable cysts would be present in domestic prime cuts during 1996 and 1997, with 8 (0-21) of them being ingested without sufficient heat treatment to kill the parasite. These cysts were expected to cause 2 (0-7) human infections.

There was no commercial serological diagnostic method available in Kashmir region which could be employed to detect pre-slaughter *Cysticercus bovis* cysticercosis in local and transported cattle and huge economic losses could be prevented and also sero epidemiological studies of cattle cysticercosis could be possible with sero diagnostics. This technique was used by Dorny *et al.* (1999) who conducted the sero-epidemiological study of *Taenia saginata* cysticercosis in Belgium cattle and found 3.09% serum samples were positive in the Ag-ELISA; while by meat inspection on the same animals cysticerci were detected in only three Carcasses (0.26%). The sero-prevalence found in this study was more than 10 times higher than the annual prevalence (0.26%)

reported by Institute for Veterinary Inspection. Echert (1996) in Workshop summary: Food Safety: meat and fish-borne Zoonoses in collaboration with WHO discusses Cysticercosis caused by *Cysticercus bovis* in cattle is still a significant problem in many parts of the world. In some countries, the infection rates of cattle with *C. bovis* have increased, for example under large-scale management conditions, sometimes reaching prevalence rates of about 50%. Jael *et al.* (1996) conducted sero-epidemiological study of *Taenia saginata* cysticercosis to determine the prevalence and distribution of the infection in three provinces of Kenya through serum samples and meat inspection records. They recorded the prevalence of *T. saginata* as 15.96% and 9.97% respectively for meat infection and serological samples. With highest prevalence of cysticercosis for North district as 31.47% and 80.42% of the animals were detected respectively. Only 9.09% of the animals were diagnosed by Ab-ELISA.

Kashmir being an agricultural state where livestock industry acts as a back bone for state economy, huge economic losses may be estimated due to cysticercosis of *C. bovis* in cattle and being a major public health problem. As Pawlowski and Schultz (1972) estimated the losses due to cysticercosis as US \$ 25 per animal in developing countries and US \$ 75 per animal in industrialized countries. Slonke *et al.* (1973) near Phoenix, AZ, reported an increased incidence of bovine cysticercosis. Approximately 10% of cattle sent to slaughtered from January to April, 1973, were infected with *Cysticercus* stage of *Taenia saginata*. One employer who worked at the feed mill and loaded hay in the feeds was also found to be infected with *T. saginata*. Onyango-Abuje and Harrison (1993) estimated the loss due to *T. saginata* cysticercosis in cattle in Kenya as Ksh 56 million per annum or Circa £ UK million. Harrison, in his project dated April 1993 to March 1996 explains Tropical developing countries suffer huge losses (1.8 billion US \$ in Africa annually) because of *T. saginata* as, current meat inspection methods were not sophisticated enough to identify all infected Carcasses. That makes eradication difficult as infected cattle populations remain undetected. The project develops new tests to identify *T. saginata* in live cattle. A MAb-ELISA antigen detection assay was successfully field-tested and will, after further refinement, accurately detect the parasite and thus allow identification pre-slaughter. This will reduce Carcasses rejection, facilitate exports and consequently boost farmer livelihoods.

These kinds of experimental studied could be excellently employed to devise control model as previously adapted by Wanzala *et al.* (2003) who devised control of *Taenia saginata* by post-mortem examination of Carcasses. The results confirmed that in spite of the time and efforts taken by meat inspectors looking for cysticerci at specified predilection sites of carcasses; this method is insensitive and inaccurate. To effectively improve meat inspection procedures, there is need to increase that area and number of predilection sites observed during inspection and vary them according to the nature of the animals, their husbandry history and the target human population for consumption. In addition, other control approaches such as vaccination, chemotherapy and immunodiagnosics should be developed and implemented to complement meat inspection procedures.

CONCLUSIONS

In the present work all the cysts found were considered as *Taenia saginata*, *Cysticercus*. As none of the cysts were

recovered from non host experimental animals (n=7) including 3 young goats, 3 young sheep, and one non-infected calf after thorough screening through total slicing technique, so, we concluded from this experimental study that there is no association between goats and sheep with the larval developmental stage (*Cysticercus*) of *Taenia saginata*, and transmission of infection cannot be through mutton but only beef being the source for this infection in Kashmir.

Two of the viable cysts of *C. bovis* obtained from the experimental calves which were fed to two human volunteers and the manifestation of symptoms i.e. passage of gravid proglottids were obtained between 85th and 93rd of infection, other symptoms included increased appetite, nausea and abdominal discomforts. These results were in accordance with Shtrom (1938) who proved by an experiment on himself that the separation of mature proglottids begins 91 days after ingestion. According to Shtrom (1938), in 1869 Oliver fed several cysticerci to two people and obtained adults after 12 weeks. .

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